# DEVELOPMENT AND EVALUATION OF AN IRON-RICH FUNCTIONAL BAR: EFFECTS ON IRON STATUS AND PHYSICAL PERFORMANCE IN FEMALE ATHLETES

**Dr. Sandeepkumar P.S[1*], Dr Baby Shijansha K P[2], Dr Mahesh K V[3], Dr Joe Joseph[4]**

[1*]Assistant Professor, College of Agriculture, Padannakkad, Kasaragod, Kerala Agricultural University, Email ID: sandeepkumar.ps@kau.in, ORCID: 0009-0006-0590-0878
[2]Assistant Professor in Physical Education, Sir Syed College, Taliparamba, Kannur University, E-mail ID: shijanshah@gmail.com, ORCID ID: 0009-0008-9252-662X
[3]Associate Professor & Head, Department of Physical Education, Sirsyed College, Taliparamba, affiliated to Kannur University, Kannur, Kerala, City Pincode: 670562, ORCID: 099-0002-3857-7609, Email ID: maheshmoolamattom@gmail.com
[4]Deputy Director of Physical Education, Directorate of Physical Education, Kannur University, Kannur-670567, Email ID: Joejoseph@kannuruniv.ac.in, ORCID id:0009-0009-8982-3807

**ABSTRACT**
**Background:** Iron deficiency is a risk factor in female athletes due to iron losses associated with menstruation, iron losses due to exercise, and the physiological needs for regular exercise. Food-based functional products could be a safe and sustainable solution to achieve better iron status and optimal sport performance.
Objective: The aim of this study was to design and test an iron-enriched functional bar and its impact on haematological, physiological, anthropometric and performance parameters in female athletes with an intermittent endurance training profile.
**Methods:** The design used was a randomized, double-blind, pre-test–post test experimental design. The first phase consisted of formulation and assessment of the iron rich functional bar for sensory, nutritional, and microbiological acceptability. Thirty intermittent-endurance trained female athletes (18-25 years) were randomly divided into an experimental (n = 15) and a placebo group (n = 15). The participants took one 30 g iron rich functional bar or placebo bar per day for six weeks. Haemoglobin, serum ferritin, body mass index (BMI), fatigue, resting heart rate, aerobic capacity ($VO_2$max), muscular endurance and physical activity were used as outcome measures. Paired-samples t-test and analysis of covariance (ANCOVA) were used to analyse the data.
**Results:** The functional bar optimized by the introduction of iron exhibited high sensory acceptability, good nutritional composition, and acceptable microbiological quality. After 6 weeks, the experimental group had significantly improved haemoglobin and serum ferritin, BMI, $VO_2$ max, muscular endurance and physical activity, and there was a significant decrease in fatigue and resting heart rate compared with the placebo group ($p < 0.05$). ANCOVA showed significantly better adjusted post intervention scores in the experimental group.
**Conclusion:** The functional iron-rich developed bar successfully improved Fe status and selected haematological, physiological and performance-related parameters, which suggests it is a viable, safe and sustainable nutritional intervention for female athletes.

**KEYWORDS:** Functional food, functional bar, Iron Rich, Female athletes, Sports nutrition, Iron status, Haemoglobin, serum Ferritin, Aerobic capacity, Muscular endurance.

## 1. INTRODUCTION

Good nutrition is crucial to good health, improved performance and recovery. Proper nutrition provides fuel for training adaptations, immune function, tissue repair, and energy metabolism, which helps to enhance exercise capacity and maintain long-term athlete health and well-being. Besides macronutrients, adequate provision of essential micronutrients is essential to support the physiological processes needed during exercise. Iron is one of these micronutrients, playing a key role due to its involvement in oxygen carrying capacity, erythropoiesis and cellular energy metabolism. The haemoglobin, which accounts for about two-thirds of all body iron, is composed of heme and globin; the non-haemoglobin iron, about one-third, is found in myoglobin and in mitochondrial enzymes involved in oxidative phosphorylation and adenosine triphosphate (ATP) formation. Therefore, sufficient iron cannot be ignored in maintaining aerobic metabolism, retarding fatigue and sustaining exercise performance (Beard & Tobin, 2000; Haas & Brownlie, 2001; Thomas et al., 2016; Burke et al., 2019).

The menstrual blood loss, haemolysis due to exercise, gastrointestinal blood loss, urinary iron loss, and increased physiological demands due to regular training and workouts are factors that make women particularly sensitive to the effects of ID. In addition, hepcidin concentrations could increase after prolonged exercise, which may lead to less iron absorption and recycling and result in limited iron availability, even in athletes who eat diets that appear to be adequate. Iron deficiency causes impaired production of haemoglobin, oxygen delivery, mitochondrial function and aerobic energy production, which in turn results in increased fatigue, impaired recovery, reduced endurance capacity, and reduced athletic performance. Importantly, these adverse effects can happen before a person reaches iron-deficiency anaemia, therefore it is important to ensure iron status is adequate during the training season (Peeling et al., 2014; Zimmermann & Hurrell, 2007; Sim et al., 2019).

Oral iron supplementation is the traditional method for treating iron deficiency; however, gastrointestinal side effects such as nausea, abdominal discomfort, constipation and poor palatability often prevent long-term compliance. Food based nutritional interventions with functional foods have, therefore, attracted increased interest, especially when it comes to increasing dietary iron intake. The functional food products contain necessary nutrients and naturally occurring bioactive compounds that are beneficial for health, as well as being easily accessible and could be used as a valuable approach to increase long-term adherence in sportspeople who need ongoing nutritional support (Granato et al., 2020; Peeling et al., 2019).

Functional nutrition bars are becoming popular for their ability to serve as a convenient, portable, shelf-stable delivery system that can deliver standardized doses of nutrients in a fixed serving size. They are ideal for athletes who need quick meal choices before or after their workouts. Thus, a functional bar made of ingredients that are naturally rich in iron could be an effective, natural alternative to traditional iron supplements, and could also contain other nutrients that promote health and recovery in athletes.

Food based approach to increase dietary iron - this will be a practical approach for the Fe intake of female athletes by using natural food ingredients to develop an Fe-rich functional bar. Cereals, millets, nuts, seeds and plant ingredients rich in vitamin C are sources of iron and can contain other bioactive compounds, dietary fibre, protein, healthy fats and antioxidants which could potentially help the uptake of iron, recovery after exercise and support energy metabolism. These types of formulations have been found to be not only nutritionally adequate but also consumer acceptable and thus are promising alternatives to the conventional iron supplementations (Devi et al., 2014; Saleh et al., 2013; Shahidi & Ambigaipalan, 2015).

With the rise in the popularity of functional foods and sports nutrition, there has been limited research that has combined product development, nutrition evaluation, sensory acceptability, microbiological safety, and placebo-controlled supplementation in one study. Furthermore, there is limited evidence on the effectiveness of a standardized iron-rich functional bar to enhance iron status and certain physiological and performance-related parameters in women athletes with a training profile of intermittent endurance. This deficiency could serve as important evidence for the potential of food-based nutrition interventions as a sustainable alternative to traditional iron supplementations.

Thus, this study was designed to develop and evaluate an iron enriched functional bar using natural food ingredients and to examine the effect of the iron rich functional bar on some haematological, anthropometric, physiological and physical performance variables among female athletes who have trained in intermittent endurance (IE) with a randomized, double-blind, placebo-controlled experimental design. It was hypothesized that supplementation with the developed functional bar, compared to placebo supplementation would significantly improve haemoglobin concentration, serum ferritin concentration and selected health- and performance-related variables after six weeks of daily supplementation.

## 2. MATERIALS AND METHODS

### 2.1 Study Design

A double blind, placebo controlled pre-test – post test experimental design was used to develop and test the functional bar containing iron and to assess its effects on selected haematological and anthropometric variables in female athletes. The study took place in two phases. During the first phase, the formulation, standardization and quality control testing of the iron-rich functional bar and placebo bar were completed, while the second phase involved a six-week supplementation study conducted in the intermittent-endurance trained female athletes.

The subjects were randomly divided into experimental and placebo groups. The iron rich functional bar was fed to the experimental group while the placebo group was fed with a placebo bar that was similar in visual and sensory characteristics. Baseline and post tests were conducted to assess supplement effects. The study protocol was developed to reduce bias by randomizing the subjects, double blinding and uniform product formulation, laboratory analysis and outcome assessment.

### 2.2 Preparation of Iron-Rich Functional Bar and Placebo Bar

The Iron-Rich Functional Bar and Placebo Bar were prepared using the same protocol. The preparation of the Iron-Rich Functional Bar and Placebo Bar was identical.

The ingredients used in the formulation of the iron rich functional bar are food grade ragi flour, whole wheat flour, black sesame powder, roasted peanut powder, almond powder, jaggery powder, ghee, amla powder and cardamom powder. Initial formulation tests were performed to improve the nutritional profile, the physical and chemical properties and the sensory acceptance of the product. The formulation used in the supplementation trial and optimized is shown in Table 1.

**Table 1. Formulation of the Optimized Iron-Rich Functional Bar**

| Ingredients | Quantity (g) |
|---|---|
| Ragi flour | 140 |
| Whole wheat flour | 90 |
| Black sesame powder | 20 |
| Roasted peanut powder | 20 |
| Almond powder | 20 |
| Jaggery powder | 80 |
| Ghee | 40 |

| | |
|---|---|
| Amla powder | 5 |
| Cardamom powder | 2 |
| Total | 417 |

Ingredients were properly weighed and mixed to ensure homogeneity in the mixture. This mixture was shaped into identical and equal sized rectangular bars.This mixture was formed into identical and equal sized rectangular bars. Each functional iron bar was formulated to contain 30 g of iron and packaged individually in a laminated food-grade airtight pouch and stored at ambient temperature for further analysis and supplementation.

An identical "placebo" bar was created using ingredients that matched the appearance and texture and energy content of the principal iron-rich ingredients, but had none of them. The iron rich (functional) bar and the placebo bar were standardized in weight, dimensions, appearance and packaging to ensure successful implementation of the double-blind supplementation trial. The formulation of the placebo bar is shown in Table 2.

**Table 2. Formulation of the Placebo Bar**

| Ingredients | Quantity (g) |
|---|---|
| Whole wheat flour | 230 |
| Jaggery powder | 110 |
| Ghee | 70 |
| Cardamom powder | 4 |
| Total | 414 |

**2.3 Nutritional, Sensory and Microbiological Evaluation**

Proper nutrition, sensory and microbiological evaluation.Proper nutrition and sensory and microbiological evaluation. The optimized formulation of the functional bar containing iron was assessed for its nutritional composition, sensory acceptability and microbiological quality by the standard analytical procedures. The proximate composition (moisture, protein, fat, ash, carbohydrate, dietary fibre and energy) and iron and calcium contents were measured by the official methods of AOAC International (2019).

Appearance, colour, aroma, taste, texture and overall acceptability were evaluated by a semi-trained sensory panel (25 members) using the 9-point Hedonic Scale (Lawless & Heymann, 2010). The microbiological quality was determined by the number of total plates, yeast and mould and coliform bacteria, as per the standard AOAC/FSSAI procedure. Each sample was analyzed three times. The optimized formulation was also subjected to a shelf-life study under ambient storage for two months to check changes in the sensory quality, moisture content and microbiological stability.

**2.3 Participants and Study Protocol**

Total 30 female athletes with intermittent endurance training background age 18-25 years were recruited from Sir Syed College, Taliparamba, Kerala, India. Female athletes were chosen because they are known to have an increased risk of iron deficiency due to menstrual blood loss, exercise induced iron loss, and the increased iron requirement due to regular athletic training (Burke et al., 2019; Sim et al., 2019). The study included participants who were regularly involved in organized sports training, appeared healthy and provided written informed consent. Participants who were iron supplemented, had a chronic disease related to iron metabolism or were allergic to any of the ingredients in the iron supplement were excluded.

After a baseline assessment, the participants were randomly divided into an experimental group (n = 15) and a placebo group (n = 15) by lottery. The study design was double blind and placebo controlled, the experimental group received the iron rich functional bar, while the placebo group received a placebo bar that looked and felt like the experimental bar but contained no iron.The study was a double blind-placebo controlled study; the iron rich functional bar was given to the experimental group for 6 weeks while the placebo bar, which appeared and felt like the iron rich functional bar, but contained no iron, was given to the placebo group for 6 weeks. Both products were packaged separately in the same type of laminated food grade pouches and labeled as Product A and Product B. An independent investigator produced and handled the allocation code and it was only shared with the outcome assessors and product distributors after the collection of data and statistical analysis.

The experimental group took 1 iron-rich functional bar (30 g) every day for 6 weeks while the placebo group took a placebo bar (30 g) every day for 6 weeks. Participants were asked to continue their usual dietary intake and training programme during the intervention and avoid taking extra iron supplements. Daily records of supplementation and weekly follow up visits were used for compliance monitoring.

**2.4 Outcome Measurements**

The six week supplementation period was followed by outcome measurements taken at baseline (pre-test) and at the end of the six weeks (post-test). The haemoglobin concentration was measured using automated haematology analyser, and serum ferritin concentration using a commercially available enzyme-linked immunosorbent assay (ELISA) kit by following the manufacturer's instructions.

Body weight was determined to 0.1 kg with a calibrated digital weighing scale while standing height was determined to the nearest 0.1 cm with a stadiometer attached to a wall. BMI was considered as body weight (kg) / height squared (m 2), following the classification of the World Health Organization (2000).
The Rating of Fatigue (ROF) Scale (Micklewright et al., 2017) was used to assess fatigue. HRR was assessed after a 10-minute seated rest with the help of a calibrated digital heart rate monitor, and BP was taken with a calibrated automated digital sphygmomanometer, as described elsewhere.
The 20-m Multistage Shuttle Run Test (Léger et al., 1988) was used for estimating aerobic capacity ($VO_2max$). The 1-minute Push-up Test was used to measure muscular endurance following the recommendations of the American College of Sports Medicine (2021). Physical activity level was assessed by the International Physical Activity Questionnaire–Short Form (IPAQ-SF) (Craig et al., 2003). Standard laboratory and field testing conditions were used for all outcome assessments and these were carried out by trained investigators.

**2.5 Statistical Analysis**
For data analysis, IBM SPSS Statistics for Windows, Version XX, (IBM Corp., Armonk, NY, USA) was used. Values are presented as mean ± standard deviation (SD) values. The normality of the data distribution was tested by Shapiro–Wilk test. The differences between the pre-test and the post-test measurements were analysed within group using paired-samples t-test, and the differences between the two groups were analysed between group using Analysis of Covariance (ANCOVA) with baseline measurement value as a co-variate. A p-value of < 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

**Table 3. Sensory Evaluation Scores of Iron-Rich Functional Bar Formulations Using a 9-Point Hedonic Scale**

| Attribute | F1 | F2 | F3 | F-value | p-value |
|---|---|---|---|---|---|
| Appearance | 7.80 ± 0.41 | 8.55 ± 0.30 | 8.00 ± 0.44 | 7.40 | 0.002 |
| Colour | 7.70 ± 0.54 | 8.42 ± 0.43 | 8.01 ± 0.48 | 5.91 | 0.002 |
| Aroma | 7.91 ± 0.52 | 8.36 ± 0.47 | 8.04 ± 0.51 | 4.88 | 0.007 |
| Taste | 7.64 ± 0.56 | 8.61 ± 0.32 | 8.10 ± 0.47 | 11.40 | <0.001 |
| Texture | 7.80 ± 0.41 | 8.47 ± 0.46 | 8.11 ± 0.46 | 5.11 | 0.003 |
| Overall Acceptability | 7.82 ± 0.43 | 8.58 ± 0.39 | 8.01 ± 0.45 | 7.89 | <0.001 |

Significance was set at $p < 0.05$.
The sensory evaluation results of the three formulations of functional bars with iron are shown in Table 3. The formulations differed significantly ($p < 0.05$) for all the sensory properties such as appearance, colour, aroma, taste, texture and overall acceptability. Formulation F2 scored the highest mean value in all evaluated attributes and overall acceptability score of 8.58 ± 0.39, followed by F3 (8.01 ± 0.45) and F1 (7.82 ± 0.43). F2 was chosen as optimized formulation for further nutritional evaluation, microbiological analysis and supplementation due to its significantly higher sensory values and overall acceptability.

**Table 4. Proximate Composition and Mineral Content of the Optimized Iron-Rich Functional Bar**

| Parameter | Value (per 100 g) |
|---|---|
| Moisture (%) | 7.88 |
| Protein (g) | 11.74 |
| Fat (g) | 12.46 |
| Ash (g) | 3.90 |
| Carbohydrate (g) | 62.65 |
| Dietary Fibre (g) | 6.13 |
| Energy (kcal) | 410.81 |
| Iron (mg) | 8.43 |
| Calcium (mg) | 260.40 |

The optimized iron-rich functional bar was subjected to proximate composition and mineral content which is shown in Table 4. The formulation contained 11.74 g protein, 12.46 g fat, 62.65 g carbohydrate, 6.13 g dietary fibre and 3.90 g ash per 100 g, with an energy value of 410.81 kcal. Furthermore, the optimized iron-rich functional bar contained 8.43 mg iron and 260.40 mg calcium/100 g.

**Table 5. Initial Microbiological Quality of the Optimized Iron-Rich Functional Bar**

| Parameter | Result | FSSAI Standard |
|---|---|---|
| Total Plate Count (CFU/g) | $2.3 \times 10^2$ | Within permissible limit |
| Yeast and Mould Count (CFU/g) | <10 | Within permissible limit |
| Coliform Count | Not Detected | Absent |

The optimized iron-rich functional bar's initial microbiological quality is detailed in Table 5. The total plate count was $2.3 \times 10^2$ CFU/g and the plate count of yeast and mould was <10 CFU/g. No coliform bacteria were found. The optimized iron-rich functional bar met all the microbiological safety standards recommended by Food Safety and Standards Authority of India (FSSAI) with all the microbiological parameters within the permissible limits.

**Table 6. Effect of Six-Week Supplementation on Study Variables**

| Variable | Group | Pre-test (Mean ± SD) | Post-test (Mean ± SD) | Mean Difference | p-value |
|---|---|---|---|---|---|
| Haemoglobin (g/dL) | EXP | 11.80 ± 0.61 | 13.72 ± 0.50 | +1.92 | <0.001 |
| | Placebo | 11.70 ± 0.64 | 11.90 ± 0.62 | +0.20 | 0.284 |
| Serum Ferritin (ng/mL) | EXP | 17.12 ± 3.11 | 21.74 ± 3.40 | +4.62 | <0.001 |
| | Placebo | 18.60 ± 3.05 | 19.40 ± 3.16 | +0.80 | 0.221 |
| BMI | EXP | 21.40 ± 1.66 | 21.71 ± 1.64 | +0.31 | 0.048 |
| | Placebo | 21.57 ± 1.71 | 21.60 ± 1.68 | +0.03 | 0.713 |
| Fatigue Score | EXP | 6.94 ± 0.73 | 4.81 ± 0.66 | −2.13 | <0.001 |
| | Placebo | 6.69 ± 0.75 | 6.43 ± 0.70 | −0.26 | 0.148 |
| Resting Heart Rat | EXP | 73.8 ± 4.6 | 70.3 ± 4.2 | −3.5 | 0.002 |
| | Placebo | 73.4 ± 4.5 | 72.8 ± 4.4 | −0.6 | 0.412 |
| $VO_2$ max | EXP | 42.84 ± 2.76 | 45.62 ± 2.54 | +2.78 | <0.001 |
| | Placebo | 42.93 ± 2.70 | 43.22 ± 2.68 | +0.29 | 0.337 |
| Muscular Endurance | EXP | 38.8 ± 3.4 | 44.9 ± 3.2 | +6.1 | <0.001 |
| | Placebo | 37.2 ± 3.5 | 38.8 ± 3.4 | +1.6 | 0.283 |
| Physical Activity | EXP | 3500 ± 428 | 3860 ± 451 | +360 | 0.008 |
| | Placebo | 3540 ± 436 | 3580 ± 440 | +40 | 0.461 |

The effect of the six week supplementation of the functional bar containing the iron on the studied variables is illustrated in Table 6. The experimental group showed significant improvement in haemoglobin concentration, serum ferritin concentration, body mass index (BMI), aerobic capacity ($VO_2$ max), muscular endurance, physical activity level as well as significant decrease in fatigue score and resting heart rate ($p<0.05$) compared to the baseline. There were no significant differences in any of the variables measured after the intervention ($p > 0.05$) in the placebo group

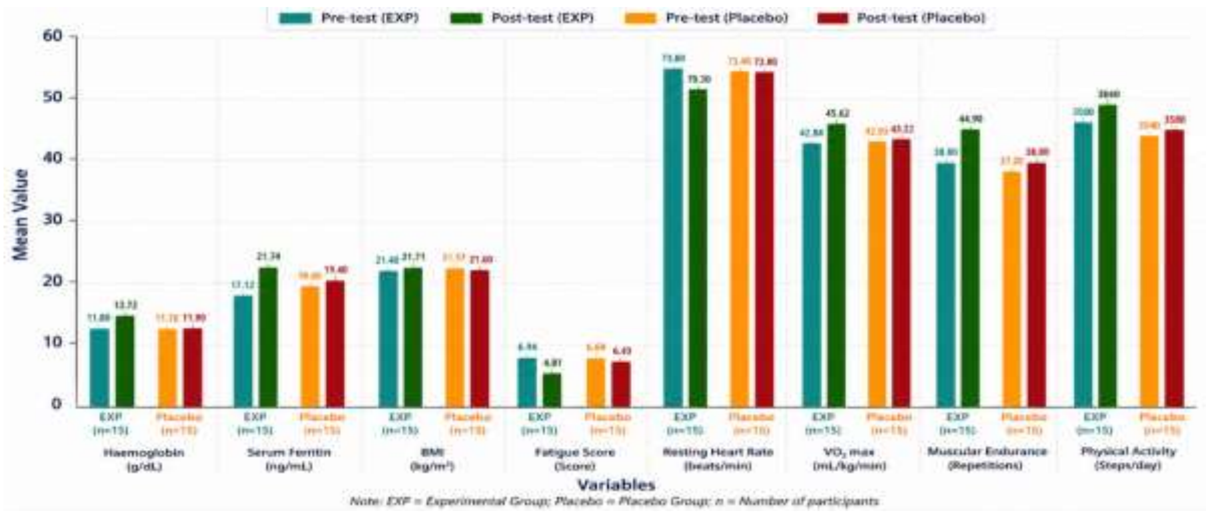


**Figure 1. Effect of Six-Week Supplementation on Study Variables**

**Table 7. ANCOVA Comparison of Post-Test Scores Between Groups**

| Variable | Experimental (Adjusted Mean) | Placebo (Adjusted Mean) | F-value | p-value |
|---|---|---|---|---|
| Haemoglobin (g/dL) | 13.60 | 11.91 | 28.54 | 0.002 |
| Serum Ferritin (ng/mL) | 21.50 | 19.32 | 20.76 | 0.002 |

| | | | | |
|---|---|---|---|---|
| BMI (kg/m²) | 21.67 | 21.58 | 5.36 | 0.002 |
| Fatigue Score | 4.82 | 6.42 | 34.83 | 0.002 |
| Resting Heart Rate (bpm) | 70.41 | 72.71 | 12.48 | 0.002 |
| $VO_2max$ ($mL \cdot kg^{-1} \cdot min^{-1}$) | 45.50 | 43.16 | 19.92 | 0.002 |
| Muscular Endurance (repetitions) | 44.74 | 38.90 | 24.64 | 0.002 |
| Physical Activity (MET-min/week) | 3850 | 3586 | 7.27 | 0.008 |

Table 7 shows the adjusted post intervention means, calculated by Analysis of Covariance (ANCOVA). The adjusted mean values of the experimental group were significantly higher than those of the placebo group in terms of haemoglobin concentration, serum ferritin concentration, BMI, $VO_2$ max, muscular endurance and physical activity level ($p < 0.05$). In contrast, the experimental group had significantly reduced adjusted mean values of fatigue score and resting heart rate compared to placebo group ($p<0.05$).

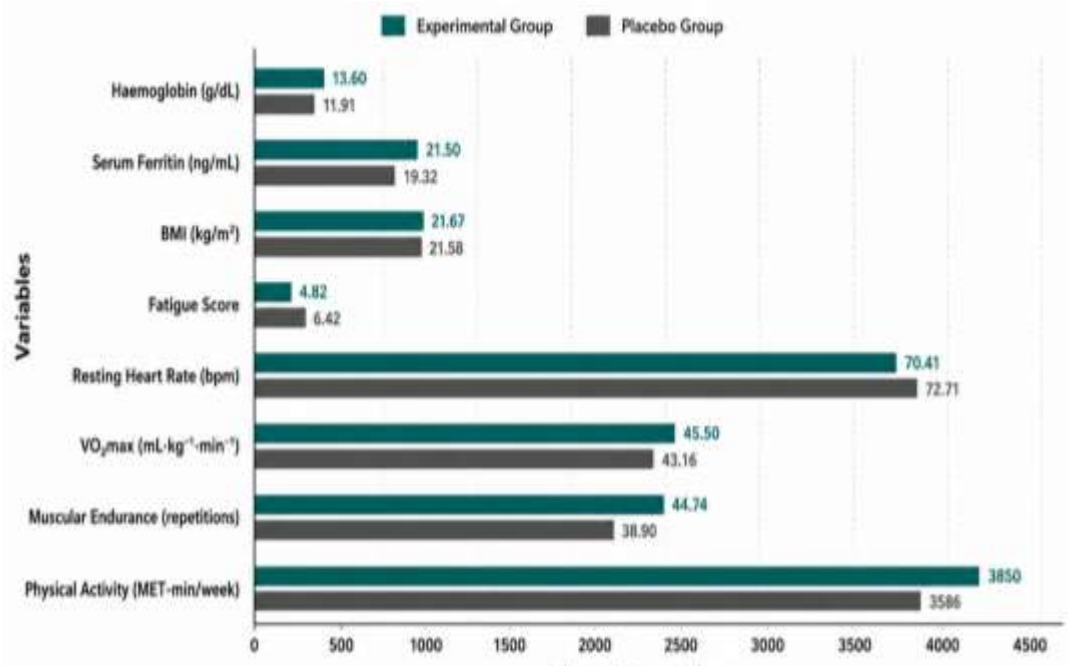


**Figure 2. Adjusted Mean Comparison between Experimental and Placebo Groups**

## DISCUSSION

The present study aimed to design and test an iron fortified functional bar made from natural food sources and to assess the influence of the bar on selected haematological, anthropometric, physiological and performance parameters in the women intermittent-endurance trained athletes. The results showed that the optimized formulation had high sensory acceptability, nutritional composition and good microbiological quality, suitable for use as a functional food for sports nutrition. More important, the haemoglobin concentration, serum ferritin concentration, BMI, aerobic capacity ($VO_2max$), muscular endurance and physical activity level were significantly improved after 6 weeks of supplementation when compared to the placebo group, whereas fatigue and resting heart rate levels were significantly lower after 6 weeks. The results substantiate the hypothesis that a food based iron intervention can be an effective way to enhance the iron status and indirectly promote the improvement of the physiological status and athletic performance of female athletes.

The development of a functional food requires the integration of the nutritional, consumer acceptability and food safety requirements. In the present study, the optimized formulation exhibited a significantly high sensory acceptability in addition to a balanced nutritional composition and satisfactory acceptability in terms of microbiological quality. In sports nutrition, high consumer acceptability is especially relevant since it enables athletes to comply with nutrition interventions if they are acceptable and easy to consume and safe in terms of regularity. The formulated bar also contained good quantities of protein, dietary fibre, iron, calcium and energy, which make it nutritious as an additional food for athletes. In the case of cereal- and millet-based functional foods, optimized formulations have been found that can successfully integrate and improve on nutritional content and sensory properties with food stability (Lawless & Heymann, 2010; Saleh et al., 2013; Devi et al., 2014; Granato et al., 2020). Thus, the desired properties of a practical sports nutrition product were met by the developed functional bar.

One of the most important findings in the present study was the remarkable increase in haemoglobin and serum ferritin levels after iron rich functional bar supplementation for 6 weeks. Menstrual blood loss, exercise-induced haemolysis, sweating, urinary iron losses, and frequent exercise training are particularly important factors that increase iron losses in women (Peeling et al., 2019; Sim et al., 2019). Better iron status leads to improved haemoglobin synthesis, oxygen transport and replacement of body iron stores, and thus improves mitochondrial oxidative metabolism and aerobic energy production (Beard & Tobin, 2000; Haas & Brownlie, 2001). However, the substantial increases in haemoglobin and serum ferritin suggest a better iron availability and nutritional status after supplementation. The results confirm previous studies

showing that iron status is improved and iron deficiency is prevented through food-based iron interventions and/or dietary strategies in physically active women and endurance athletes (Thomas et al., 2016; Burke et al., 2019; Sim et al., 2019).
Fatigue and resting heart rate significantly decreased, $VO_2$max and muscular endurance significantly increased, along with significant improvements in BMI and physical activity level, as iron status improved. These physiological changes support the well documented actions of iron in maintaining oxygen transport, aerobic energy metabolism, and cellular respiration. Better iron bio-availability helps to deliver oxygen to working muscles and helps maintain the function of mitochondria, which helps reduce fatigue and enhances exercise capacity. Thereby, athletes can train at a higher intensity while maintaining the ability to recover and perform optimally during endurance exercise. The weight gain was small but may be attributed to better nutritional status rather than weight gain, since participants were still following their normal sports program during the period of the intervention. The same exercise capacity and physiological improvement when iron status improved is seen in female athletes and endurance trained populations (Beard and Tobin 2000; Thomas et al. 2016; Peeling et al. 2019; Sim et al. 2019).
The positive results found in the current study may be due to the synergistic effect of the various nutrients given rather than iron alone. The functional bar contained iron and protein, in addition to dietary fibre, healthy fat and antioxidants, all in a natural food matrix, with the addition of calcium and vitamin C. These combinations can have beneficial effects on the utilization of nutrients, non-haem iron absorption, muscle recovery, oxidative stress, and metabolic function. A food-based functional product is a sustainable approach to long-term nutrition, convenient, palatable, and free of gastrointestinal issues, unlike traditional iron supplements. The results of this study further support the increasing evidence of the functional foods as an effective alternative to conventional supplements while also promoting the health and performance of athletes (Granato et al., 2020; Peeling et al., 2019).
The present study has a number of significant advantages. It used a randomized, double blind, placebo controlled experimental design, which helps to reduce any potential bias and increases the internal validity of the results. Moreover, the study combined functional food development and a thorough sensory, nutritional and microbiological evaluation prior to clinical supplementation. A variety of haematological, physiological and performance-related measures were analyzed using standard, laboratory- and field-based methods, which enabled a thorough evaluation of the product developed. However, there are some constraints that need to be recognized. The sample size of the study was relatively small and limited to a single institution, which could restrict the generalizability of the findings. The intervention period was only six weeks; dietary intake during the period outside of the supplementation protocol was not strictly controlled; and there was no assessment of any biomarkers, including hepcidin, inflammatory markers, and markers of oxidative stress. Further studies are warranted with larger and more diverse athletic populations, longer intervention periods and additional physiological and biochemical markers to better determine the long-term effectiveness and mechanism by which iron supplementation using food sources works.
The present study showed that a supplement naturally formulated with iron was an efficient way of improving iron status and selected physiological and performance-related variables in female intermittent endurance-trained subjects. Overall, the developed functional bar shows promise as a viable, safe, and sustainable nutritional approach for athletes, given its high sensory acceptability, nutritional profile, microbiological safety, and potential physiological effects. The results of the study add to the existing body of evidence that food-based functional nutrition is an effective means of preventing iron deficiency and enhancing the health and performance of athletes. Additional well-designed multicentre studies are recommended to validate these results and help incorporating functional food interventions into everyday sports nutrition.

## 4. CONCLUSION

The results of the present study indicated that the use of a nutritionally formulated iron-rich functional bar is a feasible food-based nutritional intervention to promote improvement in iron status and selected fitness and health parameters for female athletes with a preference for intermittent endurance type exercise. Optimized formulation also had high sensory acceptability and acceptable nutritional composition with acceptable microbiological quality, which make it a safe and acceptable functional food product.
After six weeks, the iron containing functional bar was able to significantly improve haemoglobin concentration, serum ferritin concentration, BMI, aerobic capacity ($VO_2$max), muscular endurance and physical activity level and significantly reduce fatigue and resting heart rate when compared with the placebo group. Based on these findings, it can be inferred that the improvement of iron status with a nutrient-rich functional food will improve oxygen transport, mitigate exercise-induced fatigue and improve athletic performance.
In conclusion, the formulated iron rich functional bar is a feasible, safe and sustainable nutritional approach for female athletes who are susceptible to iron deficiency. Naturally available ingredients offer an appealing alternative to traditional iron supplementation, and also provide other nutrients that can benefit health while at the same time improving exercise performance. The results of this study add to the growing body of evidence that functional foods are valuable nutritional interventions in sports nutrition, and provide a scientific foundation for the development of natural food products with the potential to improve the health and performance of athletes.

## 5. Practical Applications

The results from the present study have practical implications for athletes, coaches, sports dietitians and sports nutrition practitioners. The present study created an iron-rich functional bar which offered a convenient and food-based solution to optimize iron status and physiological performance in female athletes at risk for iron deficiency. Due to its desirable sensory acceptability, nutritional balance and microbiological safety, the formulated bar can be used as a healthy snack or nutritional supplement in the standard training diet.

The formulated functional bar combination naturally occurring iron with protein, dietary fibre, calcium, healthy fats and antioxidant rich ingredients, offer several nutritional benefits in one food product. Chronic ingestion may help to ensure normal iron stores and help lower fatigue related to exercise and to enhance aerobic performance during training and competition. The present results further offer a scientific basis for the preparation of functional sports foods based on locally available natural ingredients, fostering sustainable, nutritional and food-based strategies for the health and performance of athletes.

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The authors used an AI-assisted writing tool solely to improve the grammar and language of the manuscript. All scientific content and final responsibility remain with the authors.

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**8. Informed Consent Statement**

Written informed consent was obtained from all participants involved in the study prior to data collection.

**9. Data Availability Statement**

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**10. Conflicts of Interest**

The authors declare no conflict of interest.

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