

VARIATIONS IN IMMUNE FATIGUE AND PHYSICAL PERFORMANCE AMONG AMATEUR SOCCER PLAYERS DURING FASTING

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ABSTRACT

This study examines the impact of Ramadan fasting on immune fatigue and physical performance in amateur soccer players. Ten male players (22.8 ± 3.5 years) from the Wilaya of Bejaia participated in this longitudinal study. Creatine Phosphokinase (CPK) and C-Reactive Protein (CRP) were assessed as markers of muscle damage and systemic inflammation. Explosive muscle power was evaluated using Squat Jump (SJ) and Countermovement Jump (CMJ) tests. Data were collected before, during, and after Ramadan. CRP levels significantly decreased during fasting ($p = 0.000$, effect size = 0.75), suggesting anti-inflammatory effects. However, CPK levels showed no significant changes ($p = 0.279$, effect size = 0.35), indicating minimal muscle damage. SJ ($p = 0.937$, effect size = 0.12) and CMJ ($p = 0.426$, effect size = 0.22) performances remained stable, suggesting fasting did not impair explosive muscle power. These findings highlight Ramadan fasting's potential immune benefits without compromising performance, given proper training and nutrition. Future studies should examine training intensity, dietary habits, and compare amateur with professional athletes to better understand physiological adaptations. Larger sample sizes are recommended to improve generalizability.

Key words: Immune Fatigue, Physical Performance, Soccer, Ramadan Fasting

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INTRODUCTION

Soccer is a high-intensity sport that demands a combination of aerobic and anaerobic endurance, muscular strength, agility, and neuromuscular coordination (Stølen et al., 2005). Throughout a match, players perform repeated sprints, rapid accelerations, decelerations, directional changes, and jumps, placing substantial stress on both the cardiovascular and musculoskeletal systems (Rampinini et al., 2007). Among the key determinants of soccer performance, lower-limb explosive power plays a crucial role in sprinting, tackling, and jumping (Reilly et al., 2000). These movements are often assessed using the Squat Jump (SJ) and Countermovement Jump (CMJ) tests, which serve as reliable indicators of neuromuscular function and lower-limb power (Markovic et al., 2004). Given the intermittent high-intensity nature of soccer, any physiological factor that compromises power output can significantly impact a player's ability to perform optimally.

Ramadan fasting, observed annually by millions of Muslim athletes, involves abstaining from food and fluid intake from dawn to sunset for approximately 29–30 days. This fasting period leads to alterations in energy metabolism, hydration levels, and circadian rhythms (Chtourou et al., 2012). These physiological changes may influence hormonal balance, immune function, and neuromuscular responses, thereby affecting athletic performance and recovery (Aziz et al., 2017). Research indicates that fasting impacts various biochemical markers, including Creatine Phosphokinase (CPK) and C-Reactive Protein (CRP), both of which are critical indicators of muscle damage and systemic inflammation, respectively (Bouhlef et al., 2006).

CPK is an enzyme primarily found in skeletal muscle, cardiac tissue, and the brain, playing a vital role in muscle function and energy metabolism (Clarkson et al., 2006). Elevated CPK levels are commonly associated with muscle damage, strenuous exercise, and metabolic stress, particularly in high-intensity sports such as soccer (Brancaccio et al., 2007). Several studies have reported increased CPK levels during Ramadan fasting, suggesting a higher degree of muscle damage due to potential energy deficits and changes in protein metabolism (Zerguini et al., 2007; Souissi et al., 2012).

Similarly, CRP is an inflammatory biomarker produced by the liver in response to tissue injury, infection, or systemic inflammation (Pepys & Hirschfield, 2003). Elevated CRP levels are often linked to increased immune stress and prolonged recovery periods following intense exercise (Smith, 2000). In the context of Ramadan fasting, some studies suggest that CRP levels may rise due to dehydration, altered sleep patterns, and increased training load (Trabelsi et al., 2020). However, the precise correlation between CRP variations and neuromuscular performance remains unclear, necessitating further investigation.

Fatigue is a multifaceted phenomenon influenced by both central (neuromuscular) and peripheral (muscle-related) factors (Girard et al., 2011). Soccer players are particularly susceptible to exercise-induced fatigue, which may be exacerbated by nutritional deficits, hydration status, and metabolic shifts induced by fasting (Chtourou et al., 2012). The interaction between fasting, biochemical stress (CPK and

CRP), and neuromuscular performance is not well understood, making it a critical area for research.

While previous studies have examined the effects of Ramadan fasting on endurance, strength, and reaction time, fewer have specifically investigated the relationship between muscle damage, immune response, and explosive power (SJ and CMJ) in amateur soccer players (Aziz et al., 2017; Chaouachi et al., 2009). Addressing this research gap is essential for developing appropriate training and recovery strategies to minimize fatigue and optimize performance during Ramadan.

Despite a growing body of research on Ramadan fasting and athletic performance, its precise effects on neuromuscular function, fatigue, and immune response remain unclear, particularly among sub-elite and amateur soccer players (Aziz et al., 2010). Most existing studies have focused on elite athletes, while less attention has been given to amateur players who may exhibit different physiological adaptations to fasting (Chtourou et al., 2011).

Furthermore, while research has highlighted the effects of fasting on general endurance and strength, the specific relationship between fasting-induced muscle damage (CPK), immune response (CRP), and explosive power (SJ & CMJ) has yet to be fully explored.

Thus, this study aims to analyze the effects of Ramadan fasting on immune fatigue and neuromuscular performance by examining variations in CPK and CRP levels and their association with SJ and CMJ performance in amateur soccer players. The findings will provide valuable insights for coaches and sports scientists in designing optimized training and recovery programs for athletes fasting during Ramadan.

MATERIALS AND METHODS

Participants

This study involved ten amateur soccer players from the regional division of the Wilaya of Bejaia. The participants had an average age of 22.8 ± 3.5 years, a height of 175.4 ± 6.2 cm, a weight of 72.5 ± 5.8 kg, and a BMI of 23.6 ± 1.9 kg/m². All players were Muslim and observed fasting throughout the month of Ramadan. They followed a structured training program consisting of four training sessions per week, along with one to two official matches during the study period.

Study Design

This study employed a longitudinal design, with data collection occurring at three different time points:

- **Pre-Ramadan Baseline** → Two weeks before Ramadan.
- **During Ramadan** → Midway through Ramadan.
- **Post-Ramadan Recovery** → Beginning of the second week after Ramadan.

To ensure standardized conditions, blood samples were collected at an accredited private medical analysis laboratory, while physical performance tests were conducted once per week at the same time of day.

Training and Competition Schedule

The participants maintained a regular training routine, except for week 3, during which no training was conducted (Table1).

Table 1: training and competition schedule

Week	1	2	3	4	5	6	7	8
Training per week	3	3	0	3	3	3	3	3
Matches per week	1	2	1	1	1	1	1	1

Biochemical Analysis (Blood Tests)

To assess immune and muscle fatigue markers, three fasting blood samples (5 mL each) were collected from the antecubital vein at rest. Blood sampling was performed by two qualified nurses under controlled conditions.

Samples were taken at the following time points:

- **Before Ramadan** → March 8–9, 2023
- **During Ramadan** → April 8–9, 2023
- **After Ramadan** → May 7–8, 2023

To ensure accuracy, samples were collected at least 12 hours after the last meal and 24 hours after the last training session.

The following biochemical markers were analyzed:

- **Creatine Phosphokinase (CPK)** → A key indicator of **muscle damage**.
- **C-Reactive Protein (CRP)** → A marker of **systemic inflammation**.

CPK and CRP levels were analyzed using a SELECTRA PROM automated biochemistry system, with blood collected in dry tubes or plasma collected in lithium-heparin tubes.

Physical Performance Tests

All physical tests were conducted at Amizour Municipal Stadium every Tuesday before training under identical conditions to ensure consistency in measurement.

1. Squat Jump (SJ)

The Squat Jump (SJ) test measures pure concentric explosive strength, excluding any contribution from the stretch-shortening cycle.

Procedure: The player starts from a **90-degree knee flexion position** without swinging the arms. Three attempts are allowed.

2. Countermovement Jump (CMJ)

The Countermovement Jump (CMJ) assesses plyometric capacity, utilizing stored elastic energy for greater jump height.

- **Procedure:** The player performs a quick downward movement followed by an explosive jump. Two attempts are allowed.
- **Measurement:** The highest recorded jump (cm) is taken.

Testing Equipment: Chronojump System

A Chronojump contact platform was used to measure jump height and muscle power. This system is:

- **Reliable** → Provides consistent results in repeated measurements.
- **Valid** → Backed by scientific research (Pagaduan et al., 2013; Padullés et al., 2012).
- **Versatile** → Can be used in both laboratory and field settings.

Statistical Analysis

Statistical analyses were conducted using SPSS software. A repeated-measures ANOVA was performed to assess differences in biochemical markers (CPK, CRP) and physical performance across the three testing periods. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to check data normality, and Levene's test assessed homogeneity of variances. For non-normally distributed data, Kruskal-Wallis and Mann-Whitney U tests were applied as non-parametric alternatives. Descriptive statistics (mean, standard deviation) were also computed. Statistical significance was set at $p < 0.05$.

RESULTS

The following figure shows the box plot of CPK levels according of the three studied periods:

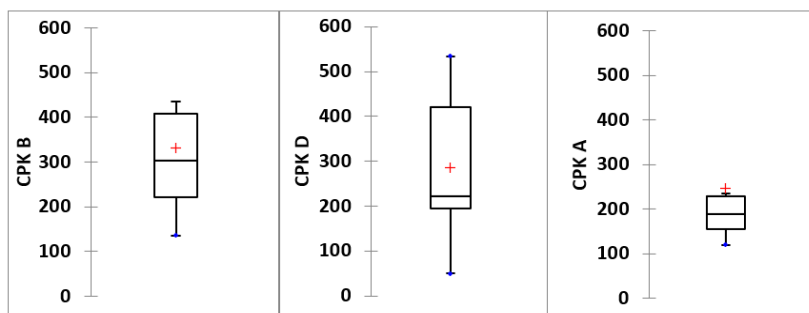


Figure 1 : Box plots of CPK levels according to the three studied periods of training; CPK B (CPK levels before Ramadan); CPK D (CPK levels during Ramadan), CPK A (CPK levels after Ramadan).

The descriptive statistics shows that CPK levels have a mean of 288.19 with a standard deviation of 163.97, indicating high variability. The levels range from 50.00 to 765.30, with percentiles at 177.38 (25th), 221.95 (median), and 417.50 (75th), suggesting a wide spread of data. Moreover, The Kruskal-Wallis test revealed no statistically significant difference in C.P.K levels across the three periods ($p = 0.279$). These results suggest that the changes in CPK levels over time are not significant.

The following figure shows the box plot of CRP levels according of the three studied periods:

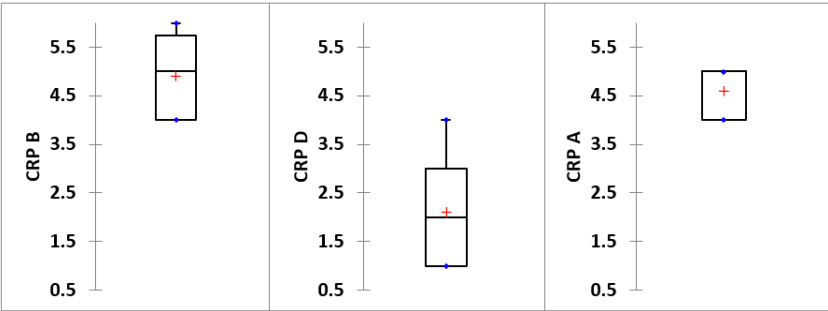


Figure 2 : Box plots of CRP levels according to the three studied periods of training; CRP B (CRP levels before Ramadan); CRP D (CRP levels during Ramadan), CRP A (CRP levels after Ramadan)

The descriptive statistics shows that CRP levels have a mean of 3.87 with a standard deviation of 1.53, indicating moderate variability. The levels range from 1.00 to 6.00, with percentiles at 3.00 (25th), 4.00 (median), and 5.00 (75th), suggesting a slightly skewed distribution. On the other hand, the non parametric Kruskal-Wallis test showed a statistically huge significant difference in CRP levels across the three periods ($p < 0.000$). These results confirm that fasting has a significant impact on CRP levels, with the most pronounced changes observed during the fasting period. In addition, The Mann-Whitney U test results indicate the following comparisons:

- *Before vs During*: A significant difference was found ($p < 0.000$), suggesting that CRP levels are significantly lower during fasting compared to before fasting.
- *Before vs After*: No significant difference was observed ($p = 0.459$), indicating similar CRP levels before and after fasting.
- *During vs After*: A significant difference was found ($p < 0.000$), indicating that CRP levels are significantly higher after fasting compared to during fasting.

Consequently, these results highlight that CRP levels decrease significantly during fasting and increase significantly after fasting, with notable differences observed between the fasting periods.

Figure 3 shows the box plot of the Squat Jump (SJ) levels according of the three studied periods:

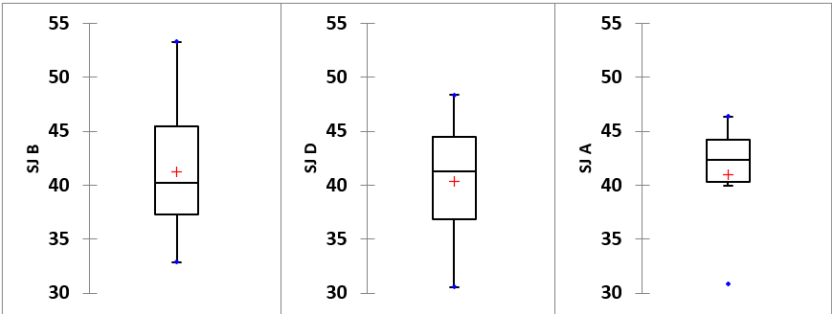


Figure 3 : Box plots of SJ levels according to the three studied periods of training; SJ B (SJ levels before Ramadan); SJ D (SJ levels during Ramadan), SJ A (SJ levels after Ramadan)

ANOVA showed a p-value ($p = 0.937$) so much greater than 0.05. Consequently, there are no statistically significant differences in Sargent Jump performance across the three periods (Before, During, and After Ramadan). This suggests that fasting during Ramadan did not have a significant impact on vertical jump performance.

Figure 4 shows the box plot of the Counter Movement Jump (CMJ) levels according of the three studied periods:

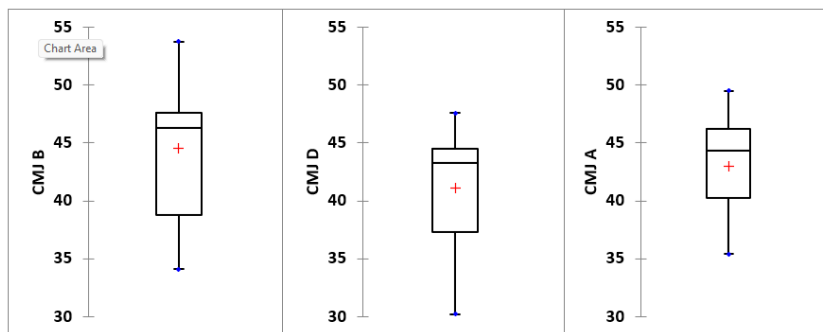


Figure 4 : Box plots of CMJ levels according to the three studied periods of training; CMJ B (CMJ levels before Ramadan); CMJ D (CMJ levels during Ramadan), CMJ A (CMJ levels after Ramadan)

ANOVA showed a p-value greater than 0.05 ($p = 0.426$). Consequently, there are no statistically significant differences in CMJ performance across the three periods (Before, During, and After Ramadan). This suggests that fasting during Ramadan did not have a significant impact on this type of physical performance.

DISCUSSION

The study results showed that creatine kinase (CPK) levels were higher before Ramadan, then decreased during fasting and continued to decline afterward. However, these changes were not statistically significant, indicating that Ramadan did not significantly affect muscle damage. This finding aligns with previous studies such as that of Zouhal et al. (2020), which reported no significant change in CPK levels among athletes during Ramadan, suggesting that fasting's impact on muscle stress may be limited under certain conditions. Similarly, Bouzid et al. (2019) found that while CPK levels increased post-exercise, there was no difference between fasting and non-fasting periods, supporting the current study's findings. Conversely, Graja et al. (2021) observed a significant rise in CPK levels towards the end of Ramadan, particularly after short and intense exercises. Likewise, Souissi et al. (2012) found that CPK levels gradually increased throughout the fasting month, indicating a cumulative effect of fasting on muscle stress. These differences could stem from variations in training load intensity, as athletes in the current study may have experienced lower physical strain than those in studies where CPK levels increased. Timing of measurement could also be a factor, as some studies recorded data in the final days of Ramadan, when physical fatigue is likely at its peak.

On the other hand, the study revealed a significant decrease in C-reactive protein (CRP) levels during Ramadan, with values dropping sharply from pre-fasting levels before rising again post-Ramadan. This outcome aligns with the findings of Zouhal et al. (2020), who confirmed that fasting has an anti-inflammatory effect by reducing CRP levels along with other inflammatory markers such as IL-6 and TNF- α . This supports the notion that fasting may contribute to enhanced immune response and reduced oxidative stress. Similarly, Trabelsi et al. (2011) found a significant reduction in CRP levels among physically active individuals during Ramadan, reinforcing the hypothesis that fasting can mitigate systemic inflammation. Hammouda et al. (2013) also observed lower inflammation levels when exercise was performed during fasting compared to non-fasting periods, highlighting the protective role of fasting against oxidative stress. Despite these similarities, some studies, such as Graja et al. (2021), reported no significant change in CRP levels during Ramadan, while Silva et al. (2022) noted a slight increase in CRP levels after pre-season training, which contrasts with the present study's findings. This discrepancy could be attributed to differences in training intensity and exercise loads, as athletes in certain studies may have undergone more strenuous physical activity, preventing the expected decline in CRP. Additionally, nutritional factors may have influenced these results, as well-balanced nutrition during Ramadan can enhance fasting's anti-inflammatory benefits and contribute to lower CRP levels.

Regarding physical performance, the study found no significant differences in vertical jump performance (SJ & CMJ) before, during, or after Ramadan, indicating that fasting did not negatively impact explosive muscle power. This finding is consistent with the work of Chtourou et al. (2012), who suggested that the effects of Ramadan on muscular performance are minimal or even negligible, particularly when athletes maintain appropriate training regimens. Likewise, Bouzid et al. (2019) reported that Ramadan had no substantial impact on muscle recovery or overall physical performance, corroborating the present study's conclusions. However, other studies documented a decline in jump performance during fasting. Graja et al. (2021) recorded a reduction in jump height during the final week of Ramadan, a trend also noted by Hammouda et al. (2013), who found that physical performance may decline slightly during fasting. Additionally, Slimani et al. (2018) emphasized that the level of competitiveness and professionalism plays a crucial role in performance variation, as elite athletes may experience greater performance declines compared to amateur players. These disparities could be explained by the level of athletic professionalism, where athletes in some studies endured higher training loads, leading to more pronounced performance decrements. Moreover, timing of assessments may have influenced the findings, as studies that reported declines in performance often conducted measurements in the final days of Ramadan, when cumulative fatigue may have been more pronounced.

Finally, the study found no significant impact of fasting on muscle fatigue or recovery, as post-exercise performance remained stable across the different phases. This outcome aligns with Ramadan et al. (1999), who observed that well-adapted athletes do not experience major negative effects on muscle function during fasting.

Similarly, Berbari et al. (2012) indicated that athletes following optimal nutritional and recovery strategies may not suffer from significant performance decrements during Ramadan. In contrast, Bouzid et al. (2019) found that muscle fatigue levels were higher post-match during Ramadan than in non-fasting conditions, while Graja et al. (2021) noted increased fatigue toward the end of Ramadan, which adversely affected muscle recovery. These differences may stem from variations in recovery protocols, as athletes in the current study may have followed less intense training regimens or consumed supportive nutrition that helped mitigate fasting's effects on muscle fatigue. Furthermore, differences in fatigue measurement methods could contribute to variations in reported findings across studies.

Overall, the study confirms that Ramadan may have anti-inflammatory benefits, particularly through reducing CRP levels, while its effects on CPK and physical performance appear to be minimal. These findings align with several previous studies, though some discrepancies exist, likely due to differences in athlete levels, training intensity, and dietary habits. Future research should investigate the impact of training intensity on CPK levels during Ramadan, analyze the role of dietary habits in mitigating fasting's effects on athletic performance, and compare fasting's impact between elite and amateur players to further understand the physiological differences between these groups.

Conclusion and Recommendations :

The results of this study indicate that fasting during the month of Ramadan has varying effects on biochemical markers and physical performance in amateur football players. A significant decrease in C-reactive protein (CRP) levels was observed during fasting, suggesting potential anti-inflammatory effects of Ramadan fasting. However, no significant changes were recorded in creatine kinase (CPK) levels, indicating that fasting did not have a notable impact on muscle damage or stress levels, which may reflect the players' ability to adapt to fasting conditions through appropriate training and nutritional strategies. Furthermore, the study found no statistically significant differences in vertical jump performance, suggesting that fasting did not adversely affect explosive strength or muscle power.

Compared to previous research, the study aligns with findings that highlight the positive effects of fasting on inflammatory responses, while differences remain regarding its impact on CPK levels and physical performance. These variations can be attributed to several factors, including player level, training intensity, recovery strategies, and dietary habits during fasting. Therefore, the effect of Ramadan fasting on athletic performance is not absolute but rather influenced by multiple environmental and individual factors that determine an athlete's ability to adapt to the physiological changes associated with fasting.

Recommendations

1. **Analyzing the Impact of Training Load Intensity:** Further studies should investigate the effects of fasting on CPK levels among athletes exposed to

varying training intensities to determine whether the magnitude of training load influences fluctuations in this biochemical marker.

2. **Evaluating Nutritional Strategies During Fasting:** Since diet plays a crucial role in enhancing performance and recovery during Ramadan, future research should examine the effects of different dietary regimens on physical performance and biochemical markers, considering the timing, quantity, and quality of nutrient intake.
3. **Comparing the Effects of Fasting on Professional and Amateur Athletes:** Given that most previous studies have focused on professional athletes, additional research should explore how fasting affects players of different competitive levels to better understand the role of **fitness level and fasting adaptation** in performance outcomes.
4. **Investigating the Impact of Fasting on Other Markers, Such as Oxidative Stress and Hormonal Balance:** Fasting may have indirect effects on muscle recovery and performance by altering **hormonal levels** (e.g., cortisol and testosterone) and modulating **oxidative stress levels**. Expanding research to include these factors could provide deeper insights into the physiological mechanisms underlying fasting's effects on athletes.
5. **Developing Training Strategies Adapted to Fasting Conditions:** It is essential to design training programs tailored to the physiological demands of fasting, accounting for changes in energy availability and nutrition while optimizing rest and recovery periods to maximize performance benefits.
6. **Examining the Long-Term Effects of Ramadan Fasting on Performance:** While most research focuses on the immediate effects of fasting during or shortly after Ramadan, future studies should assess **long-term impacts** on fitness levels and physiological adaptation to repeated fasting periods, particularly across competitive sports seasons.

Final conclusion :

Based on the findings and recommendations presented, it is evident that Ramadan fasting may have beneficial effects on inflammatory markers without necessarily compromising physical performance or causing significant muscle damage, especially when appropriate training and nutritional adjustments are made. However, further research is needed to explore the underlying **mechanisms of fasting in various athletic contexts**, ensuring optimal strategies for **performance maintenance and adaptation** during this period.

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