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The effect of soybean tempeh milk and soybean tempeh youghurt on fatigue after maximal excercise

Perbedaan pemberian susu tempe kedelai dengan yoghurt tempe kedelai terhadap indikator kelelahan setelah latihan fisik maksimal

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Abstract

Studies report skeletal muscle fatigue during repeated intense muscle contractions resulting from depletion of energy substrates, and accumulation of metabolic. *Creatine kinase* (CK) and lactic acid are enzymes that are directly related to energy metabolism. This study aims to analyze the differences of soy tempeh milk and soy tempeh yogurt on fatigue indicators, namely CK levels and lactic acid in subjects induced by maximum exercise. The subjects of this study used experimental animals, namely white rats with a total sample of 28 rats. The dose of soy tempeh milk and soy tempeh yogurt given was 4,2 ml/BW of rats while the soy tempeh yogurt was given was 4,4 ml/BW, each containing 3 grams of tempeh. Blood samples were tested using the Elisa method and the results were analyzed using SPSS version 20. The results of this study showed significant changes in serum CK and lactic acid after being given the intervention of soy tempeh milk and tempeh yogurt ($p<0,05$). Soy tempeh milk and soybean tempeh yogurt significantly suppressed the increase in CK and lactic acid in rats induced by maximum exercise. Both provide protein containing BCAAs which are a source of energy during exercise. Further clinical trials are needed to determine the effectiveness, safety, and side effects of soy tempeh milk or soy tempeh yogurt when given to humans.
Keywords: Maximum exercise, milk, tempeh, yogurt

Abstrak

Penelitian melaporkan kelelahan otot rangka selama kontraksi otot intens yang berulang terjadi akibat penipisan substrat energi, dan akumulasi produk sampingan metabolik. *Creatine kinase* (CK) dan asam laktat merupakan enzim yang berhubungan langsung dengan metabolisme energi. Penelitian ini bertujuan untuk menganalisis perbedaan pemberian susu tempe kedelai dan yoghurt tempe kedelai terhadap indikator kelelahan yaitu kadar CK dan Asam laktat pada subjek yang diinduksi latihan maksimal. Subjek penelitian ini menggunakan hewan coba yaitu tikus putih dengan total sampel 28 ekor tikus. Dosis susu tempe kedelai dan yoghurt tempe kedelai yang diberikan adalah 4,2 ml/BB tikus sedangkan yoghurt tempe kedelai adalah diberikan adalah 4,4 ml/BB, masing-masing mengandung 3 g. tempe. Sampel darah diuji dengan metode Elisa dan hasilnya dianalisis menggunakan SPSS versi 20. Hasil penelitian ini menunjukkan perubahan yang signifikan terhadap serum CK dan asam laktat setelah diberikan intervensi susu tempe kedelai dan yoghurt tempe kedelai ($p<0,05$). Susu tempe kedelai

dan yoghurt tempe kedelai signifikan menekan peningkatan CK dan asam laktat pada tikus yang diinduksi latihan maksimal. Keduanya menyediakan protein yang mengandung BCAA yang menjadi sumber energi selama latihan.
Kata Kunci: Latihan maksimal, susu, tempe, yoghurt

Introduction

Muscle fatigue is associated with a decrease in the power and working capacity of skeletal muscles (Theofilidis et al., 2018). Research reports that skeletal muscle fatigue during repeated intense muscle contractions occurs as a result of the depletion of energy substrates and the accumulation of metabolic byproducts (Kenney, Wilmore, and Costill, 1995). Fats (triacylglycerol), glycogen, phosphocreatine (PCr), and adenosine triphosphate (ATP) are found in the sarcoplasm as energy stores. Mellin AK et al. (2019) reported a 9,8% decrease in speed in the 400m swimming over 12 weeks of competition time due to energy deficiency. The decline in physical performance during maximal exercise that takes place quickly affects anaerobic power. Anaerobic energy production involves the replenishment of adenosine triphosphate (ATP) from the creatine kinase pathway (ATP-PCr system), as well as glycogenolysis and glycolysis pathways (Reaburn and Dascombe, 2009).

Creatine kinase (CK) and lactic acid are enzymes directly related to energy metabolism. Trinidad et al. (2018) mentioned that creatine kinase (CK) and lactic acid increased at the end of each exercise session, which became an acute marker for fatigue detection related to the type, duration, and intensity of exercise. (Trinidad et al., 2018). High metabolic demand under intensive exercise conditions causes ATP utilization rates to exceed capacity, and ATP-PCr in this case has a high ATP generation rate (Kennedy, Tamminen, and Holt, 2013). This condition triggers mecanime by creatine kinase (CK) in anaerobic ATP resynthesis, which provides rephosphorylation of PCr and ADP (Fedotovskaya et al., 2012). CK can come out into the blood as a result of rhabdomyolysis of muscle cell membranes after intense exercise for a long time. This inhibits PCr formation and causes mitochondrial PCr depletion (Brancaccio et al., 2010).

Muscle activity and muscle fatigue factors can also be explained through the formation of lactic acid (Yusni and Amiruddin, 2019). Blood lactic acid measurements were used to estimate energy expenditure in anaerobic metabolism and looked at athletes' resistance to fatigue during high-intensity exercise (Theofilidis et al., 2018). High lactic acid levels result in decreased metabolic capacity and impaired ATP production (Sarma, 2018). Chronic, progressive increases in lactic acid without adequate recovery time will also inhibit the energy supply of aerobic energy metabolism in muscle cells and cause intracellular metabolic acidosis (Yusni and Amiruddin, 2019).

Research recommends the intake of carbohydrates and additional protein to speed up post-workout recovery (Nielsen, Lambert, and Jeppesen, 2020). Hall et al. (2013) reported that male subjects who consumed 250 mL of carbohydrate drinks with added protein every 15 minutes during a bicycle race experienced a 1,8% increase in time-trial performance compared to trials using only carbohydrates (Hall et al., 2013). Protein provides essential amino acids (EAAs) and non-essential amino acids (Moore et al., 2014). Increased oxidation of BCAAs occurs during exercise, and BCAAs are used as an energy source for exercise (She et al., 2010). Intramuscular amino acid oxidation may account for about 2–5% of ATP regeneration during 60–90 minutes of endurance training (Stone et al., 2006). Branched-chain amino acids (BCAAs) (leucine, isoleucine, and valine) are the most relevant amino acids metabolized during exercise.

BCAA supplementation can be given in the form of sports drinks. Previous research showed that using soy juice drinks containing 3,2 g of protein per 100 ml decreased CK and lactic acid activity after exercise (Widyastuti, 2019). Protein sources that have similarities with soybeans and are potential enough to be developed, one of which is by utilizing local food, namely soybean tempeh.

Soybean tempeh is a traditional Indonesian food that has recently been in the spotlight for its superior nutritional quality and metabolic regulatory function (Jauhari et al., 2014). Tempeh milk is one of the alternatives to processed tempeh products that have not been widely developed. The nutrient content in soybean tempeh has been reported to increase due to the fermentation process (Messina, 2016). The high content of BCAAs (valine, leucine, and isoleucine) and isoflavones in tempeh has the potential to have benefits for post-workout recovery (Jauhari et al., 2014).

Another product that can be used as an alternative to tempeh processing is yogurt. Tempeh milk has potential as a basic ingredient in making yogurt (Ruben Wicaksono, 2016). Research on tempeh yogurt has begun to be developed, but there have been no studies looking at the effect of tempeh yogurt itself on fatigue in athletes. The protein content in tempeh yogurt has the potential to be a source of essential amino acids and BCCA, which is the main source of energy during exercise (She et al., 2010).

Soy tempeh milk and soy tempeh yogurt are expected to be used as an alternative that can provide protein content for contractile protein repair, thereby reducing the risk of rabdomiliosis and suppressing the increase in post-exercise blood CK, which can interfere with PCr re-formation. Consumption of soy tempeh milk and soy tempeh yogurt is also expected to suppress the increase in lactic acid concentration and the release of lactic acid from the muscles during exercise, so as to maintain capacity metabolism and ATP production.

Methods

The type of research used is a true experiment with a randomized control group. The subjects of this study were white rats (Rattus norveigicus) of the Sprague-Dawley strain with eight weeks of age obtained from the Nutrition Laboratory, Center for Food and Nutrition Studies, Inter-University Center (PAU), Gajah Mada University.

This study is a pre-clinical study that aims to see the difference in the effect of giving soy tempeh milk and soy tempeh yogurt on creatine kinase (CK) and lactic acid, which are divided into four treatment dose groups. This research covers the field of food science for nutritional interventions that will be given with maximum physical activity. The intervention of feeding soy tempeh milk and soy tempeh yogurt was carried out before the treatment of maximal physical activity in rats.

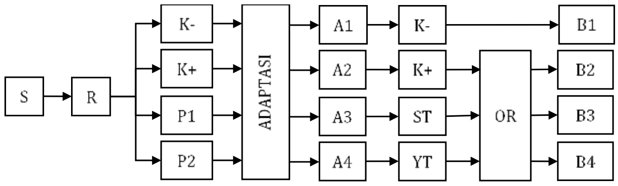

Figure 1. Research design

The minimum number of samples used under Federer's formula is 6, with the addition of 20% of samples in anticipation of dropout in each treatment group. The total sample in this study was 28 mice.

The rats used in the study were male rats with an age of 8 weeks. The body weight of the subject is 200–250 grams. The rats were conditioned in cages for 12 hours in the light and 12 hours in the dark. The room temperature is conditioned between 20 and 24 °C. Subsequent randomization is carried out after adaptation to determine the treatment group.

Table 1. Treatment groups

Rat Group	Treatment
Negative Control (K-)	Standard feed + aquades + without treatment of high physical activity
Positive Control (K+)	Standard feed + drinking ad libithum + treatment of maximum physical activity
Treatment 1 (P1)	Standard feed + soy tempeh milk drink (4.2 ml/BB) + maximum physical activity treatment
Treatment 2 (P2)	Standard feed + minunam soy tempeh yogurt (4.4 ml/BB) + maximum physical activity treatment

Conditioning before the intervention is aimed at reducing water-induced stress without promoting physiological changes in relation to physical training. Rats will swim in plastic containers filled with water with a height of 40–60 cm. The temperature of the water used for swimming is monitored in the range of 33–36° (Jones, 2007). Rats will swim for approximately 10 minutes per day until reaching a state of exhaustion for 3 days (Zhou et al., 2019). Increased weight on the tail by 6% of body weight when swimming (Gobatto et al., 2001).

The condition is indicated by the rat's head submerged in the water, which within 3 seconds shows no diving, kicking, or other grunting movements (Zhou et al., 2019). The average time obtained when the mice swam to exhaustion during the adaptation period would be used as the duration on the day of the intervention.

The formulation used for tempeh milk used a single-treatment CRD/RAL (complete randomized design) trial design. Tempeh is used as much as 1 kg, cut into 1.5 x 1.5 x 1.5 cm3, then steam blanched for 10 minutes at a temperature of 80–100 °C. Tempeh is then milled using a blender, to which is added water in a ratio of tempeh and water 1: 2 so that tempeh milk is obtained.

Soy tempeh yogurt is made using soy tempeh milk that has been pasteurized at 70–80 °C within 30 minutes. The next step is milk cooled to a temperature of 40 °C and then inoculated aseptically with a commercial yogurt stater with the Biokul brand, as much as 5% of the volume of tempeh milk, then incubated for 12 hours at a temperature of 40 °C. The yogurt contains the probiotic bacteria Lactobacillus achophilus and Lactobacillus bifidobacterium.

The intervention time for giving soy tempeh milk and soy tempeh yogurt is 7 days. The mice were previously weighed in the morning to adjust the dose to be given. Soy tempeh milk was given 10 minutes before the swimming sports intervention. The dose of soy tempeh milk and soy tempeh yogurt given was in accordance with the effective dose of tempeh in the previous study, which was 3 grams per BB rats, so the dose of tempeh milk given was 4,2 ml per BB rats, while the dose of soy tempeh yogurt given was 4,4 ml per BB rats.

$$\text{Dosage Calculation} = \frac{tempeh\ needed\ x\ supernatan}{tempeh\ used}$$

$$\text{Soy Tempeh Milk} = \frac{3\ g\ x\ 1400\ ml}{1000\ g} = 4{,}2\text{ ml}$$

$$\text{Soybean Tempeh Yogurt} = \frac{3\ g\ x\ 1470\ ml}{1000\ g} = 4{,}4\text{ ml}$$

Blood collection is done twice, before and after (pre and post) the intervention. Blood is drawn through the orbitol. A total of 5 ml using a syringe and accommodated in a vacuotainer. Blood is centrifuged at 3000 rpm for ± 5 minutes to separate serum and blood cells. A serum sample is taken using a pipette and inserted into an Eppendorf tube. The sample was then tested using the Elisa method using the General Lactic Acid ELISA Kit (LA) tool for the measurement of lactic acid levels and CK activity seen with Beckman UniCel® DxC800 Synchron by the enzymatic rate method.

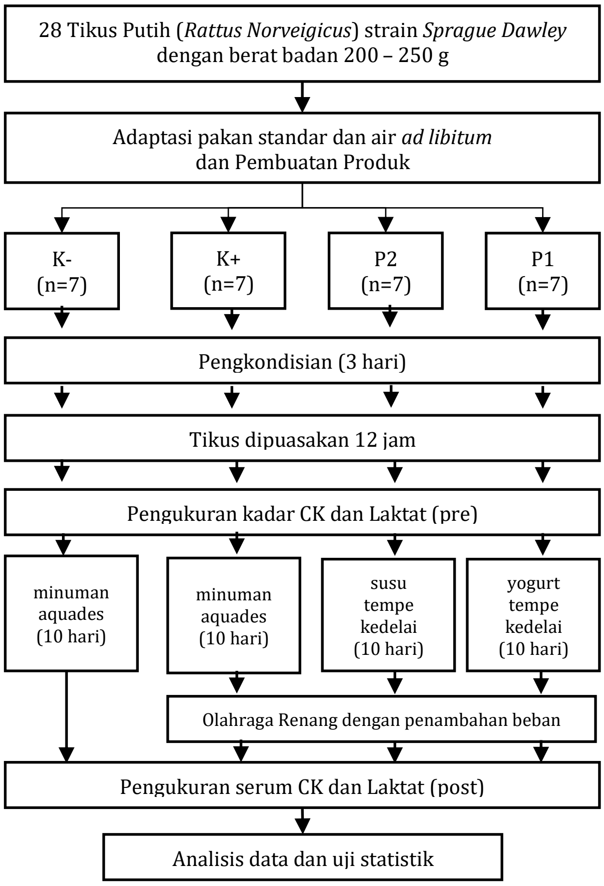

Figure 2. Research flow

The test results were then analyzed using SPSS version 20. The data taken included weight measurement data, creatine kinase, and blood lactic acid levels. The first analysis was a group analysis to see the difference in CK and lactic acid levels of each group using the one-way ANOVA test, followed by Tamhane's post hoc test. The next analysis is a different test of pre-test and post-test data using the Paired T-Test test.

Result and Discussion

The body weight of the mice before and after the intervention in each group experienced very significant changes (p = 0,000). The group that experienced the highest weight gain was the no-treatment group (K-), while the lowest weight increase was in the maximal activity treatment group (K+). The low increase in body weight in the K+ group is in line with Aaron's research (2017), which states that maximal activity treatment significantly inhibits weight gain in rats. The rats' weight gain after maximal physical activity further occurred after the intervention in the form of soy tempeh milk and soy tempeh yogurt (Harun, Susanto, and Rosidi, 2017). This is due to the high quality of soybean tempeh protein and its good digestibility, so it can play a role in the growth of rats.

Post-hoc Bonferoni test results showed a significant difference in changes in mouse weight between groups K+, P1, and P2 (p = 0,000) when compared to group K-. Significant weight differences (p = 0,000) could also be seen between groups P1 and P2 compared to K+, but no significant difference (p = 1,000) between groups P1 and P2. This is because the intervention of soy tempeh milk and soy tempeh yogurt provides additional energy during maximum physical exercise.

Maximal physical exercise increases energy expenditure and increases the catabolism of proteins and amino acids. The protein content in soybean tempeh provides essential amino acids (BCAAs: valine, leucine, and isoleucine), which are very beneficial for fatigue recovery (Setiawan, Susanto, and Kartasurya, 2020). So as to maintain a stable body weight with sufficient energy availability during maximum physical exercise.

CK levels

The most significant increase in average CK levels was seen in the K+ group, which doubled after the intervention. In P1, there was still an increase in the average CK, while in the P2 group, there was a decrease after the intervention. This is because in the K+ group, during the intervention of maximum physical activity, rats were only given aquades, while the P2 group was given soy tempeh milk yogurt.

Table 3. CK levels before and after intervention

Rat Group	CK Pre (Mean±SD)	CK Post Rate (Mean±SD)	*p* value	Δ (Mean±SD)
K-	63,12 ± 2.44	65,21 ± 2,97	0,018*	2,09 ± 1,72
K+	61,91 ± 2.39	124,40 ± 7,02	0,000*	62,50 ± 6,83
P1	62,51 ± 2.34	65,82 ± 3,58	0,024*	3,32 ± 2,93
P2	63,41 ± 2.56	60,70 ± 1,45	0,004*	-2,70 ± 1,59

p = Paired T-Test; *p'* = *One-Way ANOVA*; * = *p* <0,05 (meaningful)

The results of this study showed significant changes in serum CK after the intervention of soy tempeh milk when compared to the K+ group (p = 0,000). CK is found in the cytosol and mitochondria of tissues with high energy requirements. Muscle fatigue in general is a result of energy insufficiency and the availability of metabolites that allow muscles to increase energy demand. Soy tempeh milk and soy tempeh yogurt contain enough protein to provide BCAAs that are important for the recovery of muscle fatigue.

This is in line with research by Ra et al. (2018), which states that repeated BCAA supplementation before exercise has a beneficial effect on serum CK activity (Ra et al., 2018). Kim et al. (2013) also mentioned that BCAA supplementation (78 ml/kg body weight) significantly decreased lactic acid and CK at 30 minutes post-exercise (Kim et al., 2013). BCAAs are essential amino acids that can be oxidized in skeletal muscle. Instrumental in increasing energy expenditure and increasing BCAA oxidation. BCAAs contribute to energy metabolism during exercise as an energy source and substrate to expand the intermediate pool of the citric acid cycle and for gluconeogenesis (Shimomura et al., 2004). BCAA concentrations comprise more than one-third of the amino acids required in contact skeletal muscle proteins (Harper, Miller and Block, 1984). BCAAs stimulate muscle protein resynthesis and exhibit an adaptive response of skeletal muscle that aids in the growth and repair of contractile proteins, thus facilitating recovery (Breen et al., 2011).

Increased muscle contractiles can lower the risk of rhabdomyolysis thereby suppressing the increase in post-exercise blood CK. Increased CK needs to be controlled because rhabdomyolysis of muscle cell membranes can result in inhibiting PCr formation and cause mitochondrial PCr depletion that can disrupt the energy system (Brancaccio et al., 2010).

Table 4. Lactate levels before and after intervention

Rat Group	CK Pre (Mean±SD)	CK Post Rate (Mean±SD)	*p* value	Δ (Mean±SD)
K-	1,93 ± 0,08	1,98 ± 0,10	0,023*	0,04 ± 0,04
K+	2,15 ± 0,08	8,71 ± 0,53	0,000*	6,57 ± 0,52
P1	2,02 ± 0,09	3,50 ± 0,13	0,000*	1,48 ± 0,11
P2	1,99 ± 0,07	2,91 ± 0,09	0,000*	0,92 ± 0,06

p = Paired T-Test; *p'* = *One-Way ANOVA*; * = *p* <0,05 (meaningful)

The results of this study also showed significant changes in serum CK after being given the soybean tempeh yogurt intervention when compared to the control group (p = 0,001). The protein content in yogurt can be a source of essential amino acids and BCAAs which are a source of energy during exercise. The content of GOS in tempeh also has functional value as a prebiotic. Prebiotics are a good substrate used for yogurt that can increase probiotic bacteria. This is in line with Chen et al's research which showed that probiotic supplementation exerts an antifetrational effect by lowering serum levels of lactic acid, ammonia, and creatine kinase as indicators of exercise-induced muscle fatigue (Chen et al., 2016). Probiotic supplementation can increase glucose utilization to increase endurance exercise time by increasing energy production during exercise so as to delay fatigue.

Lactate Levels

The test results showed that there was a significant difference in lactate levels compared to K (p = 0,000). Blood lactic acid concentrations reflect muscle metabolism and muscle activity that can convey fatigue-related information (Goh et al., 2012). Lactic acid leaving muscle fibers is accompanied by hydrogen ions that have the buffer capacity effect of extraseluer. This is intended to control intramuscular pH so that it does not drop, which can interfere with cell function. High-intensity exercise can lower the pH to 6,4 (normally 7,1) when lactic acid builds up in large amounts. Low pH levels can also stimulate muscle-free nerve endings, causing pain (Maughan RJ, 2000).

Lactic acid production after long-term exercise can be significantly altered by changes in BCAA availability. Soy tempeh milk and soy tempeh yogurt contain enough protein to provide BCAAs that are important for the recovery of muscle fatigue. BCAAs provide additional substrates for energy through the direct oxidation of exogenous BCAAs to supply TCAI (via the TCA cycle) (Harper, Miller, and Block, 1984). BCAAs will enter the tricarboxylic acid cycle (TCA), and when the catabolism of BCAAs increases, BCAAs significantly suppress lactic acid production (Hormoznejad, Zare Javid, and Mansoori, 2019). These results are in line with Tang and Chan's (2017) research, which says that BCAA supplementation of 12 g/day for 15 days enhances plasma lactic acid clearance after resistance exercise, which supports rapid recovery from muscle fatigue (Tang and Chan, 2017).

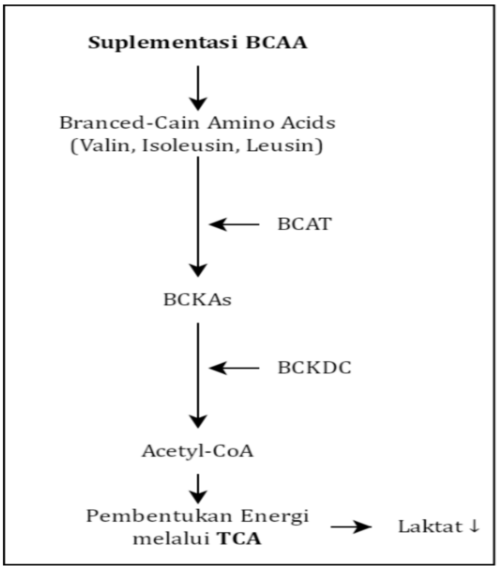

Figure 3. BCAA catabolism

The results of this study also showed significant changes in lactic acid levels after the soybean tempeh yogurt intervention when compared to the control group (p = 0,000). Soy tempeh yogurt, a derivative product of soy tempeh milk, also provides protein, which is a source of essential amino acids, and BCAAs, which are a source of energy during exercise.

The limitation of this study was that the physical freshness at the beginning of the experiment was not the same between the mice, and the daily physical activity of the mice could not be controlled. This study still requires further research by paying attention to the physical freshness of mice by controlling physical exercise before and during the study.

Conclusion

Results showed soy tempeh milk and soy tempeh yogurt significantly suppressed the increase in serum CK and lactic acid in maximally exercise-induced rats. Both provide BCAA-containing proteins that become a source of energy during exercise.

Suggestions: It is necessary to conduct further clinical trials to determine the effectiveness, safety, and side effects of soy tempeh milk products or soy tempeh yogurt if given to humans.

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