Journal of Biosciences and Medicines, 2022, 10, 18-29
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Red Beetroot Juice and Stamina: An Experimental Study

Galina Smirnova[1,2], Peteris Tretjakovs[3], Anna Fedotova[1], Raimonds Simanis[3], Svetlana Vasiļjeva[2], Olegs Suhorukovs[4], Dalija Seglina[5], Inta Krasnova[5], Vadims Bartkevics[6], Dmitry Babarykin[1*]

[1]Institute of Innovative Biomedical Technology Ltd., Riga, Latvia
[2]Institute of Biology of the University of Latvia, Riga, Latvia
[3]Riga Stradins University, Riga, Latvia
[4]Hospital of Traumatology and Orthopaedics, Riga, Latvia
[5]Institute of Horticulture, Dobele, Latvia
[6]Institute of Food Safety, Animal Health and Environment, Riga, Latvia
Email: *dmitry.b@parks.lv

How to cite this paper: Smirnova, G., Tretjakovs, P., Fedotova, A., Simanis, R., Vasiļjeva, S., Suhorukovs, O., Seglina, D., Krasnova, I., Bartkevics, V. and Babarykin, D. (2022) Red Beetroot Juice and Stamina: An Experimental Study. *Journal of Biosciences and Medicines*, **10**, 18-29.
https://doi.org/10.4236/jbm.2022.109002

Received: July 8, 2022
Accepted: August 30, 2022
Published: September 2, 2022

Abstract

Introduction: Red beet juice is increasingly used in sports to enhance the endurance of athletes. To increase the specific efficiency of red beet juice, the method of its fractionation by ultrafiltration was used for the first time. The purpose of the study was to evaluate the effect of fractionated beetroot juice on the strength and endurance of laboratory rats. **Methods:** Male Wistar rats (6 groups; n = 10) were used in the study. In addition to the standard chow, some groups of rats 2 h before the exercises received 0.5 ml of native (RBJ) or fractionated (FRBJ) *per os*. Three groups of animals were trained using a motorized wheel with a gradual speed increase over four weeks: 20 min/day for five days a week. Muscle strength of animals in all groups was measured by electronic dynamometry and the endurance of rats was evaluated once a week using electrical stimulation on a racetrack which moved at a speed of 15 m/min. The test was performed an hour after the ingestion of RBJ or FRBJ. At the end of the experiment, biochemical blood indices were determined. FRBJ was prepared by the original method. **Results:** Most significant differences in the chemical composition of RBJ and FRBJ were found for glutamic acid, of which the content was 67.2% higher than in native juice. The greatest changes during the experiment were in the mass of the rats' calf muscles. Regular running exercise caused a 29% increase in muscle mass. The additional increase in *m. gastrocnemius* was also provided y FRBJ – 12%. The combination of physical activity and the introduction of red beet derivate led to the increase of the calf muscle mass by 121% within a month. Assessing the endurance of animals by frequency of falling from the treadmill, we can conclude that compared with untrained animals, trained rats receiving FRBJ had three times higher levels of endurance. **Conclusion:** Consumption of FRBJ led to increased muscle strength in rats and the ergogenic effect of the product was significantly higher in combination with physical activity.

Keywords

Red Beetroot, Ultrafiltration, Running Trained Rats, Muscle Strength, Endurance, Anabolic Effect, Glutamic Acid

1. Introduction

The public's interest in using red beetroot as a means of increasing muscle strength and endurance manifested itself only about 20 years ago. In folk medicine, red beetroot juice is mainly used as an anti-anemic agent [1]. However, until recently, this fact practically has not been studied. In a series of experiments, it was observed that beet juice stimulates the absorption of iron (ions) in the intestine and increases blood hemoglobin in iron-deficient individuals [2]. The functional properties of red beet juice (RBJ) are usually associated with a group of red pigments-betacyanins, mainly betanin, which determines the antioxidant effect of beetroot [3]. The ergogenic effect of RBJ is associated with nitrates, previously considered as a contaminant of vegetables. A report about a significant increase in the endurance of cyclists under the influence of red beet juice has been published [4]. Since that time, beet juice has become one of the most popular juices among athletes' cost-effective drink, which exhibits anti-inflammatory, antioxidant, and vasodilatation property [5]. Studies have been done to modify the functionality of beet juice using unconventional technologies. Experiments with red beet juice water or ethanol extract gel filtration on Sephadex, which aimed to isolate "active fractions" to increase the juice functionality, did not give the desired result [6]. However, the idea of red beet juice fractionation seems promising. Recently, using diafiltration [7] and ultrafiltration [8] methods, it was possible to divide beet juice into fractions based on molecular weight and study the biological effects of the various fractions. After a series of experiments, it was found that the low-molecular fraction of red beet juice was characterized by modified biological effects. For example, the fraction of beetroot juice which was obtained by ultrafiltration at cut-off-point 20 kDa. The ability to modulate the differentiation of rat bone marrow mesenchymal multipotent cells *in vitro*, increase blood microcirculation, and stimulate active iron transport in chick's duodenum is 2.5 times more effective than native beet juice [2]. The present study evaluated the effect of fractionation based on molecular mass of red beetroot juice (FRBJ) as well as on the physical strength and endurance of rats (running) trained under aerobic loading conditions in a laboratory experiment for the first.

2. Material and Methods

2.1. Ethics Statement

All experimental procedures were approved by the Animal Ethics Committee of the Food and Veterinary Department, Ministry of Agriculture in Latvia.

2.2. Animals and Experimental Design

Laboratory male *Wistar* rats were used for the investigation. Sample of 5-week-old animals with body weight of 160 g were used in the study. Sixty rats were randomized into 6 groups of 10 heads in each. Group 1 (control)—healthy rats, which were fed standard rat chow (protein 20%, total fat 4.8%, carbohydrate 59.4%, fiber 13%, energy value 14.0 MJ/kg). Group 2 (control trained)—healthy rats, which were fed the same standard rat chow. In addition, group 3 and 4 were administered *per os* 0.5 ml fractionated red beetroot juice (FRBJ), but group 5 and 6—native red beetroot juice (RBJ) was administered 2 h before exercises. Groups 2, 4 and 6 animals were trained using a motorized wheel with gradual speed increase during the 4 weeks period: 20 min/day for five days a week. Muscle strength of animals in all groups was measured by electronic dynamometry using BIO-GS3 grip strength test version 3+ (BIOSEB, USA) and endurance of rats was evaluated once a week using electrical stimulation on a racetrack which moved at a speed of 15 m/min. The test, which lasted 20 min, was performed an hour after the ingestion of RBJ or FRBJ.

2.3. Biochemical and Physiological Assays

At the end of the experiment, biochemical blood indices, lactate, glucose, glutamate dehydrogenase, urea, creatinine, iron were determined on the analyzer ILAB 300 Plus (Instrumentation Laboratory, USA). On the last day of the experiment, heparinized blood was drawn for blood element analysis on Cell-Dyn® 3700 (Abbott, USA). Venous blood oxygen partial pressure (pO_2) was analyzed using a gas analyzer RapidLab® 1265 (Siemens, Germany). Animals were euthanized by transcervical dislocation an hour after determining the performance and blood sampling [9]. Adipose tissue was collected from the abdominal cavity and weighed, and parameters of the right and left *gastrocnemius* muscle weight and animal body weight gain were determined.

2.4. Fractionated Red Beetroot Juice (FRBJ) and Native Red Beetroot Juice (RBJ)

The tested FRBJ was prepared using previously described method [2]. The chemical composition was analyzed for the original (native) and fractionated juice. Total sugar and reducing sugar were determined by a Modification of Somogyi-Nelson Method [10], respectively. Ethanol was determined by AOAC Official Method 942.06. Protein in RBJ as well as in FRBJ was measured using KT 200 Kjeltec™, but total and free amino acids were determined using ion exchange chromatographic method by an Amino Acid Analyzer T339 (Mikrotechna Praha) AOAC Official Method 985.28. The content of the following amino acids was determined: aspartic acid, threonine, serene, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. The content of pigment betalain components (red betanine and yellow vulgaxanthin-I) in juice was determined using the spectrophotometric method [11]. Betain was determined using high performance liquid chromatography coupled with mass spectrometry [12]. The total phenol concentration was determined according to Singleton *et al.* [13]. The total phenol content was expressed as gallic acid equivalent to 100 ml of FRBJ sample (mg GAE/100 ml FW). Flavonoid concentration was measured by Čanadanović-Brunet *et al.* method [14]. Total flavonoid content was expressed as mg rutin equivalents (RuE) per 100 ml of FRBJ. Iron (Fe) concentration was measured with an atomic absorption spectrophotometer (Perkin-Elmer, Analyst 700) [15]. Nitrate concentration was analyzed following the ion chromatographic method based on EN 12014-2 "Determination of nitrate and/or nitrite content, Part 2" 2017 [16].

2.5. Statistical Analysis

All statistical analyses were performed using the software Statistica 7. Results of body weight and visceral fat mass of rats and biochemical parameters are presented as means ± SE. Multiple group comparison was done using *one-way* ANOVA and Post-hoc Tukey HSD test. Statistical significance was attributed to $P < 0.05$.

3. Results

Table 1 shows the data for chemical composition of native and fractionated red beet juice. The most significant differences in the chemical composition of RBJ and FRBJ were found for glutamic acid, of which the content was 19.3% (total content) in the ultrafiltrate and 67.2% (free amino acid) higher than the native juice (**Table 1**). The content of reducing sugars, iron and nitrates in both juice variants was the same.

During the experiment, changes in the physical strength and endurance of animals were observed. Under the influence of FRBJ (group 3), the muscle strength of the front paws increased by 22%, and all paws, by 21%. In trained animals (group 4), this indicator was 38% and 26%, respectively. The effect of native juice was less pronounced than that of fractionated juice. This was observed both with and without running performance.

Assessing the endurance of animals by the frequency of falling from the treadmill, the differences between the 3rd and 4th groups were found to be approximately double due to the influence of FRBJ. If we compare the 1st (control) and 4th groups in terms of the frequency of falling from the treadmill, we can conclude that compared with untrained animals, trained rats receiving FRBJ had three times higher level of endurance (**Table 2**).

Table 1. Chemical composition and energy value of native red beetroot juice and fractionated red beetroot juice.

Ingredient	Red beetroot juice	Fractionated red beetroot juice	P-value
Protein, g/100ml	1.51 ± 0.03	1.68 ± 0.03	0.03
Total amino acids, g/100ml including glutamic acid	1.45 ± 0.14 0.72 ± 0.07	1.71 ± 0.10 1.01 ± 0.12	0.14 0.038
Betanin, mg/100ml	59.6 ± 1.2	68.5 ± 1.8	0.04
Vulgaxanthin-I, mg/100ml	34.4 ± 1.0	32.7 ± 2.0	0.47
Betaine, mg/100 ml	158.3 ± 21.8	185.0 ±19.7	0.39
Sugar (total), mg/100ml	6.3 ± 0.7	4.6 ± 0.9	0.17
Phenols (total), GSE* mg/100ml	60.9 ± 1.2	51.4±1.4	0.014
Flavonoids (total), **RuE mg/100ml	40.8 ± 1.0	34.4 ± 0.3	0.044
Energy value, kcal/100ml	30.35	23.13	

*gallic acid equivalent; **rutin equivalent.

Table 2. Effect of native red beetroot juice (RBJ) and fractionated red beetroot juice (FRBJ) intake on physiological parameters of the trained (for 1 month) in a motorized wheel laboratory rat.

Group of rats	Muscle strength, g		Muscle strength gain**/body weight gain	Physical stamina (number of falling from a racetrack)
	Front paws	All paws together		
1 (control)	254.7 ± 17.1[c*]	554.8 ± 18.3[c]	1.05 ± 0.07[c]	69.0 ± 10.2[a]
2 (trained)	297.4 ± 10.2[b]	605.0 ± 17.9[b]	1.27 ± 0.08[b,c]	40.0 ± 6.5[b]
3 (+FRBJ)	297.8 ± 12.2[b]	612.9 ± 18.5[b]	1.22 ± 0.05[b,c]	46.0 ± 11.1[a,b]
4 (trained + FRBJ)	353.4 ± 23.8[a]	671.4 ± 22.6[a]	1.67 ± 0.16[a]	23.0 ± 4.6[c]
5 (+RBJ)	272.1 ± 14.1[b]	587.5 ± 9.8[c]	1.14 ± 0.08[c]	54.3 ± 8.3[a,b]
6 (trained + RBJ)	307.3 ± 11.9[b]	610.6 ± 14.5[b]	1.29 ± 0.05[b]	35.8 ± 7.1[b,c]

*Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$). **All paws together.

In trained rats (group 4), lactate reducing effect of FRBJ was more pronounced than for RBJ (**Table 3**).

The level of iron in the blood was stable in all groups, but in group 4, it was 19% lower than the control group (**Table 4**).

Data on the dynamics of the body weight of rats during the experiment shows that both training and consumption of both types of juice contributed to an increase in the body weight of rats. Training animals contributed to an increase in body weight, most likely due to an increase in muscle mass, as evidenced by the dynamics of changes in calf muscle mass in trained rats of group 2. Regular supplementation with FRBJ also led to an increase in body weight, mainly due to growth of adipose tissue (**Table 5**).

Table 3. The effect of native red beetroot juice (RBJ) and fractionated red beetroot juice (FRBJ) intake on blood indices in rats.

Group of rats	Hb, g/dL	RBC, M/uL	Lactate, mmol/L	pO_2, mmHg	Glucose, mmol/L
1(control)	12.9 ± 0.5[a*]	6.6 ± 0.3[b]	6.3 ± 0.4[a,b]	62.5 ± 7.5[a]	4.7 ± 0.4[a]
2 (trained)	13.1 ± 0.2[a]	7.8 ± 0.2[a]	6.9 ± 0.3[a]	60.1 ± 3.0[a]	4.3 ± 0.4[a]
3 (+FRBJ)	13.2 ± 0.1[a]	7.1 ± 0.5[a,b]	6.1 ± 0.5[a,b]	66.8 ± 4.5[a]	4.9 ± 0.3[a]
4 (trained + FRBJ)	13.4 ± 0.2[a]	7.9 ± 0.4[a]	5.9 ± 0.3[b]	70.9 ± 3.2[a]	4.9 ± 0.5[a]
5 (+RBJ)	13.0 ± 0.2[a]	7.2 ± 0.3[a,b]	6.2 ± 0.4[a,b]	64.3 ± 2.0[a]	5.0 ± 0.2[a]
6 (trained + RBJ)	13.1 ± 0.3[a]	7.3 ± 0.2[a,b]	6.1 ± 0.3[a,b]	67.1 ± 3.7[a]	4.9 ± 0.3[a]

*Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$).

Table 4. The effect of native red beetroot juice (RBJ) and fractionated red beetroot juice (FRBJ) intake on blood biochemical indices in rats.

Group of rats	Glutamate dehydrogenase, mmol/L	Urea, mmol/L	Creatinine, mmol/L	Iron, mmol/L
1 (control)	5.90 ± 0.20[a*]	8.09 ± 0.46[a]	0.06 ± 0.01[a]	68.3 ± 15.2[a]
2 (trained)	5.42 ± 0.54[a,b]	7.89 ± 0.30[a]	0.06 ± 0.02[a,b]	69.2 ± 13.1[a]
3 (+FRBJ)	5.43 ± 0.61[a,b]	7.01 ± 0.47[a,b]	0.05 ± 0.02[a,b]	70.2 ± 17.1[a]
4 (trained + FRBJ)	5.01 ± 0.30[b]	5.29 ± 0.65[b]	0.04 ± 0.01[b]	55.2 ± 14.5[a]
5 (+RBJ)	5.47 ± 0.20[a,b]	7.64 ± 0.34[a]	0.05 ± 0.01[a,b]	71.4 ± 10.7[a]
6 (trained + RBJ)	5.26 ± 0.33[a,b]	6.99 ± 0.40[a]	0.05 ± 0.02[a,b]	68.7 ± 14.5[a]

*Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$).

Table 5. Effect of native red beetroot juice (RBJ) and fractionated red beetroot juice (FRBJ) intake on calf and visceral adipose tissue mass of the trained (for 1 month) in motorized wheel laboratory rats.

Group of rats	*Mm. Gastrocnemius* weight, g	Visceral adipose tissue weight, g
1 (control)	0.52 ± 0.12[b*]	4.80 ± 0.82[a,b]
2 (trained)	0.73 ± 0.26[a,b]	4.42 ± 1.60[a,b]
3 (+FRBJ)	0.58 ± 0.13[b]	5.21 ± 0.81[a]
4 (trained + FRBJ)	1.15 ± 0.20[a]	3.04 ± 0.91[b]
5 (+RBJ)	0.59 ± 0.24[b]	5.01 ± 0.99[a,b]
6 (trained + RBJ)	0.88 ± 0.10[a]	4.15 ± 0.86[a,b]

*Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$).

The greatest changes during the experiment were due to the weight of the calf muscle of rats. Regular running exercise caused a 29% increase in muscle weight

($p < 0.05$). The additional increase in *m. gastrocnemius* weight was also provided by FRBJ – 12% ($p > 0.05$). However, the combination of physical activity and the introduction of the red beet derivate led to an increase in the calf muscle mass by 121% within a month (**Table 5**).

Combination of running performance and the introduction of FRBJ led to a significant (37%) reduction in visceral fat mass (**Table 5**).

4. Discussion

The desire to enhance the functional properties of beet juice necessitated the need for new technological approaches in its processing. We have developed a technology for ultrafiltration, which produces red beetroot derivative and allow you obtain a product that has the ability to stimulate the absorption of iron in the duodenum by a factor of 2.5 compared to native juice [17]. This study examined the ergogenic properties of FRBJ in the context of physiological and biochemical changes in rats which were trained in a motorized wheel with gradual speed increase for 4 weeks.

Changes in the physical strength and endurance of rats receiving FRBJ may be associated with the influence of betaine to a certain extent. Although, the literature data on the mechanism of betaine's influence on skeletal muscles are contradictory. On the one hand, it is reported that betaine supplementation enhances skeletal myogenesis in mice [18], however, ducks showed only an increase in antioxidant activity and muscle content [19]. A systematic review on the effect of betaine on muscle strength shows that the stimulating effect has not yet been proven [20].

Is there any reason to link the differences in the ability to stimulate the strength and endurance of native and fractionated beet juice with its amino acid composition? Among the amino acids in terms of ergogenicity, beta-alanine [21] stands out, which is included in the composition of sports nutrition products and whose effect is confirmed by a systematic review and meta-analysis [22]. However, in our study, the content of beta-alanine in the studied products was the same. A more likely stimulant is glutamic acid, as evidenced by recent studies [23].

In our experiment, running training led to an increase in the physical strength of animals, both for front and all legs at the same time due to the synergistic effect of exercise and FRBJ. The frequency of rats falling off the treadmill is affected by regular running performance (group 2) and beet product (group 3) separately, they had an equal effect, but both factors together (group 4) doubled the endurance index (**Table 2**). This phenomenon is most likely the result of several factors: an increase in skeletal muscle mass, a slowdown in aerobic oxidative deamination of amino acids, as evidenced by a decrease in glutamate dehydrogenase activity and urea content in the blood of rats in group 4 (**Table 4**). A decrease in urea levels in the blood may indicate an anabolic effect of FRBJ. This phenomenon is also described by trained people and may be a reflection of decreased glutamate dehydrogenase activity [24].

It can be assumed that FRBJ contributed to the improvement of skeletal muscle oxygenation. Trained rats which received red beetroot product were characterized by a minimum level of lactate and a maximum partial pressure of blood oxygen (**Table 3**). This fact can be explained both by a higher level of blood oxygenation under the action of FRBJ and by the influence of betaine contained in beet juice, which inhibits the growth of lactate in the blood after exercise [25]. Due to the action of nitrates of beet juice which occurred during intense exercise, the microvascular RBC muscle concentration increased [26]. This explains the conclusion of Papadoupolos *et al.* [27] that beetroot juice promotes oxygenation and increases muscle strength during sustained isomeric exercise but does not affect the effectiveness of the oxidative processes of muscle tissue during relaxation.

Endurance training improves skeletal muscle blood flow and increases oxygen supply to myocytes during muscle contraction [28]. FRBJ potentiated the anabolic effect of physical activity, and the main role is performed by nitrates, which after bioconversion into nitrites, are a source of nitric oxide—a vasoactive substance and a powerful stimulator of microcirculation. A transient decrease in oxygen availability due to a drop in PO_2 during muscle contraction is one of the main causes of reduced endurance, *i.e.* ability to perform repetitive tasks. Reduced NO bioavailability and prolonged muscle metabolic recovery are commonly observed in ageing and diseased populations [29]. In relation to stimulation of microcirculation, beetroot juice is more effective than sodium nitrate in its pure form [30].

The safe daily dose of nitrates allowed in the EU countries is 3.7 mg/kg body weight [31], although, sports nutrition containing 400 mg/100 ml of nitrates is offered on the market [32]. Taking into consideration information about possible risks [33], it is not advisable to get carried away with high doses of nitrates to achieve an ergogenic effect. The FRBJ studied in this paper contained about 120 mg/100ml. Despite the discovery of nitrate's role in red beetroot juice induced vasodilation, we are far from a complete understanding of the mechanism of red beetroot product's impact on the cardiovascular system. Bahadoran *et al.* [34] made a systematic review and meta-analysis results demonstrate the blood pressure-lowering effects of red beetroot juice and highlight its potential nitrate-independent effects. Of all nitrate rich vegetables, precisely red beetroot provides the most significant microcirculation stimulation. It seems *Beta vulgaris* specific effects are the result of a complex impact of a few biologically active substances. The degree of increased blood flow and oxygen delivery to the muscles under the action of beet nitrates depends on the type of muscle fibers. In this respect, the calf muscles of rats are more sensitive to the action of beet juice than the flounder muscle (*m. soleus*) [28]. According to modern concepts, vasodilators (nitric oxide), anaerobic energy sources (beta-alanine) and organic osmolytes (betaine) have the best effect in terms of stimulating muscle mass gain [35]. There is reason to believe that other biologically active substances, in addition to those mentioned above, can increase the strength and endurance of skeletal muscles. So, exercises are a factor that induces oxidative stress, leading to a reduction in force generation and increased muscle atrophy [36]. On the other hand, the resulting reactive oxygen species (ROS) stimulate the synthesis of antioxidants. Additional intake of antioxidants is widespread in sports. With this in mind, it is logical to use beet juice and its derivatives containing a powerful beet pigment and antioxidant betalain. The red pigment betanin is 1.5 - 2 times more active than anthocyanins [37]. The mechanism of modulating the functionality of beet juice by ultrafiltration remains unclear. The concentration of tested biologically active substances in the ultrafiltrate does not differ significantly from the native juice (**Table 1**). It is possible that as a result of deproteinization of the juice, some high molecular weight components associated with the inhibition of the corresponding biological effects of beet juice are removed. This hypothesis is supported by experiments on the antimicrobial activity of fractionation on the basis of molecular mass plant extracts and juices—red chicory, mushroom, raspberry, green and black tea, and cranberry juice, in which low molecular mass fractions were found to be significantly more active than those of high molecular mass [38]. The results of the study suggest that supplementation with FRBJ, which has an ergogenic effect, improves endurance in running trained rats. However, the mechanism of this phenomenon requires further investigation.

5. Conclusions

Consumption of FRBJ led to increased muscle strength in rats, and the ergogenic effect of the product was significantly higher in combination with physical activity. During a month of training, the weight of the calf muscle doubled, and the endurance of animals, if judged in terms of the frequency of falling from the treadmill, increased three times.

We conclude from the obtained results that regular use of FRBJ can significantly improve muscle strength and endurance not only for athletes, but also for a wide range of people suffering from diseases of the cardiovascular system, musculoskeletal system, and other related diseases.

Conflicts of Interest

The authors declare no competing interests.

References

[1] Hamedi, S. and Honarvar, M. (2019) *Beta vulgaris*—A Mini Review of Traditional Uses in Iran, Phytochemistry and Pharmacology. *Current Drug Discovery Technologies*, **16**, 74-81. https://doi.org/10.2174/1570163815666180308142912

[2] Babarykin, D., Smirnova, G., Markovs, J., Vasiljeva, S., Basova, N., Simanis, R., *et al.* (2019) Therapeutic Effect of Fractionated by Ultrafiltration Red Beetroot (*Beta vulgaris* L.) Juice in Rats with Food-Induced Fatty Liver. *European Journal of Biological Research*, **9**, 1-9.

[3] Czapski, J., Mikołajczyk, K. and Kaczmarek, M. (2009) Relationship between Anti-

oxidant Capacity of Red Beet Juice and Contents of Its Betalain Pigments. *Polish Journal of Food and Nutrition Sciences*, **59**, 119-122.

[4] Wylie, L.J., Kelly, J., Bailey, S.J., Blackwell, J.R., Skiba, P.F., Winyard, P.G., *et al.* (2013) Beetroot Juice and Exercise: Pharmacodynamic and Dose-Response Relationships. *Journal of Applied Physiology*, **115**, 325-336. https://doi.org/10.1152/japplphysiol.00372.2013

[5] Stander, Z., Luies, L., van Reenen, M., Howatson, G., Keane, K.M., Clifford, T., *et al.* (2021) Beetroot Juice—A Suitable Post-Marathon Metabolic Recovery Supplement? *Journal of the International Society of Sports Nutrition*, **18**, Article No. 72. https://doi.org/10.1186/s12970-021-00468-8

[6] Lee, J., Kim, Y., Friso, S. and Choi, S.-W. (2017) Epigenetics in Non-Alcoholic Fatty Liver Disease. *Molecular Aspects of Medicine*, **54**, 78-88. https://doi.org/10.1016/j.mam.2016.11.008

[7] Mereddy, R., Chan, A., Fanning, K., Nirmal, N. and Sultanbawa, Y. (2017) Betalain Rich Functional Extract with Reduced Salts and Nitrate Content from Red Beetroot (*Beta vulgaris* L.) Using Membrane Separation Technology. *Food Chemistry*, **215**, 311-317. https://doi.org/10.1016/j.foodchem.2016.07.132

[8] Krumina, G., Babarykin, D., Smirnova, G., Vasiljeva, S., Basova, N., Krumina, Z. and Babarikina, A. (2016) Red Beet (*Beta vulgaris*) Root Juice Membrane Ultrafiltration Use to Modify Product's Functionality. 2*nd International Conference Nutrition and Health*, Riga, 5-7 October 2016, 66.

[9] Close, B., Banister, K. and Baumans, V. (1996) Recommendations for Euthanasia of Experimental Animals: Part 1. *Laboratory Animals*, **30**, 293-316. https://doi.org/10.1258/002367796780739871

[10] Hatanaka, C. and Kobara, Y. (1980) Determination of Glucose by a Modification of Somogyi-Nelson Method. *Agricultural and Biological Chemistry*, **44**, 2943-2949. https://doi.org/10.1080/00021369.1980.10864408

[11] von Elbe, J.H. (2001) Betalains. *Current Protocols in Food Analytical Chemistry*, F3.1.1-F3.1.7. https://doi.org/10.1002/0471142913.faf0301s00

[12] Servillo, L., D'Onofrio, N., Giovane, A., Casale, R., Cautela, D., Ferrari, G., *et al.* (2018) The Betaine Profile of Cereal Flours Unveils New and Uncommon Betaines. *Food Chemistry*, **239**, 234-241. https://doi.org/10.1016/j.foodchem.2017.06.111

[13] Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. (1999) Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, **299**, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

[14] Čanadanović-Brunet, J.M., Savatović, S.S., Ćetković, G.S., Vulić J.J., Djilas S.M., Markov S.L., *et al.* (2011) Antioxidant and Antimicrobial Activities of Beet Root Pomace Extracts. *Czech Journal of Food Sciences*, **29**, 575-585. https://doi.org/10.17221/210/2010-CJFS

[15] Jorhem, L. and Engman, J. (2000) Determination of Lead, Cadmium, Zinc, Copper, and Iron in Foods by Atomic Absorption Spectrometry after Microwave Digestion: NMKL Collaborative Study. *Journal of AOAC International*, **83**, 1189-1203. https://doi.org/10.1093/jaoac/83.5.1189

[16] https://infostore.saiglobal.com/preview/293951856829.pdf?sku=872698_SAIG_NSAI_NSAI_2074987

[17] Babarykin, D., Smirnova, G., Krumina, G., Vasiljeva, S., Krumina, Z., Basova, N., *et al.* (2018) Stimulating Effect of Red Beetroot (*Beta vulgaris*) Juice, Fractioned by

Membrane Ultrafiltration, on Iron Absorption in Chicken Intestines. *Journal of Biosciences and Medicines*, **6**, 37-49. https://doi.org/10.4236/jbm.2018.611005

[18] Du, J., Shen, L., Zhang, P., Tan, Z., Cheng, X., Luo, J., *et al* (2018) The Regulation of Skeletal Muscle Fiber-Type Composition by Betaine Is Associated with NFATc1/MyoD. *Journal of Molecular Medicine*, **96**, 685-700. https://doi.org/10.1007/s00109-018-1657-2

[19] Chen, R., Wen, C., Cheng, Y., Chen, Y.P., Zhuang, S. and Zhou, Y.M. (2019) Effects of Dietary Supplementation with Betaine on Muscle Growth, Muscle Amino Acid Contents and Meat Quality in Cherry Valley Ducks. *Journal of Animal Physiology and Animal Nutrition*, **103**, 1050-1059. https://doi.org/10.1111/jpn.13083

[20] Ismaeel, A. (2017) Effects of Betaine Supplementation on Muscle Strength and Power. *Journal of Animal Physiology and Animal Nutrition*, **31**, 2338-2346. https://doi.org/10.1519/JSC.0000000000001959

[21] Goron, A. and Moinard, C. (2018) Amino Acids and Sport: A True Love Story? *Amino Acids*, **50**, 969-980. https://doi.org/10.1007/s00726-018-2591-x

[22] Saunders, B., Elliott-Sale, K., Artioli, G.G., Swinton, P.A., Dolan, E., Roschel, H., *et al*. (2017) β-Alanine Supplementation to Improve Exercise Capacity and Performance: A Systematic Review and Meta-Analysis. *British Journal of Sports Medicine*, **51**, 658-669. https://doi.org/10.1136/bjsports-2016-096396

[23] Coqueiro, A.Y., Rogero, M.M. and Tirapegui, J. (2019) Glutamine as an Anti-Fatigue Amino Acid in Sports Nutrition. *Nutrients*, **11**, Article No. 863. https://doi.org/10.3390/nu11040863

[24] Graham, T.E., Turcotte, L.P., Kiens, B. and Richter, E.A. (1997) Effect of Endurance Training on Ammonia and Amino Acid Metabolism in Humans. *Medicine and Science in Sports and Exercise*, **29**, 646-653. https://doi.org/10.1097/00005768-199705000-00010

[25] Trepanowski, J.F., Farney, T.M., McCarthy, C.G., Schilling, B.K., Craig, S.A. and Bloomer, R.J. (2011) The Effects of Chronic Betaine Supplementation on Exercise Performance, Skeletal Muscle Oxygen Saturation, and Associated Biochemical Parameters in Resistance Trained Men. *Journal of Strength and Conditioning Research*, **25**, 3461-3471. https://doi.org/10.1519/JSC.0b013e318217d48d

[26] Aucouturier, J., Boissière, J., Pawlak-Chaouch, M., Cuvelier, G. and Gamelin, F.X. (2015) Effect of Dietary Nitrate Supplementation on Tolerance to Supramaximal Intensity Intermittent Exercise. *Nitric Oxide—Biology and Chemistry*, **49**, 16-25. https://doi.org/10.1016/j.niox.2015.05.004

[27] Papadopoulos, S., Dipla, K., Triantafyllou, A., Nikolaidis, M.G., Kyparos, A., Touplikioti, P., *et al*. (2018) Beetroot Increases Muscle Performance and Oxygenation during Sustained Isometric Exercise, but Does Not Alter Muscle Oxidative Efficiency and Microvascular Reactivity at Rest. *Journal of the American College of Nutrition*, **37**, 361-372. https://doi.org/10.1080/07315724.2017.1401497

[28] Booth, F.W., Ruegsegger, G.N., Toedebusch, R.G. and Yan, Z. (2015) Endurance Exercise and the Regulation of Skeletal Muscle Metabolism. *Progress in Molecular Biology and Translational Science*, **135**, 129-151. https://doi.org/10.1016/bs.pmbts.2015.07.016

[29] Hirai, D.M., Copp, S.W., Ferreira, L.F., Musch, T.I. and Poole, D.C. (2010) Nitric Oxide Bioavailability Modulates the Dynamics of Microvascular Oxygen Exchange during Recovery from Contractions. *Acta Physiologica*, **200**, 159-169. https://doi.org/10.1111/j.1748-1716.2010.02137.x

[30] Ferguson, S.K., Holdsworth, C.T., Wright, J.L., Fees, A.J., Allen, J.D., Jones, A.M., *et*

al. (2015) Microvascular Oxygen Pressures in Muscles Comprised of Different Fiber Types: Impact of Dietary Nitrate Supplementation. *Nitric Oxide—Biology and Chemistry*, **48**, 38-43. https://doi.org/10.1016/j.niox.2014.09.157

[31] EUR-Lex-02006R1881-20200928-EN-EUR-Lex [WWW Document] (n.d.). https://eur-lex.europa.eu/legal-content/LV/TXT/?uri=CELEX:02006R1881-20200928

[32] Mosher, S.L., Andy Sparks, S., Williams, E.L., Bentley, D.J. and Mc Naughton, L.R. (2016) Ingestion of a Nitric Oxide Enhancing Supplement Improves Resistance Exercise Performance. *Journal of Strength and Conditioning Research*, **30**, 3520-3524. https://doi.org/10.1519/JSC.0000000000001437

[33] Habermeyer, M., Roth, A., Guth, S., Diel, P., Engel, K.-H., Epe, B., *et al.* (2015) Nitrate and Nitrite in the Diet: How to Assess Their Benefit and Risk for Human Health. *Molecular Nutrition and Food Research*, **59**, 106-128. https://doi.org/10.1002/mnfr.201400286

[34] Bahadoran, Z., Mirmiran, P., Kabir, A., Azizi, F. and Ghasemi, A. (2017) The Nitrate-Independent Blood Pressure-Lowering Effect of Beetroot Juice: A Systematic Review and Meta-Analysis. *Advances in Nutrition*, **8**, 830-838. https://doi.org/10.3945/an.117.016717

[35] Cholewa, J., Trexler, E., Lima-Soares, F., de Araújo Pessôa, K., Sousa-Silva, R., Santos, A.M., *et al.* (2019) Effects of Dietary Sports Supplements on Metabolite Accumulation, Vasodilation and Cellular Swelling in Relation to Muscle Hypertrophy: A Focus on "Secondary" Physiological Determinants. *Nutrition*, **60**, 241-251. https://doi.org/10.1016/j.nut.2018.10.011

[36] Steinbacher, P. and Eckl, P. (2015) Impact of Oxidative Stress on Exercising Skeletal Muscle. *Biomolecules*, **5**, 356-377. https://doi.org/10.3390/biom5020356

[37] Gliszczyńska-Świgło, A., Szymusiak, H. and Malinowska, P. (2006) Betanin, the Main Pigment of Red Beet: Molecular Origin of Its Exceptionally High Free Radical-Scavenging Activity. *Food Additives and Contaminants*, **23**, 1079-1087. https://doi.org/10.1080/02652030600986032

[38] Daglia, M., Papetti, A., Mascherpa, D., Grisoli, P., Giusto, G., Lingström, P., *et al.* (2011) Plant and Fungal Food Components with Potential Activity on the Development of Microbial Oral Diseases. *Journal of Biomedicine and Biotechnology*, **2011**, Article ID: 274578. https://doi.org/10.1155/2011/274578