

Effect of Beetroot Extract Supplementation on the Athletic Performance of Trained Rugby Athletes

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Study Protocol:

- Objective:

To evaluate the effect of nitrate supplementation, based on the consumption of beetroot juice, on athletic performance in rugby players.

- Design:

The research use a quasi-experimental design, using a non-randomized, crossover controlled trial, with the aim of minimizing the effects associated with each athlete's individual performance patterns, given that each participant acted as their own control. A double-blind protocol was implemented. Participants were divided into two groups (Beetroot-Placebo and Placebo-Beetroot); the researcher prepared waffles containing both beetroot (a source of nitrates) and placebo. A person external to the study was responsible for administering the compounds to each group, ensuring the corresponding crossover was performed in the second phase of data collection, so that all participants received both the placebo and nitrate supplementation at the end of the study.

- Methods

Beetroot and placebo (Waffles):

These are given to participants on the third day before the physical tests, to be consumed from that day onward, the following day, and on the day of the physical tests. To ensure consumption of the compounds two hours before physical activity, participants must send a photo of their consumption with their sports practice schedule, and two hours before their appointment for the physical tests.

There is then a 3-day washout period, during which participants are not given any compounds and cannot consume foods rich in nitrates (they are given a list of these foods). The process is repeated with the crossover compound (those who received placebo receive beetroot, while those who received the placebo receive beetroot).

- Physical Tests:

The protocol implemented by Sella, F. et al. (2023) is followed, with the 40 meters sprint being the first test. Prior to performing the sprints, participants completed a 10-minute standardized warm-up comprising jogging, dynamic stretches, running drills, and three stride-outs at increasing intensity. The physical tests are then performed, beginning with a 40-meter sprint. After an adequate 10-minute rest period, the Bronco test is performed. After 20 minutes, strength tests are performed, starting with the squat and then the bench press, and ending with the Borg scale.

This protocol is followed on both testing days; participants perform tests on the same day of the following week, at the same time

- **Background**

Specifically, in the case of nitrates in rugby, the evidence is scarce. There are some studies, such as the one conducted by Esen et al. (2023), which evaluated the effect of beetroot supplementation on trained male rugby players. They were given an average of 12.8 mmol of nitrates and a placebo with less than 0.08 mmol of nitrates, ingested 3 hours before physical tests. These studies revealed no improvement in the yo-yo endurance test or the countermovement jump.

On the other hand, there is the study conducted by López-Samanes et al. (2022) on semi-professional female rugby players, which studied the influence of beetroot on neuromuscular performance. Providing similar amounts of nitrates to the aforementioned study yielded a positive response in the countermovement jump exercise; however, there were no differences in speed, agility, strength, and endurance tests. Thus, despite the dose, differences are found depending on the protocol used.

However, while specific studies on rugby are limited, there is research in sports or activities with similar characteristics. An example of this is the study by Domínguez et al. (2018), who evaluated the effect of beetroot supplementation on high-intensity intermittent exercise. Their research observed an improvement in performance when rest periods between efforts were short. These improvements were primarily attributed to increased resynthesis of phosphocreatine, which contributes to delaying its depletion. Increases in muscle power and speed were also reported, as well as a possible reduction in muscle fatigue, although the specific physiological pathway through which this response occurs is not yet fully understood.

In his study, Fernandes (2021) conducted a literature search on nutritional supplements that improve performance in elite soccer players, revealing the use of various supplements such as creatine, caffeine, sodium bicarbonate, beta-alanine, nitrates (from beetroot), among others. The results, focused on nitrates, reveal that 6 mmol of NO₃, used for at least 5 days, consumed 2 hours before training, improves soccer players' performance. Furthermore, they emphasize the importance of doubling the dose on game day. This study, compared to those presented initially,

highlights the need to find an appropriate protocol, including dosage and application time, for each specific sport. Although the trend for ball sports, according to these studies, implies the need for chronic, not just acute, intake, reflecting the need for further research in the field.

Delleli et al. (2023) conducted a systematic review of randomized controlled clinical trials evaluating the effect of beetroot supplementation on combat sports performance. They concluded that there was an improvement in different aspects of athletic performance, both with a single supplementation and multiple doses, in different combat sports; mainly oxidative metabolism in isometric and isokinetic exercises; however, there is variability in the results, associated with both individual factors and the characteristics of each individual sport. There are also other systematic reviews (Murphy et al. 2022 and Tan et al. 2022) that reflect the study of nitrates in sports, including team sports, with variability in their results. Therefore, the need for further research on the subject is evident, mainly in Colombia, where no results on the study of nitrates in rugby are found.

In addition, this supplement is endorsed by the Australian Institute of Sport (AIS) for use by classifying it in category A, due to its safety and level of scientific evidence (2022).

- Human subjects protection review board

Initially, the study was considered experimental and approved by the committee of the Faculty of Medicine of the National University of Colombia in minutes B.FM. 1. 002-CE-043-24. However, as the necessary sample of volunteers for randomization was not obtained (44 people were required, but 10 were obtained), the study was changed from experimental to quasi-experimental, and the changes were reviewed and approved by the same committee in minutes 2.FM. 1. 002- CE 105-25.

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Statistical Analysis Plan (SAP):

A descriptive and exploratory analysis was performed on the collected data using the R program. Univariate tests were performed for the mean (paired Student t-test) or Wilcoxon depending on the type of variable (continuous or ordinal) and compliance with the assumptions of normality with a confidence level of 95%.

$$d = \frac{\bar{d}}{s_d}$$

Where:

\bar{d} = mean of the differences (Beetroot – Placebo)

s_d = standard deviation of the differences

Thus, for the Wilcoxon test, the following was used:

$$r = \frac{Z}{\sqrt{n}}$$

Where:

Z = Z statistics of the test

n = number of participants with data in both conditions