Effect of Whey Protein Supplementation on Performance Determinants and Inflammatory Biomarkers of Male Football Players: A Randomized Parallel-Group Placebo-Controlled Trial

Kingshuk Ghosh , Jasmine Kaur Chawla , Indranil Manna .

Abstract

Introduction: Resistance training (RT) is beneficial in developing strength, power, and speed in footballers. Taking protein supplements combined with RT is expected to cause a more significant muscular hypertrophy over time. Also, the intermittent high-intensity sprints, rapid changes of direction, and powerful eccentric decelerations, the biomechanical demands of football induce significant micro-trauma to the skeletal muscle fibres. Choice of Whey-Protein supplementation combined with RT can significantly dictate adaptations to resistance training and the rate of post-match physiological restoration. But the practice of periodized resistance training and intake of protein supplements is rare in non-elite football players in rural areas.

Objectives: The present study was designed to find out the impacts of resistance training combined with whey protein supplementation on different performance determinants and inflammatory biomarkers of non-elite football players from rural areas.

Methods: A total of 96 football players who have been playing football at the district level for more than 2 years were selected for the study. Then they were subdivided into two groups: (i) Whey Protein Supplement Group (WPSG, n=48), where a supplement of whey protein was provided in 1.00 gm/ kg BM/day, and (ii) Placebo Control Group (PCG, n=48), which received a maltodextrin placebo. Both groups followed a resistance training schedule for two mesocycles of 6 weeks (2 sessions/wk, for 12 weeks). Assessment of body composition, physical fitness, physiological variables, and inflammatory biomarkers was measured at 0 weeks, 6 weeks, and 12 weeks.

Results:Significantly greater improvements in lean body mass, muscular strength, vertical jump, anaerobic power, sprint speed, agility, and anaerobic capacity were established in WPSG compared with the PCG ($p \le 0.05$). Whey protein supplementation also significantly diminished the increases in inflammatory biomarkers, including CRP, CK, and LDH.

Conclusion: Whey protein supplementation in combination with resistance training effectively enhances performance determinants, lean body mass, and recovery while reducing exercise-induced inflammation in non-elite male football players.

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Resistance training, Strength, Power, Speed, Whey Protein, Inflammatory biomarkers. Football players.

Introduction

A complex physiological profile, including high aerobic capacity, muscular strength, anaerobic power, and metabolic capacities, is the main endeavour of playing football. Consequently, one of the most important objectives in preparing football players for competition is developing high levels of muscular strength. Resistance training (RT) is known to improve performance-enhancing qualities like strength, power, speed, agility, and endurance, reducing the risk of injury. It has been demonstrated that prolonged periodized RT could be effective for increasing the size of skeletal muscles and, eventually, their ability to generate force. Therefore, resistance training must be incorporated into the training regimens of Football [Tabassum et al.,2023].

RT is only maximized when accompanied by an adequate supply of essential amino acids (EAAs) to stimulate muscle protein synthesis (MPS). The intermittent high-intensity sprints, rapid changes of direction, and powerful eccentric decelerations, the biomechanical demands of football induce significant micro-trauma to the skeletal muscle fibres. The modern football athlete operates within a narrow recovery window where the choice of protein supplementation can significantly dictate adaptations to resistance training and the rate of post-match physiological restoration. The Endurance athletes involved in various team sports have to maintain a positive nitrogen balance, so the recent recommendation for football players indicates potential benefits from higher amounts, specifically 1.6–2.2 g/kg body mass/day [Williamson et al., 2019]. High-quality protein sources are crucial, as they contain a high proportion of essential amino acids (EAAs) necessary for MPS [Sousa et al., 2022; Jacinto et al., 2022]. Recent evidence suggests that Leucine may reverse the net negative whole-body protein balance and can improve the recovery of inflammatory biomarkers like Creatine Kinase (CK), Lactate dehydrogenase (LDH), and C-Reactive protein (CRP) [Jackman et al., 2017; Wang & Wu, 2023]. Among the protein sources available, whey protein (WP) is considered the most popular one. Whey protein is a byproduct of cheese production. Due to its high concentration of branched-chain amino acids (BCAAs), particularly leucine, whey protein has been the gold standard for athletes for a long time [Griffen et al., 2022].

Indian football players have lower muscle mass than Europeans [Pomeroy et al., 2019]; therefore, they lack in muscular strength and power and cannot keep up with the physical and physiological capabilities of other international players [Kulkarni et al., 2013]. Lack of proper resistance training and protein supplementation also aids in this problem. But the practice of periodized resistance training and intake of protein supplements is rare in non-elite football players in rural areas. However, in the case of the availability of resistance training, there is no adequate weight selection through the 1RM test for athletes and no proper training load monitoring, which ultimately leads to overtraining associated with reduced muscular strength and sometimes injury. Dietary protein supplementation should be given scientifically and periodically. In this regard, the present study was designed to find out the impacts of resistance training and whey protein supplementation on different performance determinants and inflammatory biomarkers of non-elite football players from rural areas.

Even though whey protein supplementation has been widely studied in athletes, limited research has directly examined its impact on both performance determinants and inflammatory biomarkers among male football players. The previous studies mainly focused on strength and recovery outcomes, with incomplete information about biomarkers such as LDH, CRP, and CK. Additionally, several studies lack randomized placebo-controlled designs and fail to assess the sports-specific physiological demands of football. Research on South Asian or Indian football players is also equally limited. Therefore, the current study aims to investigate the effect of whey protein supplementation combined with RT on performance determinants and inflammatory biomarkers among male football players using a randomized parallel-group placebo-controlled design.

II. METHODS

A. Participants

This study was conducted by the Department of Physiology, Midnapore College Research Centre, Vidyasagar University, W.B., India. Male football players (age: 18–20 years) who participated in district-level competitions for the last two years were randomly selected for this study. G*power software [Kang, 2021] was used to compute the sample size. A minimum of 52 subjects was required to carry out the study, as computed from the software. A total of 96 football players were selected for the study. Then they were subdivided into two groups as (i) Placebo Control Group (PCG, n=48) and (ii) Whey Protein Supplement Group (WPSG, n=48). Participants unwilling to take part in the study, had a record of illness or disease for at least three months before the research study started, were excluded from the study.

B. Ethical Consideration, CTRI Registration and Informed Consent Statement

Written information was given to all the participants about the objectives and possible complications of the study, and written consent was taken from them. The Institutional Ethics Committee approval was obtained for the present study [Registration No – CE/2023-24/05; Date: 15/10/24]. The study is also registered in the Clinical Trials Registry India [CTRI No-CTRI/2025/08/092533, Register on 06/08/25].

C. Resistance Training Protocol

Participants of both groups followed a periodization of resistance training schedule. The total training program was divided into two different 6-week mesocycles: (i) 1st mesocycle of Muscular Endurance Phase (MEP, 1-6 weeks, 1hr. 30mins. /session), and (ii) 2nd mesocycle of Maximal Strength Phase (MSP, 6-12 weeks, 1hr.15mins. /session) [Bompa & Buzzichelli, 2021]. Other football-specific training will continue in the remaining days for 2 hours/day, 4 days/week. The first mesocycle focused mainly on muscular endurance, characterized by short rest periods between sets and high training volume (3 sets of 10 repetitions – 2 minutes of rest), designed to help the body adjust anatomically and become ready for a more demanding training session during the maximal strength phase. Subjects were engaged in resistance training at a moderate level (65-75% of 1RM) during this period. The second mesocycle, to acquire maximum strength, training volume (4 sets of 5 repetitions – 3 minutes of rest) was reduced, and training load increased (85-95% of 1RM) during this phase [Barjaste & Mirzaei, 2017]. 1-repetition maximum (1-RM) was used to monitor training intensity [Kim et al., 2023]. The training programme was followed according to the suggestions of the qualified coaches.

D. Nutritional Intervention:

Participants were advised to stick to their regular nutritional routine and avoid taking any extra supplements beyond those that were supplied throughout the intervention to reduce the possibility of dietary confounding outcomes. Participants of the Whey Protein Supplement Group (WPSG) were supplied with a Whey protein (NUTRABAY PURE, India: contain 77.9 gm of protein/ 100 gm) supplement (1.00 g/kg BM whey protein powder + 0.2 g/kg BM sucrose powder). The Placebo Control Group (PCG) was provided Maltodextrin supplement (1.00 g/kg BM Maltodextrin powder + 0.2 g/kg BM sucrose powder). The composition of the two supplements is given in Table 02. The subject consumed two doses of the supplement (0.6 g/kg BM mixed supplement powder) dissolved in water after their morning and afternoon training sessions. Sucrose powder was mixed with protein supplement to ensure that the supplements and placebo would be comparable in energy content, appearance, taste, and texture. The supplement will be taken by the subjects around half an hour after each training session [Macnaughton et al., 2016].

E. Study Design

Nutritional status of the subjects was assessed at baseline (0 week). The selected Body composition, Strength, Power, Speed, Physiological, and Biochemical variables were measured in each group at the beginning of the Muscular Endurance Phase (0 week) and the end of the Muscular Endurance Phase (MEP, 6 weeks), and the Maximal Strength Phase (MSP, 12 weeks).

F. Assessments of Nutritional Status:

The nutritional status of the subjects was assessed by questionnaire method using the 24-hour recall method, at baseline (0week). An attempt was made to find out whether the subjects consume protein as per the recommendation of the Indian Council of Medical Research (ICMR) [Castell et al., 2015].

F. Measurement of body composition variables

Height (stature) and body mass were measured, and body mass index (BMI) and body surface area (BSA) were determined [Chandrasekar et al., 2023]. A skinfold caliper (Cescorf, USA) was used to determine percent body fat, total fat mass, and lean body mass (LBM) following standard methods [Siri, 1961]. The mid-upper arm circumference (MUAC) was measured using standard procedures [Chandrasekar et al., 2023].

G. Measurements of physical fitness variables

A hand grip dynamometer, back and leg dynamometer (Baseline, USA) were used for measurement of hand grip strength, back, and leg strength following standard methods. The vertical jump (VJ), standing broad jump (SBJ), agility and sit-up (abdominal strength), and agility tests were performed using standard procedures. The 30-m sprint times were taken, and speed was determined [Chandrasekar et al., 2023]. The running-based anaerobic sprint test (RAST) was performed to determine the anaerobic power [Burgess et al., 2016].

H. Measurements of physiological variables

The resting heart rate of the subjects was measured after fifteen minutes of rest [McArdle et al., 2015]. The Yo-Yo Intermittent Recovery Test 1 (YYR1) was used for the determination of maximum oxygen uptake (VO2max) of the subjects [Bangsbo et al., 2008].

I. Measurement of Inflammatory Biomarkers

A 5 ml of 12-hour fasting blood sample was drawn from an antecubital vein 24 hours after the last exercise bout for the determination of selected biochemical parameters such as Creatine Kinase (CK), C-Reactive protein (CRP), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Urea, and Uric Acid [Manna, Khanna, & Dhara, 2010].

J. Statistical Analysis

Descriptive statistics were computed for all variables. A two-way ANOVA analysis was performed to find out the effects of group, training, and their interaction (group × training) on all the variables. Bonferroni post-hoc multiple comparison tests were used to detect within-group differences. An independent samples t-test was used to determine between-group differences. For each dependent variable, the main effects of training and group, as well as the interaction effect (group × training), were reported along with their corresponding F-values, degrees of freedom, and partial eta squared ($\eta 2p$) as a measure of effect size. Pearson's correlation analysis was used to investigate the relationship between variables. The statistical significance was chosen at $p<0.05$ [Banerjee, 2018]. All statistical analyses were carried out using SPSS software (version 27.0; IBM Corp., Armonk, NY, USA).

III. RESULT

A. Nutritional Assessment of Two Groups at Baseline

The nutritional assessment of both the Placebo control group (PCG) and the Whey protein supplement group (WPSG) showed no significant differences ($p \le 0.05$) between the groups. But both PCG (53.33 ± 7.39) and WPSG (54.01 ± 6.40) consumed less protein, as per the recommendation of the Indian Council of Medical Research (ICMR) [Castell et al., 2015].

B. Effect of Whey Protein Supplementation Combined with Resistance Training on Body Composition

After a 12-week intervention, both the Placebo Control (PCG) and the Whey Protein Supplement Group (WPSG) showed changes in body composition. The WPSG demonstrated a significant increase in total body weight. After 12 weeks, it rose to 59.30 ± 4.02 kg from the baseline of 54.80 ± 4.28 kg, which was significantly higher than the PCG. Simultaneously, Lean Body Mass (LBM) in the WPSG increased considerably from 50.49 ± 4.10 kg to 55.92 ± 3.78 kg. Body fat percentage (BF%) significantly reduced in both groups. WPSG also achieved a superior increment in mid-upper arm circumference (MUAC), concluding the study at 22.27 ± 2.85 cm.

C. Effect of Whey Protein Supplementation Combined with Resistance Training on Physical and Physiological Determinants

The strength adaptation in both groups yielded resistance training, but the WPSG consistently achieved a greater magnitude of improvement. Both right and left grip strength increased after the intervention. In PCG, the Right grip strength (GSTR) increased from 30.41 ± 3.15 kg to 40.47 ± 3.48 kg, whereas after 12 weeks, WPSG achieved a significantly higher GSTR, increasing from 31.30 ± 2.95 kg to 42.83 ± 2.20 kg. GSTL in WPSG increased from 29.72 ± 3.09 kg to 40.41± 2.15 kg, which is significantly higher than the PCG, which rose from 29.16 ± 3.37 kg to 36.57 ± 3.16 kg. After 12 weeks of training, leg strength reached 141.35 ± 7.11 kg, and the back strength increased up to 116.64 ± 9.91 kg in WPSG when compared to PCG. Abdominal strength (repetitions/minute) increased significantly in both groups. After 12 weeks, the abdominal strength of PCG improved from 40.29 ± 4.16 rep/min at baseline to 43.97 ± 2.96 rep/min at week 12. While the WPSG recorded a significantly greater improvement, rising from 40.52 ± 4.78 rep/min to 45.85 ± 1.96 rep/min.

Both groups resulted in significant improvements in jumping performance after a 12-week resistance training intervention. Vertical Jump (VJ/CMJ) in the Placebo Control Group (PCG) increased from a baseline of 39.12 ± 2.78 cm to 43.56 ± 2.89 cm at 12 weeks. A significantly greater enhancement in VJ/CMJ, rising from 39.20 ± 2.87 cm to 46.55 ± 1.88 cm, could be seen in WPSG, statistically outperforming the PCG. In addition, the Standing Broad Jump Test (SBJT) was enhanced in the PCG from 6.26 ± 0.72 ft. to 7.55 ± 0.84 ft. The WPSG exhibited a superior increase in SBJT, progressing from 6.30 ± 0.70 ft. to 7.90 ± 0.69 ft. by the conclusion of the trial.

Both groups showed improvements in their anaerobic power outputs after 12 weeks. But average power (A-Power) improved significantly in WPSG (183.18 ± 22.11 W to 269.56 ± 35.10 W) compared to PCG (190.79 ± 38.17 W to 243.50 ± 69.99 W). At 12 weeks, WPSG showed an increase in their Fatigue Index, concluding

Table 01. Changes in body composition variables among the Football players

Parameters	Placebo Control Group (n=48)			Whey Protein Supplement Group (n=48)			Two-Way ANOVA		
	0 Wk	6 Wk	12Wk	0 Wk	6 Wk	12 Wk	Group F1 (ηp^2) [p value]	Training F2(ηp^2) [p value]	Interaction F3(ηp^2) [p value]
BMI (kg/m^2)	20.03 ± 1.19	20.08 ± 1.17	19.97 ± 1.00	19.91 ± 0.40	20.43* ± 0.45	21.72* #$ ± 0.46	36.9 (0.116) [p≤0.001]	17.81 (0.11) [p≤0.001]	35.22 (0.20) [p≤0.001]
BF%	7.18 ± 1.59	7.12 ± 1.61	5.62*# ± 1.29	7.87 ± 1.52	6.47$* ± 1.26	5.69*# ± 1.24	0.298 (0.001) [0.585]	51.61 (0.268) [p≤0.001]	3.08 (0.21) [0.047]
LBM (kg)	51.67 ± 4.86	51.19 ± 4.74	51.72 ± 4.43	50.49 ± 4.10	52.18* ± 3.98	55.92* $ ± 3.78	7.52 (0.26) [0.006]	11.14 (0.07) [p≤0.001]	8.67 (0.058) [p≤0.001]
MUAC (cm)	20.04 ± 2.95	20.04 ± 2.94	21.01 ± 3.06	20.50 ± 2.84	21.43 ± 2.88	22.27* $ ± 2.85	9.03 (0.003) [0.03]	5.41 (0.03) [0.05]	0.71 (0.005) [0.49]

[Data are presented as Mean ± SD. Two-way ANOVA was performed to examine the effects of Group, Training, and Group × Training interaction. The Bonferroni post-hoc test was applied for within-group comparisons. * Indicates significant difference compared with 0 week (p≤0.05), # indicates significant difference compared with 6 weeks (p≤0.05). F values, p values, and partial eta squared (ηp²) are reported. An independent samples t-test was used to determine between-group differences. $ indicates a significant difference compared with the PCG (p≤0.05). Degrees of freedom for the independent t-test = 94; critical t = 1.986. SD = standard deviation, ANOVA= Analysis of Variance, PCG= placebo control group, WPSG= whey protein supplement group; BMI= body mass index, BF%= body fat percentage, LBM = lean body mass, MUAC = mid upper arm circumference,]

Table 02. Changes in Physical and Physiological determinants among the football players

Parameters	Placebo Control Group (n=48)			Whey Protein Supplement Group (n=48)			Two-Way ANOVA		
	0 Wk	6 Wk	12Wk	0 Wk	6 Wk	12 Wk	Group F1 (ηp^2) [p value]	Training F2(ηp^2) [p value]	Interaction F3(ηp^2) [p value]
GSR (kg)	30.41 ± 3.15	38.75* ± 3.07	40.47*# ± 3.48	31.30 ± 2.95	38.64* ± 2.78	42.83*#$ ± 2.20	8.91 (0.003) [0.03]	338.70 (0.706) [p≤0.001]	4.21 (0.016) [0.029]
GSL (kg)	29.16 ± 3.37	34.69* ± 2.77	36.57*# ± 3.16	29.72 ± 3.09	36.08*$ ± 2.38	40.41*#$ ± 2.15	32.83 (0.104) [p≤0.001]	248.71 (0.638) [p≤0.001]	8.56 (0.057) [p≤0.001]
Back Str. (kg)	94.97 ± 10.45	107.81* ± 9.84	111.72* ± 8.77	95.47 ± 10.46	108.54* ± 10.42	116.64*#$ ± 9.91	3.02 (0.11) [0.08]	90.14 (0.39) [p≤0.001]	1.48 (0.01) [0.23]
Leg Str. (kg)	113.35 ± 12.42	129.62* ± 9.86	134.10* ± 9.81	112.06 ± 11.94	128.52* ± 11.90	141.35*#$ ± 7.11	1.65 (0.006) [0.199]	136.19 (0.491) [p≤0.001]	5.01 (0.034) [0.00]
VJ / CMJ (cm)	39.12 ± 2.78	43.15* ± 2.91	43.56* ± 2.89	39.20 ± 2.87	44.06* ± 1.97	46.55*#$ ± 1.88	18.77 (0.062) [p≤0.001]	134.52 (0.48) [p≤0.001]	8.06 (0.054) [p≤0.001]
SBJT (f.inch)	6.26 ± 0.72	7.21* ± 0.83	7.55* ± 0.84	6.30 ± 0.70	7.26* ± 0.69	7.90*#$ ± 0.69	2.91 (0.10) [0.89]	91.64 (0.39) [p≤0.001]	1.36 (0.010) [p≤0.001]
Abdominal Strength (rep/min)	40.29 ± 4.16	42.39* ± 3.60	43.97* ± 2.96	40.52 ± 4.78	43.33* ± 3.21	45.85*#$ ± 1.96	5.82 (0.02) [0.016]	38.56 (0.21) [p≤0.001]	1.28 (0.009) [0.277]
Agility (sec.)	19.06 ± 1.13	17.80* ± 1.19	16.62*# ± 1.04	19.35 ± 1.29	17.35* ± 1.19	15.65*#$ ± 0.65	8.45 (0.029) [0.004]	186.05 (0.56) [p≤0.001]	7.77 (0.52) [p≤0.001]
Speed (sec)	6.49 ± 0.33	6.21* ± 0.44	6.10* ± 0.43	6.40 ± 0.34	5.79*$ ± 0.31	5.61*#$ ± 0.31	57.25 (0.16) [p≤0.001]	66.01 (0.31) [p≤0.001]	7.55 (0.051) [p≤0.001]
AP (watt)	190.79 ± 38.17	224.77* ± 55.70	243.50* ± 69.99	183.18 ± 22.11	239.04* ± 29.97	269.56*#$ ± 35.10	4.29 (0.015) [0.04]	59.86 (0.29) [p≤0.001]	3.51 (0.024) [0.03]
FI (watt/sec)	1.87 ± 0.72	2.15 ± 1.11	1.67# ± 0.88	2.12 ± 0.68	2.57*$ ± 0.77	2.52*$ ± 0.43	29.09 (0.094) [p≤0.001]	5.37 (0.03) [0.005]	3.51 (0.024) [0.03]
VO_{2max} (ml/kg/min)	43.54 ± 2.38	43.62 ± 2.22	44.70*# ± 1.55	43.52 ± 6.35	43.60* ± 2.47	44.80 ± 2.30	0.005 (0.00) [0.944]	8.75 (0.05) [p≤0.001]	0.23 (0.00) [0.97]

Data are presented as Mean ± SD. Two-way ANOVA was performed to examine the effects of Group, Training, and Group × Training interaction. The Bonferroni post-hoc test was applied for within-group comparisons. * Indicates significant difference compared with 0 week (p≤0.05), # indicates significant difference compared with 6 weeks (p≤0.05). F values, p values, and partial eta squared (ηp^2) are reported. An independent samples t-test was used to determine between-group differences. $ indicates a significant difference compared with the PCG (p≤0.05). Degrees of freedom for the independent t-test = 94; critical t = 1.986. SD = standard deviation, ANOVA= Analysis of Variance, PCG= placebo control group, WPSG= whey protein supplement group; GSR= grip strength in right hand, GSL= grip strength in left hand, SBJ= standing broad jump, VJ= vertical jump, AP= average power, FI= fatigue index, VO_{2max} = maximum oxygen consumption]

Maximal sprint speed, reflecting on Sprint time, significantly decreased in the WPSG after 12 weeks, from a baseline of 6.40 ± 0.34 seconds to 5.61 ± 0.31 seconds. The decreased sprint time indicates a significant improvement in sprinting speed compared to the PCG. A similar pattern followed by the agility performance, WPSG completed the agility test in 15.65 ± 0.65 seconds by the conclusion of the study, indicating superior neuromuscular adaptation.

After the 12-week resistance training intervention, VO2max (Aerobic capacity) resulted in a positive from 43.54 ± 2.38 ml/kg/min at baseline to 44.70 ± 1.55 ml/kg/min. The WPSG also demonstrated an increase, shifting from 43.52 ± 6.35 ml/kg/min at baseline to 44.80 ± 2.30 ml/kg/min at 12 weeks. However, a direct comparison between the groups showed no significant difference in the magnitude of increased VO2max; the whey protein supplementation did not yield a statistically superior aerobic outcome compared to the placebo.

D. Effect of Whey Protein Supplementation Combined with Resistance Training on Inflammatory Biomarkers

Table 03. Changes in Inflammatory biomarkers among the football players

Parameters	Placebo Control Group (n=48)			Whey Protein Supplement Group (n=48)			Two-Way ANOVA		
	0 Wk	6 Wk	12Wk	0 Wk	6 Wk	12 Wk	Group F1 (ηp^2) [p value]	Training F2(ηp^2) [p value]	Interaction F3(ηp^2) [p value]
CRP (mg/l)	1.57 ± 0.62	1.64 ± 0.58	2.62*# ± 0.68	1.63 ± 0.63	1.68 ± 0.29	1.82$ ± 0.52	12.125 (0.041) [p≤0.001]	34.83 (0.19) [p≤0.001]	17.77 (0.11) [p≤0.001]
CK (U/L)	310.64 ±112.69	322.81 ± 113.38	392.04*# ± 89.80	315.20 ± 113.20	319.95 ± 108.93	340.97$ ± 94.26	1.74 (0.188) [0.06]	7.11 (0.04) [p≤0.001]	1.95 (0.014) [0.143]
LDH (U/L)	349.08 ± 87.46	364.68 ± 84.42	444.02*# ± 83.46	349.97 ± 94.95	361.00 ± 100.99	396.54$ ± 96.34	2.41 (0.008) [0.121]	16.20 (0.10) [p≤0.001]	02.04 (0.014) [0.13]
Total protein (gm/dl)	6.92 ± 0.75	7.10 ± 0.87	7.67*# ± 0.83	6.86 ± 0.74	7.92*$ ±0.84	8.47*# $ ± 0.95	27.34 (0.088) [p≤0.001]	47.54 (0.25) [p≤0.001]	8.56 (0.057) [p≤0.001]
Urea (mg/dl)	25.86 ± 7.53	34.13* ± 8.40	37.78* ± 7.77	25.33 ± 7.43	25.86$ ± 7.53	44.93*#$ ± 9.37	0.33 (0.001) [0.565]	98.27 (0.41) [p≤0.001]	22.07 (0.135) [p≤0.001]
Uric Acid (mg/dl)	6.34 ± 1.16	6.75 ± 1.09	7.90*# ± 0.87	6.23 ± 1.26	6.34 ± 1.16	7.67*# ± 1.11	3.57 (0.013) [0.060]	48.91 (0.25) [p≤0.001]	0.452 (0.637) [0.00]
AST (U/L)	35.84 ± 10.64	35.90 ± 10.51	35.87 ± 10.55	35.60 ± 10.87	35.84 ± 10.64	35.81 ± 10.33	0.009 (0.00) [0.924]	0.00 (0.00) [0.99]	0,00 (0.00) [0.99]
ALT (U/L)	39.63 ± 10.57	39.68 ± 11.04	48.06*# ± 10.34	39.51 ± 10.80	39.63 ± 10.57	45.38*# ± 9.53	0.592 (0.002) [0.442]	14.67 (0.94) [p≤0.001]	0.48 (0.00) [0.614]

[Data are presented as Mean ± SD. Two-way ANOVA was performed to examine the effects of Group, Training, and Group × Training interaction. The Bonferroni post-hoc test was applied for within-group comparisons. * Indicates significant difference compared with 0 week (p≤0.05), # indicates significant difference compared with 6 weeks (p≤0.05). F values, p values, and partial eta squared (ηp^2) are reported. An independent samples t-test was used to determine between-group differences. $ indicates a significant difference compared with the PCG (p≤0.05). Degrees of freedom for the independent t-test = 94; critical t = 1.986. SD = standard deviation, ANOVA = Analysis of Variance, PCG = placebo control group, WPSG = whey protein supplement group; CK=creatine kinase, CRP = C-Reactive Protein, LDH=Lactate Dehydrogenase, AST=Aspartate Aminotransferase, ALT=Alanine Aminotransferase]

Aspartate Aminotransferase (AST) levels showed no significant changes across the 12 weeks for either the PCG (35.84 ± 10.64 to 35.87 ± 10.55 U/L) or the WPSG (35.60 ± 10.87 to 35.81 ± 10.33 U/L). While the Alanine Aminotransferase (ALT) increased significantly from baseline to week 12 in both groups (PCG: 39.63 ± 10.57 to 48.06 ± 10.34 U/L; WPSG: 39.51 ± 10.80 to 45.38 ± 9.53 U/L), there was no significant difference found between the two groups.

In PCG, the systemic inflammation increased significantly as the C-Reactive Protein (CRP) of PCG increased to 2.62 ± 0.68 mg/l at week 12 from 1.57 ± 0.62 mg/l at baseline. On the contrary, the WPSG maintained significantly lower levels, ending the trial at 1.82 ± 0.52 mg/l. After 12 weeks, a significant elevation was shown in CK levels of PCG (310.64 ± 112.69 U/L to 392.04 ± 89.80 U/L). Whereas the WPSG showed an inhibited response (315.20 ± 113.20 U/L to 340.97 ± 94.26 U/L), which indicates significantly lower CK levels compared to the placebo group. A similar pattern was also observed in Lactate Dehydrogenase (LDH). A significant increase, rising from 349.08 ± 87.46 U/L to 444.02 ± 83.46 U/L, can be shown in PCG. The WPSG reduced the increase from 349.08 ± 87.46 U/L to 444.02 ± 83.46 U/L, which was significantly lower than the PCG.

A significant increase in total protein was found after 12 weeks among the WPSG (6.86 ± 0.74 gm/dl to 8.47 ± 0.95 gm/dl) compared to PCG (6.92 ± 0.75 to 7.67 ± 0.83). Compared to the PCG (25.86 ± 7.53 to 37.78 ± 7.77), urea levels also exhibited a significant increase in the WPSG, rising from 25.33 ± 7.43 mg/dl to 44.93 ± 9.37 mg/dl at week 12. Uric acid increased significantly after only 3rd phage in both groups, but between groups, there was no difference.

III. DISCUSSION

The study's objective was primarily fixed to evaluate the synergistic effect of a 12-week structured resistance training program and whey protein supplementation on body composition, performance determinants, and inflammatory biomarkers in male football players. Given the critical role of physical fitness and recovery in elite football, understanding the specific benefits of protein sources is of paramount importance. The data obtained from the present study exhibit that the strategic integration of whey protein significantly enhanced the growth and increase in lean body mass, maximal strength, speed, and power, while modulating systemic inflammation and cellular turnover. A notable increased Boby Weight (BW), LBM, and MUAC observed among the WPSG demonstrates the efficacy of protein supplementation in optimizing the anabolic environment. Performance-enhancing qualities can be improved by RT; however, protein is crucial for boosting muscle protein kinetics and promoting muscle protein synthesis (MPS). A total daily protein intake of approximately 1.6 to 2.2 g/kg body mass/day seems to be the most important factor for optimizing muscle mass accretion [Kerksick et al.,2018]. Whey protein is well known for its high leucine content, a primary amino acid agonist that stimulates MPS [Joy et al., 2013; Park et al., 2019; Wang & Wu, 2023].

The grip strength (GSR and GSL) is considered a reliable proxy of total peripheral strength. On the other hand, the abdominal strength indicates core muscular endurance. Both types of strength are important for maintaining balance, succeeding in physical duals, and avoiding injuries in football. In the current study, a significantly greater gain in GSR and GSL, along with abdominal strength, demonstrates the whey protein's potent anabolic efficiency when it is combined with structured muscular overload. Both the leg and back strength of PCG and WPSG had improved significantly and progressively. However, the strength adaptation of the lower-body and posterior chain was significantly improved by Whey protein ingestion. Previous studies also observed these types of localized strength improvements due to the effects of whey protein ingestion. Obradović et al. (2020) demonstrated that male college athletes taking whey protein supplementation whey protein supplementation in male college athletes obtained significantly greater increases in localized strength measures compared to a placebo [Obradović et al., 2020].

The integration of whey protein alongside a 12-week structured resistance training program successfully maximized the explosive capabilities, evidenced by the significantly superior Highest Power, Average Power, and Anaerobic Capacity observed in the whey-supplemented footballer. Vertical and horizontal jumping parameters (CMJ and SBJ) are critical indicators of lower-body explosive power and force production. The current data demonstrate that while resistance training alone enhances explosive power, the addition of whey protein significantly magnifies these adaptations. In the present study, performance variables critical to football match-play, such as sprint speed, agility, and anaerobic power, responded highly favourably to the combined intervention of RT and whey protein supplementation. Sprint time significantly decreased, and agility test times improved in the whey group. Interestingly, these robust improvements contrast with some previous literature. Previous studies also indicated that protein supplementation (whey, soy, or plant-derived) frequently has little to no significant impact on indirect measures of explosive strength, such as countermovement jump (CMJ) or 30-meter sprint timings, specifically when overall dietary protein intake is already sufficient [Kritikos et al., 2021; Teixeira et al., 2022]. In another comparative study between whey protein and rice protein, in combination with structured RT, whey protein significantly increases the strength performance in college-aged males [Joy et al., 2013]. The greater adaptability in power and speed performance observed in the current study could be linked to the restricted 12-week RT protocol. Previous studies suggested that the prolonged structured RT significantly affects the force-generating ability of skeletal muscle. The combination of essential amino acids from whey is likely to be beneficial for athletes to maximize their mechanical output during training, because it enhances inter-session recovery. This greater recovery helps athletes to generate higher anaerobic capacity and force [Kritikos et al., 2021; Teixeira et al., 2022].

A central finding of this study is the divergent inflammatory and physiological responses between the two groups. Disruption or damage to muscle fibres is consistently caused by rigorous resistance training. In the present study, this was evidenced by the significant rise in C-Reactive Protein (CRP) in the placebo group. The whey protein group, however, successfully blunted this systemic inflammatory spike. This supports the concept that adequate protein intake is crucial for decreasing undesirable inflammatory reactions and aiding in the recovery of strength.

Rigorous resistance training causes structural disruption and damage to muscle fibres, elevating circulating systemic markers [Morton et al.,2018]. A critical and highly functional outcome of this study is the protective effect of whey protein against this cellular damage. The placebo group experienced massive, compounding elevations in Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) by week 12. In stark contrast, the whey protein group successfully attenuated these markers, maintaining significantly lower levels of both CK and LDH. These findings strongly align with established sports nutrition literature. As Nieman et al. (2020) demonstrated, whey protein supplementation effectively decreases the rise of muscle damage biomarkers like CK and myoglobin compared to a control following intense exercise [Nieman et al., 2020]. Similarly, Lollo et al. (2014) established that whey protein administration significantly decreases both CK and LDH, preserving normal muscle function during the intense training regimes of a championship season [Lollo et al.,2014]. While some acute trials, such as Mor & Ipekoglu (2018), suggested that a single dose of whey protein might not immediately lower muscle damage values (including LDH and CK) post-exercise, our 12-week longitudinal data confirms that chronic, consistent whey supplementation provides a powerful, cumulative protective effect, reducing undesirable structural disruption and expediting muscle recovery in football players [Mor & Ipekoglu, 2018].

Finally, hepatic and systemic stress markers (AST and ALT) provided further insight into the metabolic toll of the intervention. While AST levels remained stable, ALT levels increased significantly over the 12 weeks for both the whey and placebo cohorts, with no statistical difference between them. Previous research by Mor & Ipekoglu (2018) noted that acute whey protein consumption does not produce any immediate positive effect on measured muscle damage values, including ALT and AST [Mor & Ipekoglu, 2018]. Furthermore, recent comparative trials by Loureiro et al. (2023) demonstrated that pea protein actually resulted in significantly lower ALT and CK levels post-game compared to whey protein, suggesting plant proteins might offer unique systemic recovery advantages. However, our data indicates that over a 12-week chronic resistance training block, whey protein does not disproportionately elevate liver transaminases (ALT) compared to a carbohydrate placebo, affirming its safety and metabolic viability alongside its superior anabolic benefits [Loureiro et al.,2023].

IV.CONCLUSSION

The present study concludes that the supplementation of whey protein with a combination of RT can significantly promote the development of body composition, strength, speed, power, and agility among male football players. Whey protein enhanced the training-induced adaptation by improving the LBM, compared to resistance training alone. The supplementation also effectively supports improved recovery and reduced physiological stress by considerably attenuating exercise-induced inflammatory responses and muscle damage markers such as CRP, CK, and LDH. Despite the ALT levels increasing in both groups, the whey protein did not show any adverse hepatic effect, confirming it is safe during the prolonged training period. Broadly, the findings of the current study suggest that the chronic supplementation of whey protein combined with periodized resistance training is a highly efficient nutritional strategy for improving performance determinants and recovery of non-elite football players, specifically from rural areas with limited access to nutritional strategies and scientific training.

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