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Effects of beta-alanine supplementation on Yo-Yo test performance: a meta-analysis

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Running title: Beta-alanine and Yo-Yo

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Abstract

Objective: The aim of this meta-analysis was to explore the effects of beta-alanine supplementation on Yo-Yo test performance.

Methods: Nine databases were searched to find relevant studies. A random-effects meta-analysis of standardized mean differences (SMD) was performed for data analysis. Subgroup meta-analyses were conducted to explore the effects of beta-alanine supplementation duration on Yo-Yo test performance, and the effects of beta-alanine supplementation on performance only in Yo-Yo level 2 test variants.

Results: Ten study groups were included in the meta-analysis. All studies included athletes as study participants. When considering all available studies, there was no significant difference between the placebo/control and beta-alanine groups (SMD: 0.68; 95% confidence interval [CI]: –0.30, 1.67). When considering only the studies that used supplementation protocols lasting between 6 and 12 weeks, there was a significant ergogenic effect of beta-alanine (SMD: 1.02; 95% CI: 0.01, 2.05). When considering only the studies that used the level 2 variants of the Yo-Yo test, there was a significant ergogenic effect of beta-alanine (SMD: 1.41; 95% CI: 0.35, 2.48).

Conclusions: This meta-analysis found that beta-alanine is ergogenic for Yo-Yo test performance in athletes when the supplementation protocol lasts between 6 and 12 weeks and when using the level 2 variants of the Yo-Yo test.

Keywords: carnosine; L-histidine; sport; buffering;

1. Introduction

Beta-alanine and L-histidine are precursors of carnosine synthesis [1]. Recent research established that beta-alanine (but not L-histidine), is the rate-limiting factor for carnosine synthesis [1]. Therefore, the primary aim of supplementation with beta-alanine is to increase muscle carnosine levels [1, 2]. Carnosine is identified to have multiple functions in the human body (e.g., antioxidant activity, effects on calcium sensitizing) [3, 4]. However, from an exercise performance perspective, the largest interest is in carnosine's effects on intracellular pH regulation [4]. A decrease in pH levels (i.e., acidosis) during high-intensity exercise has been associated with muscle fatigue and a decline in exercise performance [4, 5]. Therefore, supplements that influence pH regulation (such as beta-alanine) may be ergogenic for exercise tasks in which acidosis is a contributing factor to fatigue [4].

The Yo-Yo test was developed in the 1990s and has become one of the most popular tests to evaluate high-intensity intermittent exercise performance [6]. The Yo-Yo test has four variants, Yo-Yo intermittent recovery level 1 and level 2, and Yo-Yo intermittent endurance level 1 and level 2 [6-9]. Level 1 versions of the Yo-Yo test predominately rely on the aerobic system. Level 2 versions start at a higher intensity and require a greater contribution from the anaerobic system [6]. Variations of the Yo-Yo test are reported to have good-to-excellent test-retest reliability and can discriminate between top-elite, moderate-elite, and sub-elite soccer players [6-9]. As noted previously, beta-alanine may enhance exercise performance because it increases muscle carnosine levels, and one of carnosine's functions is intracellular pH regulation [4, 10]. During resting conditions, muscle pH is around 7.1 [4]. However, at the end of the Yo-Yo test, previous research has reported that muscle pH is reduced to 6.8 [8]. Due to its physiological demands, it seems plausible that supplementation with beta-alanine may be ergogenic for performance in the Yo-Yo test. This area of research may be of importance as performance in this test may impact sport-specific outcomes. For example, Yo-Yo test performance of elite female soccer players is associated with the amount of high-intensity running performed at the end of both halves of a soccer match [11].

In a 2012 study, Saunders et al. [12] found that supplementation with beta-alanine throughout 12 weeks was ergogenic for Yo-Yo test performance. Specifically, the group that consumed beta-alanine improved performance by around ~34%, whereas the group that consumed

placebo experienced a decrease in performance by ~8%. In a more recent study [13] the group consuming beta-alanine (n = 5) improved Yo-Yo test performance by 54%; however, this improvement was not statistically different from the changes observed in the placebo group (+10%; n = 6). This suggests that some of the studies conducted on the topic might have been underpowered to observe statistically significant differences, subsequently leading to a type II error. One way to overcome this limitation of primary studies is to conduct a meta-analysis. Even though previous meta-analyses explored the effects of beta-alanine on exercise performance [14, 15] none of them specifically focused on the Yo-Yo test. Therefore, this review aimed to conduct a meta-analysis examining the effects of beta-alanine on Yo-Yo test performance.

2. Materials and methods

For the purpose of this review, the following databases were searched: Academic Search Elite, ERIC, CINAHL, *OpenDissertations*, PubMed/MEDLINE, SPORTDiscus, Open Access Thesis and Dissertations, Scopus, and Web of Science. The following combination of keywords and Boolean operators was used in all of these databases: (beta-alanine OR β-alanine OR carnosine) AND (yo-yo OR yoyo OR yo yo OR "intermittent recovery" OR "intermittent endurance"). In addition to the primary search, secondary searches were performed, which consisted of checking the studies that cited the included studies (using Google Scholar) and screening the reference list of the included studies. The search for studies was carried out on December 6th, 2020.

Studies that satisfied the following criteria were included: (a) written in English; (b) provided isolated beta-alanine supplementation; and (c) evaluated performance in any variant of the Yo-Yo test. This review considered both peer-reviewed literature such as journal articles as well as unpublished documents in the form of theses, dissertations, or conference abstracts.

From all included studies, the following data were extracted: (a) names of authors and year of publication; (b) sample characteristics (e.g., age, sex, training status); (c) protocol of beta-alanine supplementation; (d) variant of the Yo-Yo test utilized; and (e) main findings. When studies presented data in figures, the Web Plot Digitizer software was used for data extraction.

The methodological quality of the included studies was evaluated using the PEDro checklist [16]. The PEDro checklist has 11 items and evaluates different aspects of the study design, including: eligibility criteria, randomization, blinding, attrition, and data reporting. Each item is scored with "1" with the criterion is satisfied, or with "0" if the criterion is not satisfied. The first item on the checklist does not contribute to the total score. Therefore, the maximum number of points on the checklist is 10. Studies that scored from 9 to 10 points were categorized as "excellent" quality; studies that scored from 6 to 8 points were categorized as "good" quality; studies that scored from 4 to 5 points were categorized as "fair" quality; studies that scored ≤3 points were categorized as "poor" methodological quality.

Meta-analyses were performed using standardized mean differences (SMDs). SMDs were calculated using pre-intervention and post-intervention mean ± standard deviation (SD) data from the beta-alanine and placebo/control groups and their respective sample sizes. SMDs were calculated as pre-post intervention mean change, divided by the pooled SD. In addition to the main analysis, two subgroup analyses were also performed. In one subgroup analysis, data were analyzed after excluding one study [17] that used a 3-week supplementation protocol, which is shorter than other studies, as they used 6 to 12 weeks of supplementation. In another subgroup analysis, data were analyzed after excluding two studies that used the intermittent recovery level 1 version of the Yo-Yo test, as it has been suggested that greater effects of beta-alanine may be observed in the level 2 version [17, 18]. SMDs were interpreted as: "trivial" (<0.20), "small" (0.20–0.39), "medium" (0.40–0.59), "large" (0.60–0.80), and "very large" (>0.80). Heterogeneity was explored using the I^2 statistic and interpreted as "low" (<50%), "moderate" (50–75%), and "high" (>75%) levels of heterogeneity. Funnel plot asymmetry was not explored, given that there were less than ten included studies. All meta-analyses were performed using the random-effects model. The statistical significance threshold was set at $p < 0.05$. All analyses were performed using the Comprehensive Meta-analysis software, version 2 (Biostat Inc., Englewood, NJ, USA).

3. Results

There was a total of 226 results across the primary and secondary searches. Out of this pool of references, ten full-text papers were read, and five studies [12, 13, 17-19] with a total of 10 groups satisfied the outlined inclusion criteria (Figure 1).

All five studies included athletes as participants. Four studies were conducted among soccer players and one study among basketball players (Table 1). Sample sizes in the individual studies ranged from 11 to 24 participants. Supplementation protocols lasted between 3 and 12 weeks. The dose of beta-alanine ranged between 3.2 g/day and 6.4 g/day. Two studies used the Yo-Yo intermittent recovery level 1 test, two studies used the Yo-Yo intermittent recovery level 2 test, and one study used the Yo-Yo intermittent endurance level 2 test (Table 1).

Out of the five included studies, two were classified as excellent methodological quality, one study was classified as good methodological quality, and two studies were classified as fair methodological quality (Table 1).

When considering all available studies, there was no significant difference between the placebo/control and beta-alanine groups (SMD: 0.68; 95% CI: –0.30, 1.67; $p = 0.172$; $I^2 = 4\%$; Figure 2). When considering only the studies that used supplementation protocols lasting between 6 and 12 weeks, there was a significant ergogenic effect of beta-alanine (SMD: 1.02; 95% CI: 0.01, 2.05; $p = 0.048$; $I^2 = 0\%$; Figure 2). When considering only the studies that used the level 2 variants of the Yo-Yo test, there was a significant ergogenic effect of beta-alanine (SMD: 1.41; 95% CI: 0.35, 2.48; $p = 0.010$; $I^2 = 0\%$; Figure 2).

4. Discussion

In the primary analysis that considered data from all available studies, there was no significant difference between beta-alanine and placebo/control in Yo-Yo test performance. However, this meta-analysis found a significant ergogenic effect of beta-alanine when considering data from studies that used supplementation protocols lasting between 6 and 12 weeks. Additionally, it was also found that beta-alanine increases performance when considering studies that used level 2 variants of the Yo-Yo test. Overall, these results suggest that beta-

alanine may enhance Yo-Yo test performance when supplementation protocols last between 6 and 12 weeks and when a higher intensity variant of the Yo-Yo test is used.

The results presented in this review suggest that supplementation duration might be a modifying factor for the ergogenic effect of beta-alanine. It might be that shorter duration supplementation protocols (e.g., 3 weeks) are insufficient to reach the threshold of increases in muscle carnosine levels needed to improve performance in the Yo-Yo test. In support of this idea, one study [20] demonstrated that supplementation with beta-alanine and placebo was comparably effective over the first three weeks. However, for the subsequent three weeks, an increase in total work performed during a high-intensity interval exercise protocol was observed only in the group that consumed beta-alanine [20]. These data might explain why the present meta-analysis found an ergogenic effect of beta-alanine when considering studies that used supplementation protocols lasting between 6 and 12 weeks. Still, future research is needed to explore the time course of the effect of beta-alanine on Yo-Yo test performance. To explore this topic, future studies may consider employing a 12-week supplementation protocol where Yo-Yo test exercise performance is evaluated every 3 weeks.

Another variable that needs to be considered when discussing the effects of beta-alanine on Yo-Yo test performance is the variant of the test used. Specifically, this meta-analysis found an ergogenic effect of beta-alanine when considering data from studies that used the level 2 versions of the Yo-Yo test. In two studies [17, 18] that used the level 1 version of the Yo-Yo test, there were no ergogenic effects of beta-alanine. Moreover, in one study [17] the data actually favored the placebo group—even though the difference between the groups was not statistically significant. From a physiological perspective, there is a logical rationale why beta-alanine would be ergogenic for level 2, but not level 1 test variant. Krustrup et al. [7] found that, when recorded at exhaustion, muscle pH in the level 1 variant was 6.98. However, muscle pH recorded at exhaustion in level 2 variant was lower and amounted to 6.80 [8]. Given that supplementation with beta-alanine enhances exercise performance due to the increase in muscle carnosine levels and carnosine regulates intracellular pH, it seems reasonable that this supplement would have a greater effect on exercise tests that have a larger influence on pH levels [2, 4, 12]. While indicative, future research on the topic may consider exploring the effects of beta-alanine supplementation on different Yo-Yo test variants within

the same group of participants. This would be important because the current comparisons between the effects of beta-alanine on level 1 vs. level 2 test variants are made from different studies that also varied in other methodological aspects besides the Yo-Yo test level utilized.

Interestingly, in the analyses where an ergogenic effect of beta-alanine was observed, the SMD was large (i.e., 1.02 and 1.41). This effect is larger than the effect of other supplements on the Yo-Yo test performance. For example, one recent meta-analysis demonstrated that both caffeine and sodium bicarbonate are acutely ergogenic for performance in the Yo-Yo test, with SMDs of 0.17 (95% CI: 0.08, 0.32) and 0.36 (95% CI: 0.10, 0.63), respectively [21]. Currently, there are no available studies that explored the effects of combined ingestion of beta-alanine with other supplements such as caffeine and sodium bicarbonate on Yo-Yo test performance. Future studies should explore this area as athletes often consume multiple supplements at once, and beta-alanine is commonly added to multi-ingredient pre-workout blends or energy drinks [22].

Based on the PEDro checklist, studies were classified as fair-to-excellent methodological quality. Still, it should be mentioned that some studies [12, 18] did not utilize a double-blind study design, which needs to be considered as this study design is the “gold standard” in sports nutrition. Additionally, recent findings have reported that correct supplement identification may influence a given exercise task's outcome and lead to bias in the results [23]. Out of the studies that used a blinded design, none of them explored the effectiveness of blinding. This should be mentioned, given that beta-alanine is associated with side-effects such as paresthesia [24], which may lead the participants to correct supplement identification. This limitation should be addressed in future studies.

5. Conclusion

When considering all available studies, there was no significant difference between beta-alanine and placebo/control in Yo-Yo test performance. However, beta-alanine was ergogenic when considering data from studies where supplementation protocol lasted between 6 and 12 weeks. Additionally, beta-alanine was ergogenic when considering studies that used level 2 variants of the Yo-Yo test. In summary, the results of this review suggest that beta-alanine is

ergogenic for Yo-Yo test performance when the supplementation protocol lasts between 6 and 12 weeks and when a higher intensity variant of the Yo-Yo test is used.

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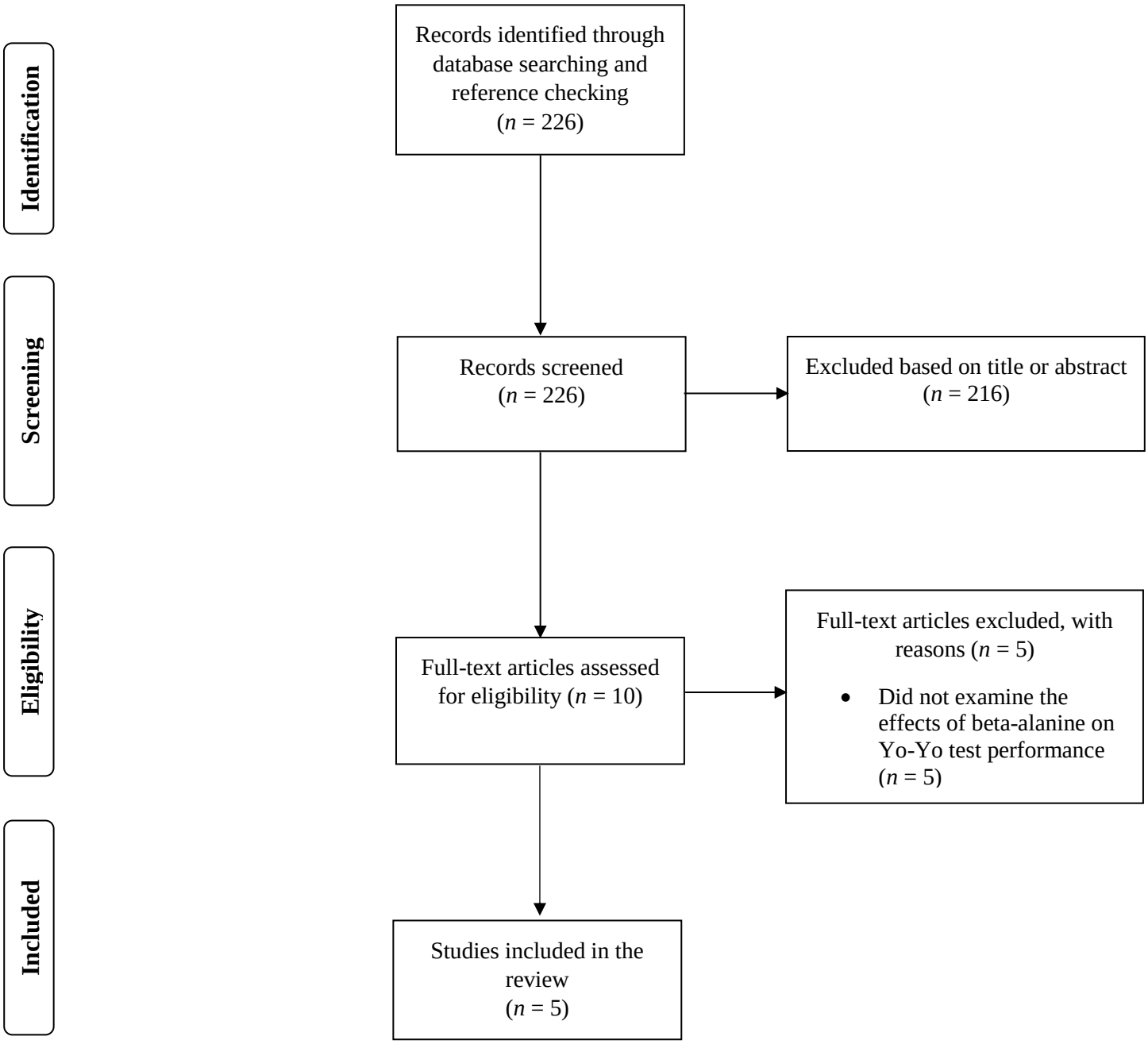
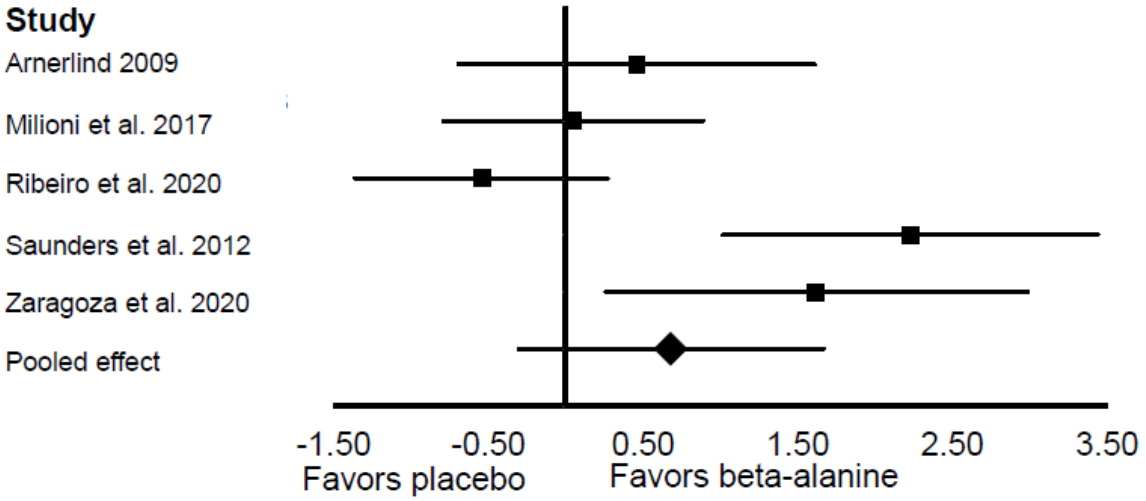
Figure 1. Flow diagram of the search process

Figure 2. Forest plot presenting the results of the random-effects meta-analysis comparing the effects of placebo/control vs. beta-alanine on Yo-Yo test performance while considering all studies (upper section), while considering only data from studies that used protocols of supplementation lasting between 6 and 12 weeks (middle section), or while considering only level 2 test variants (lower section). Data are reported as standardized mean differences (SMD) and 95% confidence intervals (CIs). The diamond at the bottom presents the overall effect. The plotted squares denote SMD and the whiskers denote their 95% CIs.


Identification
Screening
Eligibility
Included
Records identified through database searching and reference checking (n = 226)
Records screened (n = 226)
Excluded based on title or abstract (n = 216)
Full-text articles assessed for eligibility (n = 10)
Full-text articles excluded, with reasons (n = 5)
Did not examine the effects of beta-alanine on Yo-Yo test performance (n = 5)
Studies included in the review (n = 5)


Study
Arnerlind 2009
Milioni et al. 2017
Ribeiro et al. 2020
Saunders et al. 2012
Zaragoza et al. 2020
Pooled effect
-1.50
-0.50
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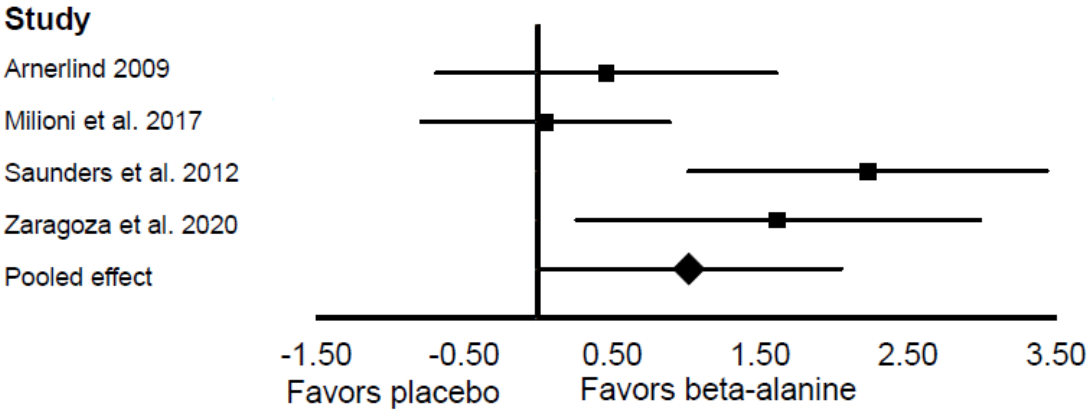
3.50
Favors placebo
Favors beta-alanine


Study
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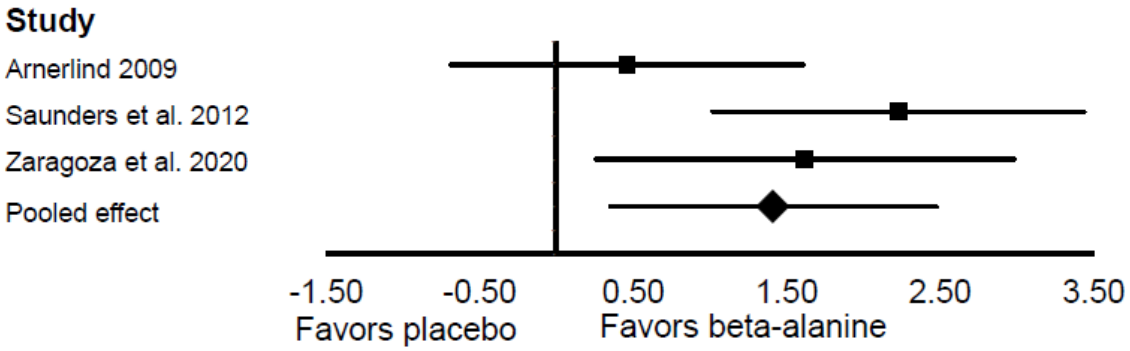
Study
Arnerlind 2009
Saunders et al. 2012
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Pooled effect
-1.50
-0.50
0.50
1.50
2.50
3.50
Favors placebo
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Table 1. Summary of the studies included in the review

Study	Sample	Beta-alanine supplementation protocol	Variant of the Yo-Yo test	PEDro checklist score
Arnerlind (2009)	12 male soccer players	5 g/day for 8 weeks	IEL 2	5 points – FQ
Milioni et al. (2017)	22 male basketball players	6.4 g/day for 6 weeks	IRT 1	6 points – GQ
Ribeiro et al. (2020)	24 female soccer players	6.4 g/day for 3 weeks	IRT 1	9 points – EQ
Saunders et al. (2012)	17 male soccer players	3.2 g/day for 12 weeks	IRT 2	5 points – FQ
Zaragoza et al. (2020)	11 male soccer players	6.4 g/day for the first 6 weeks and 3.2 g/day for the second 6 weeks	IRT 2	9 points – EQ
EQ: excellent quality; FQ fair quality; GQ: good quality; IEL: intermittent endurance level; IRT: intermittent recovery test				