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The acute supplementation of combination juice of yellow watermelon *(citrullus lanatus thunb.)* - plantain (*musa paradisiacal* var. *Sapientum l.*) suppress post-exercise blood lactic acid production in rats

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ABSTRACT

Background: Yellow watermelon contains citrulline, which can suppress lactic acid production, while plantains contain potassium which is important for muscle performance. The yellow watermelon and plantain combination juice potential to be a natural sports drink that delays muscle fatigue by suppressing lactic acid production after exercise.
Objectives: To determine the effect of yellow watermelon-plantain juice on lactic acid in rats after swimming test.
Materials and Methods: This true experimental study used a post-test-only with controlled group design. Thirty Sprague Dawley rats, eight-week-old, male, were divided into five groups, namely positive control (C+), negative control (C-), dose 1 (P1), dose 2 (P2), and dose 3 (P3). The C (+) group received no juice and was not tested swimming, the C (-) group received no juice but was tested swimming, P1 received combined juice up to 1.8 g and tested swimming, P2 received combined juice up to 3, 6 g and tested swimming, P3 received combined juice up to 1.8 g with the addition of 0.27 g granulated sugar and tested swimming. The juice is given 30 minutes before the test. The swim test was performed for three minutes; after that, the blood was taken to test the lactic acid levels. The data were analyzed using the one-way ANOVA and the advanced post-hoc with the least significant difference test.
Results: The lactic acid levels in C (+), in C (-), P1, P2, and P3 groups after swimming test were 1.38 mMol / L; 7.14 mMol / L; 3.74 mMol / L; 1.66 mMol; and 2.91 mMol/L. There were differences in levels of lactic acid ($p <0.05$) in each group after the combination juice intervention was given.
Conclusion: Combination juice of yellow watermelon-plantain has an effect on lactic acid levels after swimming test. Dose 2 (3.6 g) was the best because it produces the lowest lactic acid after the swimming test.

Keywords: Yellow watermelon; Plantain; Lactic acid; Swimming test

BACKGROUND

Energy metabolism during anaerobic exercise is accentuated exclusively from muscular strength with high explosive power.[1] This process begins with the glucose breakdown process (glycolysis) as well as the glycogen breakdown process (glycogenolysis) and is independent of oxygen availability during ATP (Adenosine Tri Phosphate) formation.[2] Thus, it is important to acknowledge glucose availability in the muscle since its metabolic advantage is providing speed and strength in a short period.

Low performance in exercise can be caused by lactic acid accumulation which faster than it should be. However, exercising has an unavoidable side effect that is the production of lactic acid, which might induce muscle fatigue. L-citrulline is one of the most popular types of nutritional supplements that are legal ergogenic aids. L-citrulline has beneficial functions such as accelerating metabolite waste removal like lactic

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acid, improving endurance performance, and promoting faster recovery after exercise.[3] L-citrulline also acts as a source of energy during exercise.[4] A findings by Pérez-Guisado and Jakeman reported that supplementation of 8 g of citrulline malate can reduce muscle soreness at 24 and 48 hours following anaerobic exercise.[5] Supplementation with 8 g of citrulline 1 hour before exercise also can increase the number of reps lifting weights.[6]

Previous studies related to nutritional ergogenic have investigated that some fruits potentially be a source of nutritional ergogenic. Watermelon is one type of fruit that naturally rich in amino acids including L-citrulline. Watermelon also contains carbohydrates, potassium, phytonutrients such as carotenoids (lycopene and beta carotene), polyphenolics, and vitamins.[7] The rind watermelon juice contains 45.02 mg/g L-citrulline and flesh watermelon juice contains 43.81 mg/g.[8] Rind watermelon juice contains a higher L-citrulline content compared to flesh watermelon, and L-citrulline in yellow watermelons is higher than red watermelons.[9] Nevertheless, the findings may suggest that both flesh and rind watermelon juices potentially offer similar benefits to pure L-citrulline for improving exercise performance.[10] A study by Ridwan, *et al*., showed that supplementation with 100% flesh watermelon juice improves endurance in swimming performance in rats.[8]

Another type of fruit that has a beneficial effect as nutritional ergogenics is plantain. Plantain is one type of banana fruit, contains about 31.15 g of carbohydrates and 564 mg potassium.[11] Potassium is an electrolyte that acts as a body fluid balancer, to deliver nerve impulses and muscle contractions. Potassium plays role in muscle relaxation and also promoting muscle fatigue delay.[12] A previous study by Ustafia, *et al*., showed that bananas were more effective than banana milkshakes in removing fatigue after exercise.[13] A findings by Faturochman also showed that banana was effective to prevent muscle fatigue in an anaerobic sprint.[14] The carbohydrates both in watermelon and plantain are a good source of energy during exercise, so they can be promoting fatigue delay. Increasing the amount of glycogen storage by 25-100% can be done by consuming the carbohydrates before the exercise. It can delay fatigue during exercise up to 20%.[15] Many evidence suggest that L-citrulline give a beneficial effect for improving exercise performance. However, there is little evidence to support that watermelon juices could provide such improvements. Cutrufello, *et al*., reported that acute watermelon juice supplementation appears to be ineffective in improving exercise performance.[16] Ridwan *et al*., who reported that supplementation with 100% flesh watermelon juice improves endurance in swimming performance did the experiment in the longer term to avert such drawbacks.[8] Nevertheless, this study design used acute term supplementation by considering that the nutritional ergogenics from both the fruits will give the effect in a short period. This study used rats as subjects not in athletes directly, because the formula with the best effect has not been found. This study needs many subjects to investigate which the best formula with optimal effect.

MATERIALS AND METHODS

Design, location, and time

This study was a true experimental with a post-test-only randomized controlled group design. The experiment was conducted in Food and Nutrition Research Centre Inter-Laboratory Universitas Gadjah Mada (UGM) in May 2019. Ethical research for this study was approved by the research ethics committee with reference number 2190/KEPK/V/2019.

Materials and juices preparation

The yellow watermelon was classified as Black Orange type (*Citrullus lanatus ‘Black Orange’*), which was obtained from yellow watermelon plantation, Nusawungu, Cilacap, Central Java. with approximately weighing between 4.0 – 5.0 kg. The plantain banana used in this study was classified as *Musa paradisiaca Linn*, which was obtained from plantain plantation, Kalimanah, Purbalingga, Central Java. The plantain was characterized as yellow-colored, weighing 150 – 200 grams each fruit. Total glucose and potassium content from yellow watermelon and plantain in this study were measured in Food and Nutrition Research Centre

Inter-Laboratory UGM with analysis certificate number PS/157/V/2019. The total glucose contents for yellow watermelon and plantain were 5.19% and 15.40%, and the potassium was 111.921 mg/kg and 438.910 mg/kg respectively. The watermelons and plantains peeled to obtain the yellow flesh and white rind watermelons and the flesh of plantains. The 100 g watermelons (flesh and rind) and 100 g flesh plantains processed using a commercial blender to obtained combination juice. The juice is prepared freshly.

Animal models and treatment doses

Thirty-five, healthy, eight-week-old male Sprague-Dawley rats were obtained from the Food and Nutrition Research Centre Inter-Laboratory UGM. The animals were acclimatized for three days with a normal pellet diet and filtered tap water *ad libitum*. Rats were assigned into 5 groups of 7 rats: negative control (C-) group (not treated with combination juice before exercise), positive control (C+) group (no combination juice administration and no exercise), single-dose (P1) group (treated with single-dose of combination juice, 1.8 grams of solid forms diluted with water, before exercise), double dose (P2) group (treated with double-dose of combination juice, 3.6 grams of solid form diluted with water, before exercise), and single-dose with sugar (P3) group (treated with single-dose of combination juice 1.8 grams of solid form diluted with water and added with 0.27 grams of granulated sugar, before exercise). Determination of the combination juice doses administered to the rats was based on the calculation of mean fruit consumption servings number in humans. In humans, these fruits can be consumed separately as a fruit or as a juice with the addition of water. The details for determining the dose of combination juice in this study are shown in Table 1 below.

Table 1. Determination of The Combination Juice Doses

Groups	Treatment Dose	Explanation
Treatment 1 (P1)	1.8 g combination juice	In humans, equal with consumption of 50 g yellow watermelon + 50 g plantain
Treatment 2 (P2)	3.6 g combination juice	In humans, equal with consumption of 100 g yellow watermelon + 100 g plantain
Treatment 3 (P3)	1.8 g combination juice + 0.27 g granulated sugar	In humans, equal with consumption of 50 g yellow watermelon + 50 g plantain + 13 g granulated sugar

Exercise treatment

The rats were fed with combination juice as the source of energy, L-citrulline, and potassium before exercising. Thirty minutes after the combination juice was administered, the exercise was conducted. The anaerobic exercise was set as a swimming test, where the rats were drowned in a water pool and were left to swim for three minutes. Three minutes is the maximum duration of a lactic acid system in anaerobic exercise.[17] After three minutes of swimming, the rats were pulled up from the pool, and the post-exercise blood sample was taken.

Serum Analysis

The blood sample was collected immediately after exercise. Blood serum was attained through rats' orbital sinus to obtain lactic acid levels. Blood serum analysis was required to analyze lactic acid level after exercise resulted from a different dose of yellow watermelon-plantain juice administration specifically in treatment groups, and compared between treatment groups and control groups.

Data Analysis

Data obtained were analyzed using SPSS 20 for Windows (SPSS Inc., Chicago, IL). The data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed with post-hoc LSD test were applied to identify statistical differences between groups. Statistically, a significant result was considered at a p-value < 0.05.

RESULTS

The mean body weight of rats in this study was 209 g. Since the study design only used a post-test, data from the positive control group were used as a comparison standard. Rats are considered to be due to factors that can affect lactic acid production, such as training volume or physical activity, as homogeneous. Figure 1 shows the mean lactic acid levels in the blood of rats after swimming for three minutes. As a normal standard, the results of the C (+) group lactic acid test were used when the mice received no treatment, and their blood lactic acid levels were monitored simultaneously with the other treated groups. The normal blood lactic acid levels in this study were 1.38 mmol / L, which corresponds to the normal blood lactic acid concentrations (at rest) in humans which are < 2 mmol / L.

The highest lactic acid levels were in the C (-) group, which received no juice but did a swim test. The blood lactic acid level of group C (-) was five times higher than C (+). Meanwhile, in the treated group, the highest lactic acid levels were produced by the P1 group, almost three times higher than normal levels. The P2 group scored was the lowest of the treatment groups, almost equal to the normal group. The P3 group, with the same juice dose as P1 with added sugar, produced lower lactic acid levels than P1. The addition of sugar affects lactic acid production after swimming. In general, however, the results of statistical tests showed that there were significant differences between groups of dose variations $p < 0.05$.

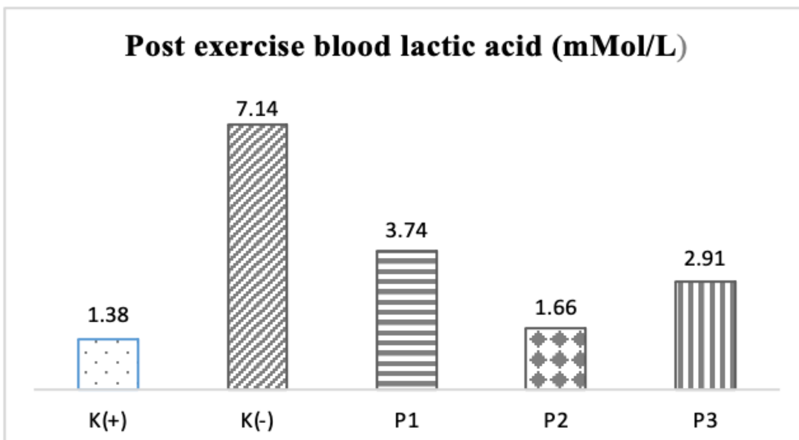

Figure 1. Comparison of mean lactic acid levels

Table 2. Mean difference lactic acid levels

Groups	Mean Difference (mMol/L)	IK 95% Minimum (mMol/L)	IK 95% Maximum (mMol/L)	*p**
C (+) vs C (-)	-5.76	-6.03	-5.49	0.00
C (+) vs P1	-2.35	-2.62	-2.08	0.00
C (+) vs P2	-0.28	-0.54	-0.01	0.04
C (+) vs P3	-1.53	-1.80	-1.26	0.00
C (-) vs P1	3.40	3.13	3.67	0.00
C (-) vs P2	5.48	5.21	5.75	0.00
C (-) vs P3	4.23	3.96	4.50	0.00
P1 vs P2	2.08	1.80	2.34	0.00
P1 vs P3	0.83	0.55	1.09	0.00
P2 vs P3	-1.25	-1.52	-0.98	0.00

* *Post hoc LSD test* (p<0,05)

The largest mean difference between the groups was between C (+) and C (-), with both standard comparisons being under opposite conditions, resting and receiving a swim test. The largest inter-group difference between treatment groups was between P1 and P2. The P2 juice dose was twice the juice dose on P1 and the result was posted swimming lactic acid production was suppressed by more than 50%. The smallest difference was between groups P1 and P3. With the addition of 0.27 g of sugar, the P3 group produced less lactic acid than P1 after swimming. The addition of 0.27 g of granulated sugar has a protective effect on lactic acid production after swimming, as the addition of sugar increases the availability of glucose for energy. However, the effect was not as good as a double dose of juice.

DISCUSSION

Muscle fatigue occurs more quickly in high-intensity and short-duration sports that require rapid energy over a short period, while oxygen uptake is very limited. This condition causes the metabolism to produce a byproduct called lactic acid. Lactic acid, which accumulates, inhibits muscle contraction and causes pain. This condition is known as muscle fatigue.[18]

The results of this study show that the administration of a combination juice at different doses to Sprague Dawley rats caused a significant difference in the mean blood lactic acid levels. The main difference was in C (+) and C (-) groups, both of which were the same control group that did not receive juice, but C (-) got three minutes of swimming activity while C (+) rested. The C (+) groups as the normal inactivity standard had a blood lactic acid level of 1.38 mmol / L. This value corresponds to the average lactic acid level of normal human blood under inactivity conditions of 1-2 mmol / L.[19] Meanwhile, C (-) group as the standard for full activity, which has the three-minute swim test without prior consumption, had blood lactic acid levels of 7.14 mmol / L.

In the treatment group, the P2 group with a juice dose twice the dose of P1 had the lowest blood lactic acid levels after exercise of all treatment groups. The lactic acid levels in the P2 group were even close to normal lactic acid levels such as C (+) under resting conditions and when the person during physical inactivity. This mean was not met by P1 and P3 groups, which means that the other two treatment groups have standard blood lactic acid levels as if they were active. Thus, it can be said that the dose of P2 has a very good protective effect in suppressing the production of lactic acid, a byproduct in the formation of energy in exercise with high intensity and short time where O2 supply for the metabolism is very limited. The P2 which administered with a combined juice of up to 3.6 g / 200 g body weight of rats had higher citrulline values than the P1 dose, which only administered of 1.8 g / 200 g body weight of rats or P3 dose that the combination juice was given 1.8 g / 200 g body weight rats with the addition 0.27 g sugar.

Citrulline is a type of amino acid that plays a role in improving athlete performance by suppressing lactic acid production. Citrulline is classified as an ergogenic non-essential amino acid involved in three metabolic pathways: detoxification of ammonia in the urea cycle, synthesis of glutamine to arginine in the gut and kidneys, and synthesis of nitric oxide.[20] During the formation of ATP for energy production in intense exercise, dehydrates AMP (adenosine monophosphate) produces ammonia. Ammonia activates phosphofructokinase and facilitates the production of lactic acid.[21] Citrulline detoxifies ammonia so that lactic acid production can be controlled.[22] Citrulline accelerates the process of elimination of ammonium and lactate in plasma and other muscle metabolites.[23] Watermelon is known to have a fairly high content of citrulline, especially in the mesocarp. Seedless yellow watermelon varieties have a higher citrulline content than the other varieties. Administering 2 g of yellow watermelon mesocarp extract can reduce lactic acid production.[24]

In P1 and P3 groups, citrulline contained in the combined juice was relatively equal, as the groups which received a juice dose of 1.8 g per 200 g of rat body weight respectively. The difference was 0.27 g of sugar was added to the P3 group. The results of blood lactic acid levels of both groups after exercise showed that P3 was better than P1. It because 0.27 g of sugar was added to the P3 group, which means that sugar absorption was increased. The addition of a simple sugar intake increases the energy source that is ready to be used in the body for activity. The availability of more ready-to-use energy sources increases the ability to perform longer activities so that muscle fatigue does not occur in a short period.[25] Previous studies have also shown a significant difference in the lactic acid levels in the blood of football players before and after given brown sugar. The treatment group showed a decrease in blood lactic acid level before and after administration of brown sugar from 11.5 mmol / L to 7 mmol / L. In the control group, there was an increase in blood lactic acid levels from 9 mmol / L to 10.8. mmol / L.[26]

This study used a combination of yellow watermelon and plantain. Juicing with a combination of two types of fruits was intended to increase nutritional value. The role of plantains in contributing to carbohydrates and high potassium. While the role of the yellow watermelon is to contribute carbohydrates and citrulline. The function of potassium as an electrolyte regulates the pH balance, the cofactor of the enzyme pyruvate kinase, Na + K + -ATPase which plays a role in energy production, synthesis of glycogen. In addition, potassium can also help train muscles and prevent muscle spasms and cramps.[12] Giving bananas before exercise greatly prevents muscle fatigue in the anaerobic phase.[27] Meanwhile, citrulline have beneficial functions accelerating metabolite waste removals such as ammonium and lactate from the plasma and the other results of muscle metabolism so the muscle fatigue does not occur quickly.[3]

The dose of combination juice was supplemented varied in each group. The doses were given according to watermelon and banana household standards consumption, 100 g each fruit. The dose was converted into mice with a conversion number of 0.018. In this study, the P2 group which received the juice dose of 3.6 g / 200 g body weight showed the lowest blood lactic acid production. It showed that the dose of combination juice made from 100 g of yellow watermelon and 100 g of plantain is most effective in suppressing lactic acid production in the blood during high-intensity short-term activities.

CONCLUSION

The combination juice of yellow watermelon-plantain affected suppressing the production of post-exercise blood lactic acid. The best dose was 3.6 g per 200 g rats body weight. It produced optimal suppression of post-exercise lactic acid production with the lowest blood lactic acid levels as if there was no activity.

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