OPEN ACCESS
Turkish Journal of Kinesiology

ORIGINAL RESEARCH

Circadian rhythm–dependent effects of acute caffeine consumption on hydration and performance in football players

Özgür Taylan Ökke[1], Ahmet Mor[2]

[1] Department of the Coaching Education, Graduate School of Education, University of Sinop, Sinop, Türkiye. [2] Department of the Coaching Education, Faculty of Sport Sciences, University of Sinop, Sinop, Türkiye.

Received: September 17, 2025

Accepted: November 29, 2025

Published: December 30, 2025

Keywords: Caffeine, circadian rhythm, football players, gastrointestinal symptoms, hydration, performance.

Abstract

The aim of this study was to investigate the effects of acute caffeine (CAF) consumption at different time-of-day, in relation to circadian rhythm, on selected performance parameters and hydration status in football players, as well as to assess gastrointestinal responses following CAF intake. The study included twelve trained male football players aged between 18-25 years. Within the experimental design, which employed a randomized, single-blind approach, the familiarization session was conducted on the first day, followed by three separate test days. Sixty minutes before the initiation of testing, the football players ingested CAF at a dose of 6 mg/kg^{-1}. The tests, measurements, and analyses were conducted at 10:00 a.m. on day 1, 14:00 p.m. on day 2, and 18:00 p.m. on day 3. Differences between variables were analyzed using repeated-measures ANOVA. No significant difference was found in vertical jump, anaerobic power, change of direction (COD), 10 and 20m sprint, ball speed, rating of perceived exertion (RPE), and visual analogue scale (VAS) between time-of-day after CAF intake ($p>0.05$). There were significant main effects for the 30m sprint between time-of-day after CAF intake ($p<0.05$). CAF intake did not induce a significant difference in hydration levels ($p>0.05$). There was a significant difference in the Gastrointestinal Symptom Rating Scale (GSRS) total between the time-of-day after CAF intake ($p<0.05$). In conclusion, this study revealed that acute CAF intake in relation to circadian rhythm had no significant effect on performance in football players. Additionally, the findings indicated a slight decline in performance during the morning hours. This outcome is thought to be primarily attributable to the athletes' habitual training and competition schedules rather than to CAF intake. Furthermore, gastrointestinal symptoms associated with morning CAF intake may have contributed to the observed performance decrease. Overall, the data suggested a tendency toward more favorable outcomes in the evening measurements.

Introduction

Football is a team sport that actively requires the use of various motor skills such as low- and high-intensity running, acceleration–deceleration, reactive agility, strength, and balance (Tessitore et al., 2006). Depending on the overall intensity of play and positional demands, football players cover approximately 10–13 km during a match (Bangsbo, 2014). Particularly in high-level matches, fatigue-induced performance declines are frequently observed among players (Bangsbo et al., 2006). Athletes often resort to ergogenic supplementation before and during matches to prevent this. Among these, CAF is one of the most widely used ergogenic aids across different sports (Grgic & Del Coso, 2021). In sports such as soccer, where sudden acceleration and deceleration, agility requirements, and high-intensity efforts are paramount, it has been reported that caffeine may enhance cognitive performance under fatigue conditions but may not have a direct, significant effect on physical performance (Nana et al., 2025). In this regard, it is thought that low-dose caffeine supplementation may provide more pronounced benefits, particularly in cognitive performance components. The effects of caffeine on cognitive and physical performance in various sports

✉ A. Mor, E-mail: amor@sinop.edu.tr
Turk J Kinesiol, 11(6), 342-353. doi: 10.31459/turkjkin.1786020

disciplines and with different methods of administration—especially in gum and tablet form—require further investigation (Bougrine et al., 2022). Studies conducted on male soccer players typically examine the effects of caffeine on performance, focusing on its potential to raise the fatigue threshold and enhance cognitive functions (Nana et al., 2025). Furthermore, studies have shown that caffeinated gum can enhance neuromuscular performance indicators, including vertical jump height, isokinetic strength, and lower extremity power (Venier et al., 2019). CAF exerts its performance-enhancing effects through several psycho-physiological mechanisms. Research has indicated that CAF enhances voluntary muscle contraction by improving neuromuscular transmission (Behrens et al., 2015) and increasing intramuscular calcium levels (Tarnopolsky, 2008). Moreover, Davis and Grey (2009) reported that CAF binds to adenosine receptors, thereby influencing the central nervous system and reducing the perception of fatigue through its stimulatory effects. Due to these diverse mechanisms of action, numerous studies have examined the impact of CAF consumption on various components of sports performance requiring physical and physiological stimulation, including jump performance, sprint speed, ball speed, COD, anaerobic capacity/power, and hydration status (Mor et al., 2024; Mor et al., 2015). Accordingly, studies on athletic performance have examined caffeine use at low doses (1-3 mg/kg), moderate doses (3-6 mg/kg), and high doses (6-9 mg/kg). Moderate doses of caffeine were used most frequently in these studies. To achieve the ergogenic effect of caffeine during performance, it must be taken in appropriate doses (3-9 mg/kg). Although the optimal dose is unknown, studies show that consumption of around 3-9 mg/kg is sufficient (Bayraktar and Taşkıran, 2019; Grgic et al., 2019). Caffeine's absorption and transport vary depending on the route of administration. It can be absorbed through the buccal mucosa, stomach, or—most commonly—the small intestine (Guest et al., 2021). Among administration methods, absorption typically occurs fastest with gum, followed by drinks and capsules. Owing to its nearly complete bioavailability, caffeine appears in the bloodstream within minutes and has an average half-life of 4–6 hours (Blanchard & Sawers, 1983). Although capsule and gum forms exhibit similar bioavailability, gum enables much faster absorption (approximately 5 minutes) compared with capsules (45–60 minutes) (Cox et al., 2002; Kamimori et al., 2002; Ryan et al., 2013).

Athletes' performance profiles can vary across different time-of-day. Research has demonstrated that athletes tend to perform better during afternoon and evening hours, whereas greater performance decrements are observed in the morning and late-night periods (Kline et al., 2007; Sedliak et al., 2008). Some studies have sought to counteract these performance declines occurring at specific time-of-day by implementing different strategies, such as passive or active warm-up protocols (Racinais et al., 2005; Souissi et al., 2010). In one study, the ergogenic effects of ingesting 6 mg/kg of CAF 60 minutes before testing, conducted either in the morning or afternoon, were examined in trained male athletes. The findings revealed that CAF ingestion enhanced performance when consumed in the morning (Mora-Rodriguez et al., 2015). In another study involving female athletes, researchers investigated the effects of CAF, administered in varying doses at different time-of-day, on physical performance. The results indicated that caffeine's efficacy depended on both the timing of ingestion and the dosage (Bougrine et al., 2024). Similarly, another investigation administered 6 mg/kg of CAF to both trained and untrained cyclists in the morning and evening to assess its impact on performance. The researchers reported that CAF enhanced performance across all participants in both periods, with trained and untrained individuals benefiting from improved morning performance. However, only the untrained participants exhibited greater cycling speed in the evening. Based on these findings, the authors concluded that trained athletes may derive greater ergogenic benefits from CAF intake in the morning compared to the evening. Conversely, untrained individuals appear to achieve more pronounced performance gains from CAF consumption in the evening relative to trained athletes (Boyett et al., 2016).

Since football matches are played at different time-of-day, players' performance levels may be influenced by circadian rhythms. Previous studies have included participants from various sports (Bougrine et al., 2024; Boyett et al., 2016; Mora-Rodriguez et al., 2015; Souissi et al., 2013); however, to the best of our knowledge, no research has specifically examined how CAF intake at different time-of-day affects the objective and subjective performance outcomes of football players. As research on CAF progresses, increasing emphasis has been placed on evaluating dosage and methods of administration. Nevertheless, studies focusing on the optimal timing of CAF ingestion remain limited (Zhang et al., 2024). Therefore, the aim of this study was to investigate the effects of acute CAF consumption at different time-of-day, in relation to circadian rhythm, on selected performance parameters and hydration status in football players, as well as to assess gastrointestinal responses following CAF intake. Considering the positive effects of nutritional supplementation on health and performance in football players, the outcomes of this research are expected to provide valuable insights for future studies on supplementation and contribute to more well-guided supplement use among athletes. Although existing studies address CAF consumption and circadian rhythms in athletes, the number of investigations considering specific subject groups, research designs, and selected parameters remains limited. Given the research protocol and chosen parameters, this study is expected to make a meaningful contribution to the existing literature. Accordingly, this study hypothesized that CAF consumption prior to exercise at different time-of-day would result in variations in both hydration status and performance.

Methods

A quantitative research design was employed in this study. A randomized, crossover, and single-blind experimental design was implemented. The study was approved by the Sinop University of Human Research Ethical Committee (2024/81) and it was carried out in accordance with the Code of Ethics of the World Medical Association also known as a declaration of Helsinki.

Participants

The study included twelve trained male football players aged between 18 and 25 years. A priori power analysis indicated that a sample size of 12 participants was necessary and sufficient (Effect size: 0.50, Confidence interval: $1-\beta = 0.95$, Error probability: $\alpha = 0.05$, Actual power: 0.95). The study was conducted in Sinop, and the athletes were selected from students enrolled in the Faculty of Sport Sciences at Sinop University. To ensure the reliability of the findings and eliminate potential confounding effects of different training regimens, all participants were required to be engaged in the same training program. All participants underwent the same tests, measurements, and analyses under standardized nutritional protocols at three separate time points, each conducted on non-consecutive days and at the same time of day. Participants completed a minimum of four football training sessions each week (8 h per week), and the players were in their post-season period during the measurements. Moreover, all participants verified that they had abstained from the use of any ergogenic aids that could potentially affect hydration status or exercise performance during the three months prior to the initiation of the study. No nutritional survey was conducted, and participants' habitual dietary practices remained unchanged during the study. Inclusion criteria required that participants be in good health, free from any chronic or acute illnesses, and without any movement limitations due to injury. Participants were removed from the study if any health problems were reported or observed. Informed consent was obtained from all participants prior to the commencement of the study.

Procedure

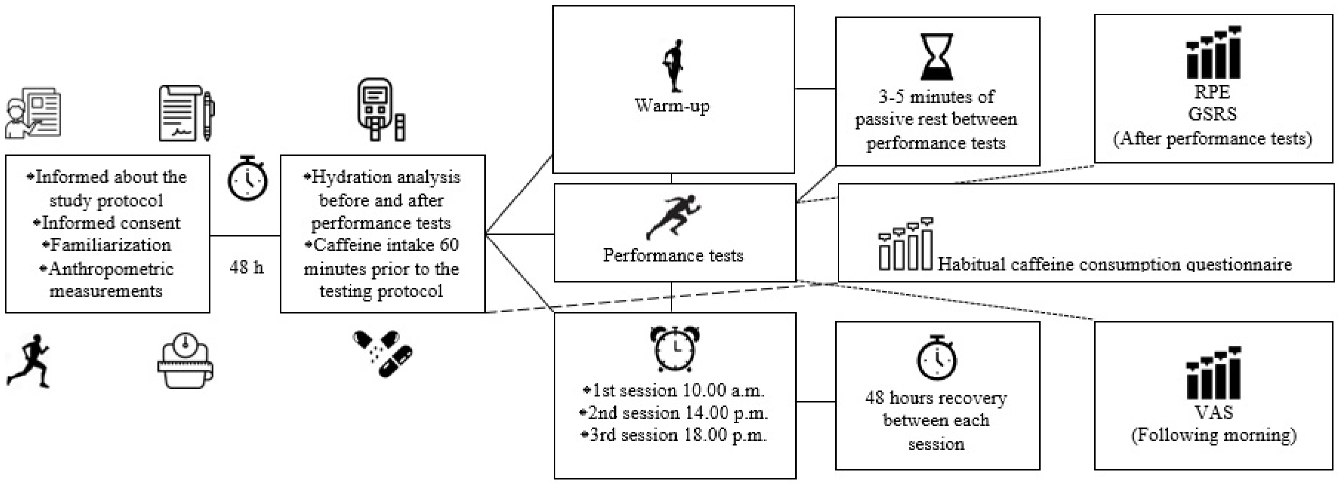
All athletes included in the study were thoroughly informed about the research protocol, and its procedures were explained in detail. Moreover, two days prior to the commencement of the study, participants underwent a familiarization session in which they were instructed to perform the tests at a low intensity without exerting themselves excessively. Within the experimental design, which employed a randomized, and single-blind approach, the familiarization session was conducted on the first day, followed by three separate test days. All tests, measurements, and analyses were carried out in the Faculty of Sport Sciences laboratories at Sinop University and on the FIFA-standard artificial turf football field of Sinop University. In this study, participants were first briefed regarding the tests, measurements, and analyses to be conducted. Anthropometric data, including height and body weight, were collected, and body mass index (BMI) values were calculated. All tests, measurements, and analyses were administered at 48-hour intervals to allow for adequate recovery. The study employed a single-blind design, whereby athletes were not informed of their specific nutritional supplementation. On separate test days and at different time points, various performance parameters of the football players—including vertical jump, anaerobic power, agility, 10-, 20-, and 30-meter sprint performance, as well as ball speed—were assessed. The tests were administered in the same order each day, with 3–5 minutes of passive rest allocated between trials. Participants completed a standardized, sport-specific warm-up protocol before each measurement and test. Sixty minutes before the initiation of testing, the football players ingested CAF at a dose of 6 mg/kg^{-1}. Saliva samples were collected both pre- and post-testing to assess hydration status. The tests, measurements, and analyses were conducted at 10:00 a.m. on day 1, 14:00 p.m. on day 2, and 18:00 p.m. on day 3. The study sought to determine whether CAF exerted an influence on body hydration status and performance at different time-of-day. Participants were instructed to abstain from food intake for three hours prior to the performance assessments. RPE was measured using the Borg scale, while delayed onset muscle soreness (DOMS) was evaluated with the visual analog scale. Participants' habitual CAF consumption frequency was recorded, and a GSRS was employed to identify symptoms commonly associated with gastrointestinal problems. The football players were instructed to rest before the tests and to refrain from engaging in strenuous exercise. All tests, measurements, and analyses were conducted under similar environmental conditions (ambient temperature: 22.33 ± 1.53 °C; relative humidity: 75.33 ± 3.06%; atmospheric pressure: 1013.33 ± 1.53 mbar; mean ± SD) (Figure 1).

Supplementation Protocol

Participants arrived at the laboratory 90 minutes before the commencement of testing, during which the research protocol was again explained to them in detail. Before participation, they were also asked to report any food allergies. Sixty minutes before the tests, the football players ingested a CAF at a dose of 6 mg/kg^{-1} (Nature's Supreme, Istanbul, Türkiye), dissolved in 200 ml of water, under laboratory conditions. Previous literature indicates that peak blood CAF concentrations typically reach approximately 60 minutes after ingestion (Graham & Spriet, 1995). Supplementation in this study was implemented in a single-blind design, with the daily administration method and dosage determined in accordance with the existing literature. Participants were instructed to refrain from strenuous physical activity, alcohol consumption, and stimulant intake for 24 hours before the study and to maintain proper nutrition and rest. Apart from these restrictions, the athletes' parameters remained unaltered in order to ensure a precise assessment of the supplementation effects. Their habitual diet, daily routines, and training programs were kept consistent throughout the study. During the testing sessions, athletes were permitted to consume only water—according to their usual drinking habits and in self-determined amounts—in addition to the supplementation. The CAF doses were prepared at room temperature by a researcher using a digital laboratory balance (FLY 300) with a precision of 0.001 g.

Anthropometric Measurements

The height of the football players were measured in centimeters using a Seca S213 stadiometer (Hamburg, Germany), while their body weight were measured in kilograms using the InBody 120 bioelectrical impedance body composition analyzer (Seoul, South Korea). Body mass index (BMI) was calculated by dividing body weight by the square of height in meters (kg/m²), following the measurement of height and body weight (Mor et al., 2021).



RPE = Rating of perceived exertion; GSRS = Gastrointestinal Symptom Rating Scale; VAS = Visual Analogue Scale

Figure 1. Study Protocol.

Vertical Jump and Anaerobic Power Test

The vertical jump performance of the football players was measured using a digital vertical jump device (Takei 5406 Jump-MD Vertical Jump Meter, Tokyo, Japan). The vertical jump test is a common method used to evaluate and measure lower extremity strength. Each participant was given two attempts with a 1-minute passive rest interval, and the best result displayed on the digital screen was recorded in centimeters with ±1 cm accuracy (Mor et al., 2022). Anaerobic power was calculated using the Lewis formula, based on each participant's body weight and vertical jump height: Anaerobic Power (W) = $\sqrt{4.9} \times$ [Body Weight (kg)] $\times$ $\sqrt{\text{Vertical Jump Height (m)}}$ (Fox et al., 2012).

Agility T-test

The test setup followed the original protocol, with four cones positioned to form a T-shaped layout: cone B was placed 9.14 meters from the starting cone (A), while cones C and D were placed 4.57 meters to the left and right of cone B, respectively. Participants were instructed to sprint from cone A to cone B (9.14 meters) and touch it with their right hand, then perform a lateral movement to cone C (4.57 meters to the left) and touch it with their left hand. Subsequently, they moved laterally to cone D (4.57 meters to the right) and touched it with their right hand, returned laterally to cone B and touched it with their left hand, and finally backpedaled to cone A to complete the test. Timing commenced as the athletes departed from cone A and stopped upon their return to cone A. A trial was deemed invalid if the participant failed to touch any cone, executed the lateral sliding steps incorrectly, or did not maintain a forward-facing position throughout the test (Mor et al., 2022; Okudur and Sanioğlu, 2012).

Sprint Test

The football players' 10-, 20-, and 30-meter sprint performances were assessed on an artificial turf field using a photocell system with a precision of ±0.01 seconds (Seven, SE-165 photocell stopwatch, Istanbul, Turkey). Participants initiated the sprint with a standing start from a line positioned one meter behind the initial photocell, beginning at their own readiness. Timing commenced automatically when the photocell at the start line was triggered, with intermediate times recorded as the athletes passed through the 10- and 20-meter gates, and final times captured upon crossing the 30-meter finish line. Athletes were instructed to sprint at maximum effort and completed two trials of the test, separated by a three-minute period of passive rest. Sprint performance was recorded in seconds and hundredths of a second, with the best result of the two trials retained for analysis (Mor et al., 2021).

Ball Speed Test

Ball speed was assessed from a distance of 11 m (penalty mark) from the goal using a radar gun (Bushnell Velocity Speed Gun, Overland Park, KS, USA), capable of measuring speeds within a range of 16–177 km/h with an accuracy of ±2 km/h. Prior to testing, participants' dominant legs were identified through subjective self-report to ensure maximal kicking performance. Subsequently, participants executed shots employing the instep kick technique. All kicks were performed using a size 5 ball (appropriate for individuals aged 12 years and older) in accordance with FIFA regulations. The same researcher conducted all measurements to maintain consistency and enhance reliability by positioning the radar gun behind the goal, directly aligned with the penalty spot from which the ball was kicked. Participants were instructed to aim for accuracy (targeting) while simultaneously achieving maximal ball speed. Each player was given two trials to obtain the best score, and the results were recorded in km/h (Mor et al., 2022; Mor et al., 2025).

Hydration Analysis

The portable hydration test device (MX3 Diagnostics LAB Pro, Melbourne, Australia) was used to assess participants' body hydration levels. The device was convenient and easy to use for outdoor measurements and analyzed the hydration level from the saliva taken directly from the tongue with a hydration test strip. The saliva sample was taken from the tongue under the sterilization rules. The saliva sample was collected and analyzed with a hydration test strip attached to the device without waiting or undergoing any other procedure. The values and their assigned hydration status follow as ≤65 = Hydrated, 65-100 = Mildly Dehydrated, 101-150 = Moderately Dehydrated, >150 = Severely Dehydrated (Mor et al., 2024).

Borg Scale (Rating of Perceived Exertion)

The Rating of Perceived Exertion (RPE) is a method used to assess athletes' perceived exertion during physical activity. In the scale developed by Borg, the highest value is expressed as 10, and the lowest as 0 at rest. An increasing score between 0 and 10 corresponds to an increasing level of exertion experienced during the test (Borg, 1998). After each measurement, athletes were asked to rate the level of exertion they felt in their bodies using a numerical value, which was then recorded.

Visual Analogue Scale (VAS)

The Visual Analogue Scale (VAS), developed by Albersnagel (1988), is a measurement tool that allows for the assessment of perceived pain or fatigue on a 10 cm horizontal line divided into equal intervals. The scale ranges from "no pain" to "worst imaginable pain" (Albersnagel, 1988). In this study, the VAS was used to numerically evaluate athletes' levels of physical fatigue.

Gastrointestinal Symptom Rating Scale (GSRS)

The Gastrointestinal Symptom Rating Scale (GSRS) was developed by Revicki et al. (1997) to assess the severity of symptoms commonly associated with gastrointestinal problems. The Gastrointestinal Symptom Rating Scale (GSRS) consists of 15 items and is structured as a 7-point Likert-type scale, with response options ranging from "no discomfort" to "very severe discomfort." Based on factor analysis, the 15 items of the GSRS are grouped into five subcategories: abdominal pain, reflux, diarrhoea, indigestion, and constipation. Items 1, 4, and 5 of the scale assess abdominal pain; items 2 and 3 assess reflux; items 11, 12, and 14 assess diarrhoea; items 6, 7, 8, and 9 assess indigestion; and items 10, 13, and 15 assess constipation. Evaluation is carried out accordingly. The total score obtained from the responses ranges from 15 to 105. Higher scores on the scale indicate more severe gastrointestinal symptoms (Revicki et al., 1997).

Data Analyses

The normality of the data was checked using the Shapiro-Wilk test. Differences between variables were analyzed using repeated-measures ANOVA. Post-hoc comparisons between groups were evaluated using the Bonferroni correction. Between-group comparisons were analyzed with a paired sample t-test. Effect sizes were calculated using the eta-squared (η^2) coefficient, ranging from 0.00 to 1.00, and interpreted as follows: 0.01 = small, 0.06 = medium, 0.14 = large, and 0.20 = very large (Alpar, 2022). The results of the study are presented as mean ± standard deviation (M ± SD), and statistical significance was set at $p<0.05$. All statistical analyses were conducted using SPSS version 22.0 software.

Results

The descriptive characteristics of the football players are presented in Table 1, the comparison of performance parameters between groups is presented in Tables 2 and 3, the comparison of hydration levels is presented in Tables 4 and 5, and the comparison of the GSRS is presented in Table 6.

Table 1
Descriptive characteristics of the football players.

Variables	Mean ± SD
Age (yr)	20.33 ± 1.87
Training Age (yr)	11.33 ± 1.94
Height (cm)	172.44 ± 6.62
Body Weight (kg)	71.90 ± 8.98
Body Mass Index (BMI) (kg/m^2)	24.14 ± 2.28
Habitual consumption of CAF (mg/day^{-1})	216.28 ± 120.24

Performance Tests

No significant difference was found in vertical jump ($F = 2.213$, $p = 0.142$, $\eta2 = 0.22$), anaerobic power ($F = 2.096$, $p = 0.155$, $\eta2 = 0.21$), COD ($F = 3.587$, $p = 0.052$, $\eta2 = 0.31$), 10m sprint ($F = 4.187$, $p = 0.062$, $\eta2 = 0.34$), 20m sprint ($F = 2.297$, $p = 0.164$, $\eta2 = 0.22$), and ball speed ($F = 2.829$, $p = 0.089$, $\eta2 = 0.26$) between time-of-day after CAF intake. There were significant main effects for the 30m sprint ($F = 4.400$, $p = 0.030$, $\eta2 = 0.35$) between time-of-day after CAF intake.

RPE and VAS

No significant difference was found in RPE ($F = 2.195$, $p = 0.144$, $\eta2 = 0.22$), and VAS ($F = 0.914$, $p = 0.421$, $\eta2 = 0.10$) between time-of-day after CAF intake.

Hydration Levels

No significant difference was found in hydration levels before performance tests ($F = 2.445$, $p = 0.118$, $\eta2 = 0.23$), and hydration levels after performance tests ($F = 0.573$, $p = 0.575$, $\eta2 = 0.07$) between the three groups at three times of day after CAF intake. There was no significant difference between the pre- and post-performance tests at 10.00 a.m. ($t = 0.494$, $p = 0.635$), 14.00 p.m. ($t = -0.805$, $p = 0.444$), and 18.00 p.m. ($t = 0.412$, $p = 0.691$) time-of-day after CAF intake.

Gastrointestinal Symptoms

No significant difference was found in abdominal pain ($F = 4.159$, $p = 0.074$, $\eta2 = 0.34$), reflux ($F = 1.000$, $p = 0.347$, $\eta2 = 0.11$), diarrhoea ($F = 2.286$, $p = 0.169$, $\eta2 = 0.22$), indigestion ($F = 4.000$, $p = 0.081$, $\eta2 = 0.33$) and constipation ($F = 1.000$, $p = 0.347$, $\eta2 = 0.11$) between time-of-day after CAF intake. There was a significant difference for GSRS (total) ($F = 6.269$, $p = 0.036$, $\eta2 = 0.43$) between the time-of-day after CAF intake.

Table 2
Comparison of football players' performance parameters according to circadian rhythm (Mean ± SD).

Variables	10.00 a.m.	14.00 p.m.	18.00 p.m.	F	p	η^2
Vertical Jump (cm)	51.89 ± 5.80	54.00 ± 4.27	53.11 ± 4.86	2.213	0.142	0.22
Anaerobic Power (W)	1142.16 ± 132.57	1165.94 ± 123.31	1156.73 ± 136.90	2.096	0.155	0.21
COD (s)	9.77 ± 0.55	9.67 ± 0.56	9.42 ± 0.39	3.587	0.052	0.31
10m Sprint (s)	1.68 ± 0.05	1.73 ± 0.11	1.64 ± 0.05	4.187	0.062	0.34
20m Sprint (s)	3.11 ± 0.19	3.29 ± 0.28	3.23 ± 0.12	2.297	0.164	0.22
30m Sprint (s)	4.29 ± 0.13ɫ	4.25 ± 0.22	4.15 ± 0.10ǂ	4.400	0.030*	0.35
Ball Speed (km/h)	88.69 ± 9.05	89.40 ± 10.05	93.16 ± 8.68	2.829	0.089	0.26

COD = Change of direction.
*Effect sizes (η^2): 0.01 = small, 0.06 = medium, 0.14 = large, and 0.20 = very large. * p<0.05; ǂ Indicates a statistically significant difference from the 10.00 a.m.; ɫ Indicates a statistically significant difference from the 18.00 p.m.*

Table 3
Comparison of football players' RPE and VAS scores according to circadian rhythm (Mean ± SD).

Variables	10.00 a.m.	14.00 p.m.	18.00 p.m.	F	p	η2
RPE	2.33 ± 0.50	2.44 ± 0.53	1.78 ± 1.09	2.195	0.144	0.22
VAS	0.89 ± 0.78	1.11 ± 1.27	0.67 ± 1.00	0.914	0.421	0.10

Effect sizes (η^2): 0.01 = small, 0.06 = medium, 0.14 = large, and 0.20 = very large.
RPE: Rating of perceived exertion; VAS: Visual analogue scale.

Table 4
Comparison of football players' hydration status according to circadian rhythm (Mean ± SD).

Variables	10.00 a.m.	14.00 p.m.	18.00 p.m.	F	p	η2
Hydration BPT (mOsm/l)	70.56 ± 23.55	50.67 ± 14.93	70.78 ± 19.61	2.445	0.118	0.23
Hydration APT (mOsm/l)	66.56 ± 6.00	58.56 ± 23.37	66.44 ± 22.90	0.573	0.575	0.07

Effect sizes (η^2): 0.01 = small, 0.06 = medium, 0.14 = large, and 0.20 = very large.
BPT: Before performance tests; APT: After performance tests.

Table 5
Comparison of football players' hydration status before and after the tests according to circadian rhythm (Mean ± SD).

Time	BPT (mOsm/l)	APT (mOsm/l)	t	p
10.00 a.m.	70.56 ± 23.55	66.56 ± 6.00	0.494	0.635
14.00 p.m.	50.67 ± 14.93	58.56 ± 23.37	-0.805	0.444
18.00 p.m.	70.78 ± 19.61	66.44 ± 22.90	0.412	0.691

BPT: Before performance tests; APT: After performance tests.

Table 6
Comparison of GSRS scores among football players (Mean ± SD).

Variables	10.00 a.m.	14.00 p.m.	18.00 p.m.	F	p	η^2
Abdominal Pain	1.48 ± 0.69	1.04 ± 0.11	1.00 ± 0.00	4.159	0.074	0.34
Reflux	1.17 ± 0.50	1.00 ± 0.00	1.00 ± 0.00	1.000	0.347	0.11
Diarrhoea	1.07 ± 0.15	1.00 ± 0.00	1.00 ± 0.00	2.286	0.169	0.22
Indigestion	1.17 ± 0.25	1.00 ± 0.00	1.00 ± 0.00	4.000	0.081	0.33
Constipation	1.04 ± 0.11	1.00 ± 0.00	1.00 ± 0.00	1.000	0.347	0.11
GSRS (total)	1.19 ± 0.22#ɫ	1.01 ± 0.02ǂ	1.00 ± 0.00ǂ	6.269	0.036*	0.44

GSRS = Gastrointestinal Symptom Rating Scale.
Effect sizes (η^2): 0.01 = small, 0.06 = medium, 0.14 = large, and 0.20 = very large.
**(p<0,05); ǂ Indicates a statistically significant difference from the 10.00 a.m.; # Indicates a statistically significant difference from the 14.00 p.m.; ɫ Indicates a statistically significant difference from the 18.00 p.m.*

Discussion

This study aimed to investigate the effects of acute CAF intake at different time-of-day, in relation to circadian rhythm, on selected performance parameters and hydration levels, as well as to evaluate gastrointestinal symptoms experienced following CAF intake in football players. The integration of circadian rhythm–based performance tests with the assessment of athletes' hydration status and the application of a gastrointestinal symptom rating scale represents a distinctive feature of this research, setting it apart from similar studies. The study's main findings revealed that pre-training CAF intake at different time-of-day—particularly in the morning—was associated with gastrointestinal discomfort in football players, which in turn negatively impacted performance. No statistically significant difference was observed in vertical jump, anaerobic power, COD, 10 m sprint, 20 m sprint, ball speed, RPE, or VAS scores. The only significant difference was observed in the 30 m sprint. In addition, no statistically significant changes were detected in the athletes' hydration levels. These findings demonstrate partial consistency with our hypothesis and indicate that CAF intake prior to exercise at different time-of-day may induce performance differences, potentially attributable to gastrointestinal effects.

In the present study, morning CAF intake induced adverse gastrointestinal symptoms in football players and was associated with a decline in overall performance, although no statistically significant differences were observed except for the 30 m sprint. CAF intake in the afternoon did not differ from morning or evening intake, with the highest performance values recorded in the evening. Bougrine et al. (2022) examined performance parameters at 8:00 a.m. and 6:00 p.m., 60 minutes after the ingestion of CAF (6 mg/kg^{-1}) or a placebo (cellulose) in young female handball players. The study findings indicated that CAF intake enhanced both cognitive and physical performance while reducing intraday variation of performance parameters. The authors concluded that a 6 mg/kg^{-1} dose of CAF may be advantageous for improving cognitive and physical performance and represents an effective ergogenic aid for mitigating the morning decline. Similarly, Mora-Rodríguez et al. (2012) investigated whether CAF intake in male athletes engaged in high-resistance training could counteract morning neuromuscular performance decrements linked to circadian rhythm. The study demonstrated that CAF consumption at 10:00 a.m. reversed the reduction in morning neuromuscular performance and elevated outcomes to levels exceeding those observed in the afternoon. Conversely, a study reporting different findings investigated whether CAF intake in trained male athletes could reduce the adverse effects of diurnal variation in repeated sprint performance and whether afternoon ingestion would enhance performance relative to the morning. The researchers reported that repeated sprint performance (10 × 6 s cycle sprints), assessed 60 minutes after ingestion of either 5 mg/kg^{-1} CAF or placebo, was influenced by the time-of-day, with lower performance in the morning than in the afternoon. However, researchers concluded that CAF supplementation did not prevent the morning decline nor improve afternoon performance (Lopes-Silva et al., 2019). Notably, circadian rhythm is recognized to play a crucial regulatory role in many aspects of physiology, energy metabolism, and sleep (Sherman et al., 2011). Nevertheless, caffeine's wakefulness-enhancing and sleep-disrupting properties are also well established in the literature. A study has demonstrated that CAF influences human circadian timing (Burke et al., 2015). In research examining parameters similar to those in our study, Bougrine et al. (2024) administered a CAF side effects questionnaire in addition to some objective and subjective assessments, including the counter movement jump (CMJ), modified agility T-test (MATT), repeated-sprint ability (RSA), and rating of perceived exertion (RPE). In the study, athletes ingested CAF capsules 60 minutes prior to testing in both the morning and evening. The findings indicated that the efficacy of CAF was dependent on both the time-of-day and the dosage, though no significant effect on RPE was observed. Notably, the incidence of side effects increased significantly following evening caffeine intake at both doses, with the most pronounced effect observed at the medium dose (6 mg/kg^{-1}). Souissi et al. (2015) investigated the impact of afternoon CAF intake on mood states, simple reaction time, and muscle power during the Wingate Anaerobic Test (WanT) in elite judokas. Their results revealed that CAF consumption increased anxiety and vigour while reducing reaction time. However, muscle power and fatigue remained unaffected, leading the authors to conclude that CAF ingested in the afternoon exerted no ergogenic effect on anaerobic performance. In another investigation, Souissi et al. (2019) evaluated the effects of CAF consumption on diurnal fluctuations in cognitive performance (reaction time and attention) and 5-m multiple shuttle run test performance. The performance of 15 trained male athletes was assessed at six different times of day following ingestion of either CAF (6 mg/kg^{-1}) or a placebo. The researchers found that CAF significantly improved both cognitive and physical performance compared to placebo at all time-of-day. Although performance outcomes were time-of-day dependent, CAF was identified as an effective ergogenic aid, particularly in mitigating declines and enhancing performance during periods when the lowest values were typically observed. Similarly, Mora-Rodríguez et al. (2015) examined whether ingesting 6 mg/kg^{-1} of CAF 60 minutes prior to testing influenced neuromuscular performance in trained male athletes during morning and afternoon sessions. The findings demonstrated that moderate (6 mg/kg^{-1}) CAF intake prevented the decline in muscle contraction velocity commonly observed in the morning under varying loads, with the effect being most evident in lower extremity muscles. Conversely, evening CAF ingestion produced minimal improvements in neuromuscular performance, and adverse side effects were more frequently reported during afternoon trials. Contrasting evidence was provided by Souissi et al. (2012), who reported that morning CAF consumption enhanced strength performance, increased anxiety and vigour, and reduced simple reaction time, indicating clear ergogenic potential. Furthermore, Souissi et al. (2013) observed that CAF intake in both the morning (07:00 a.m.) and evening (17:00 p.m.) elevated anxiety, vigour, and fatigue, while also reducing simple reaction time compared to placebo. Based on these findings, the researchers recommended that CAF ingestion be strategically timed in the morning to minimize circadian rhythm-related fluctuations in short-term maximal performance.

There is considerable debate regarding whether morning or evening exercise is more advantageous. While some individuals consider training sessions as an effective way to conclude the day, others report difficulty resting after late-evening exercise. This may initially appear unexpected when considered in the context of normal circadian rhythms. In both strength and endurance training, peak performance levels are typically observed in the afternoon and evening. This phenomenon is primarily attributed to diurnal rhythmic fluctuations in core body temperature. Such rhythmicity is regulated by alterations in blood flow and, consequently, by changes in distal skin temperature, which reaches its maximum in the late evening and its minimum in the morning. The same physiological mechanisms that regulate core body temperature are also responsible for thermoregulation during exercise (Haupt et al., 2021). Circadian rhythms play a critical role in the variability of both physical and cognitive performance, with the degree of variability increasing as task complexity rises. The circadian rhythm of performance exhibits fluctuations of approximately 10% to 30% relative to daily averages, underscoring the importance of carefully considering the timing of athletic activities. Research evidence indicates that these rhythmic fluctuations are evident across sensory-motor, psychomotor, and perceptual functions that influence athletic performance. Additionally, factors such as workload, physiological stressors, motivation, arousal levels, chronotype, environmental lighting, sleep patterns, and the so-called "post-lunch dip" have been shown to modulate this rhythmicity in performance outcomes (Drăgoi et al., 2024). Accordingly, circadian rhythms appear to play a decisive role in shaping athletic performance, often contributing to reduced performance capacity in the morning hours. CAF ingestion has been demonstrated to mitigate these adverse effects, yielding ergogenic benefits and performance enhancements comparable to those observed in the evening. Although the precise mechanisms by which CAF influences circadian rhythms and athletic performance remain to be fully elucidated, its established ergogenic effects have been attributed to adenosine receptor antagonism, enhanced intracellular calcium release, and modulation of catecholamines. Moreover, CAF may indirectly influence circadian rhythms by increasing sensitivity to light-induced phase shifts (Zhang et al., 2024).

Limitations

The findings of this study offer important contributions, but certain limitations should be considered. First, as the sample size consisted of only 12 male soccer players, the generalizability of the results is limited. Studies involving different age groups, female athletes, or athletes from different sports could allow for more comprehensive conclusions regarding the interaction between caffeine and circadian rhythms. Furthermore, selecting all participants from students in the sports science faculty of the same university created a homogeneous sample but limited external validity. Second, the study design was single-blind. Potential expectation effects related to caffeine consumption among participants could not be fully controlled. Double-blind and placebo-controlled designs may help to obtain stronger evidence in future studies. Third, the study evaluated only acute caffeine intake. The effects of regular and long-term caffeine use on circadian rhythm and performance are unknown. Furthermore, a single dose (6 mg/kg⁻¹) was used, and the differential effects of different dose ranges on performance and gastrointestinal side effects were not investigated. Fourth, hydration status was assessed only through saliva analysis. While this method is practical and rapid, the use of additional biomarkers such as urine specific gravity and plasma osmolality could have enabled more accurate and reliable measurement of hydration status. Fifth, performance assessments were limited to laboratory and field tests. The effects of caffeine intake on performance and physiological responses may differ under actual match conditions. Furthermore, the measured parameters (sprint, agility, ball speed, etc.) do not reflect all performance dimensions in soccer. Finally, individual factors that could affect circadian rhythm, such as participants' sleep patterns, chronotype characteristics, psychological states, and dietary habits, were not controlled. As these variables may have influenced performance and caffeine responses, it is recommended that such individual differences be considered in future research.

Conclusion

In conclusion, the evaluation of the performance parameters in this study revealed that acute CAF consumption in relation to circadian rhythm had no significant effect on vertical jump, anaerobic power, speed, agility, or ball speed in football players. Nevertheless, the findings indicated a slight decline in performance during the morning hours. This outcome is thought to be primarily attributable to the athletes' habitual training and competition schedules rather than to CAF intake. Indeed, the participants were not accustomed to morning training or match routines. In this regard, although it was expected that CAF would enhance or at least maintain performance during these hours, the results did not support this assumption and instead demonstrated that CAF exerted only a limited effect during the period when it was anticipated to be most beneficial. Additionally, gastrointestinal symptoms associated with morning CAF consumption may have contributed to the observed performance decrease. Overall, the data suggested a tendency toward more favorable outcomes in the evening measurements. Conversely, the results indicated that CAF consumption at different time-of-day did not influence body hydration levels, with players' hydration status remaining within normal ranges. Furthermore, daily CAF intake did not produce significant differences in RPE or VAS. Together, these findings suggest that CAF may be used as a pre-exercise nutritional supplement to support performance in football players; however, particular attention should be given to potential gastrointestinal side effects, especially when consumed in the morning.

Authors' Contribution

Study Design: ÖTÖ, AM; Data Collection: ÖTÖ, AM; Statistical Analysis: AM; Manuscript Preparation: ÖTÖ, AM; Funds Collection: ÖTÖ, AM.

Ethical Approval

The study was approved by the Sinop University of Human Research Ethical Committee (2024/81) and it was carried out in accordance with the Code of Ethics of the World Medical Association also known as a declaration of Helsinki.

Funding

This study was supported by the TÜBİTAK 2209-A University Students Research Projects Support Program (Project Number: 1919B012333984, Project Period: 2023/2).

Conflict of Interest

The authors hereby declare that there was no conflict of interest in conducting this research.

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