

Bovine colostrum improves neutrophil phagocytosis and oxidation in recreational runners

El calostro bovino mejora la fagocitosis y la oxidación de los neutrófilos en corredores recreativos

Manuel A. Vázquez-Bautista¹, Mónica Reséndiz-Sandoval¹, Alma E. Robles-Sardin¹, María Jossé Navarro-Ibarra¹, Carla E. Villegas-Granados, Aracely Angulo-Molina², Graciela Caire-Juvera¹, Jesús Hernández^{1*}

¹ Coordinación de Nutrición, Centro de Investigación en Alimentación y Desarrollo, A.C., 83304, Hermosillo, México.

² Departamento de Ciencias Químico-Biológicas, Universidad de Sonora, 83000, Hermosillo, México.

ABSTRACT

Vigorous and prolonged physical training can impair immune function, such as neutrophil and monocyte activity, including phagocytosis and oxidative burst, which are critical for pathogen defense. These impairments can result in immunosuppression, increasing athletes' susceptibility to illnesses, such as upper respiratory tract symptoms. Bovine colostrum has been proposed as an efficient nutritional strategy to reverse immunosuppression due to its immunomodulatory effects. This work evaluated the effects of bovine colostrum supplementation on the phagocytic and oxidative activity of neutrophils and monocytes from recreational athletes. A pilot randomized, controlled, double-blind clinical trial was designed. Twenty-seven recreational athletes were supplemented with bovine colostrum ($n = 16$) or placebo ($n = 11$) for four weeks. Phagocytosis and oxidative burst in neutrophils and monocytes were assessed before and after intervention using flow cytometry. Upper respiratory tract symptoms were evaluated daily using WURSS-11 questionnaire. The results revealed a significant increase in neutrophil and monocyte phagocytic and oxidative functions in the bovine colostrum ($p = 0.02$) but not in the placebo ($p = 0.19$) group. No significant difference was observed in upper respiratory tract symptoms between the groups at the end of the intervention. In conclusion, bovine colostrum supplementation enhances neutrophil and monocyte function but does not affect the incidence of upper respiratory tract symptoms (ClinicalTrials.gov Identifier: NCT06123910).

Keywords: Immunonutrition; sports nutrition; immunosuppression.

RESUMEN

El entrenamiento físico intenso y prolongado puede afectar la función inmunitaria, como la actividad de los neutrófilos y los monocitos, incluidos la fagocitosis y el estallido oxidativo, que son fundamentales para la defensa contra patógenos. Estas alteraciones pueden provocar inmunosupresión, lo que aumenta la susceptibilidad de los deportistas a enfermedades, como los síntomas del tracto respiratorio superior. El calostro bovino se ha propuesto como una estrategia nutricional eficaz para revertir la inmunosupresión debido a sus

efectos inmunomoduladores. Este trabajo evaluó los efectos de la suplementación con calostro bovino sobre la actividad fagocítica y oxidativa de los neutrófilos y monocitos de deportistas recreativos. Se diseñó un ensayo clínico piloto aleatorizado, controlado y doble ciego. Veintisiete deportistas recreativos recibieron suplementos de calostro bovino ($n = 16$) o placebo ($n = 11$) durante cuatro semanas. Se evaluó la fagocitosis y el estallido oxidativo en neutrófilos y monocitos antes y después de la intervención mediante citometría de flujo. Los síntomas del tracto respiratorio superior se evaluaron diariamente mediante el cuestionario WURSS-11. Los resultados revelaron un aumento significativo de las funciones fagocíticas y oxidativas de los neutrófilos y monocitos en el grupo del calostro bovino ($p = 0.02$), pero no en el grupo del placebo ($p = 0.19$). No se observaron diferencias significativas en los síntomas del tracto respiratorio superior entre los grupos al final de la intervención. En conclusión, la suplementación con calostro bovino mejora la función de los neutrófilos y monocitos, pero no afecta a la incidencia de los síntomas del tracto respiratorio superior (ClinicalTrials.gov Identifier: NCT06123910).

Palabras clave: Inmunonutrición; nutrición deportiva; inmunosupresión.

INTRODUCTION

Regular exercise benefits health, but vigorous, prolonged training may impair immunity. An "open window of infection" can follow intense exercise, lasting hours (Walsh, 2019; Williams *et al.*, 2018). Recent research suggests that the immune response to exercise is a complex and nuanced process. Intense and prolonged exercise sessions have been associated with transient decreases in phagocytic and oxidative burst functions of neutrophils and monocytes, effects that are modulated by training status, exercise intensity, and recovery practices (Balogh *et al.*, 2026; Shi *et al.*, 2025). These alterations may increase the susceptibility to diverse illness such as upper respiratory tract symptoms (URTS). Soligard *et al.* (2017) reported that 47 % of Olympic athletes in 2016 had respiratory symptoms, indicating a high incidence of URTS in elite sport due to airway inflammation. More recent research has revealed that elite athletes show patterns of neutrophilic or mixed inflammation and a high prevalence of respiratory dysfunction (Jaeger *et al.*, 2025).

*Author for correspondence: Jesús Hernández
e-mail: jhdez@ciad.mx

Received: September 22, 2026

Accepted: February 12, 2026

Published: xxxxx, 2026

Nutritional interventions, including carbohydrate, fatty acids, vitamin, and antioxidant supplementation, have been proposed to mitigate immunosuppression (Bermon *et al.*, 2017). Furthermore, bovine colostrum (BC) has been suggested as an effective alternative. BC is a compound secreted by cow mammary glands during the first hours after parturition. The BC contains many components that can modulate immune function (Bermon *et al.*, 2017; Davison, 2021; Rathe *et al.*, 2014). BC consumption improves some neutrophil functions in individuals under prolonged exercise training (Davison and Diment, 2010; Jones *et al.*, 2013, 2015; Shing *et al.*, 2007). Nevertheless, the results have been contradictory, and the effect of BC supplementation on the phagocytic activity of neutrophils in athletes is still unknown. Moreover, research on the impact of BC supplementation on URTS incidence has also been contradictory (Brinkworth and Buckley, 2003; Crooks *et al.*, 2010, 2006; Jones *et al.*, 2013; Shing *et al.*, 2007). Therefore, the primary aim of this study was to evaluate the effect of BC supplementation on the function of neutrophils and monocytes in endurance athletes. A secondary aim was to determine whether this nutritional intervention influences the incidence of URTS in these participants.

MATERIALS AND METHODS

Participants and study design

A pilot, double-blind, placebo-controlled, randomized, parallel design study was conducted with an allocation ratio of 1:1. Participants were assigned to either the BC group (BC) or the placebo (PLA) group (ClinicalTrials.gov Identifier: NCT06123910). The study lasted four weeks. Recruitment took place between June and August 2018. Fifty-two healthy recreational endurance athletes, aged 18 to 50 years and training for the Half Marathon in November 2018, the Grand Marathon in December 2018, or the 21k Trial track in April 2019, were invited. Thirty-two agreed to participate (Figure 1).

The participants had at least three years of exercise training, were non-smokers, had no history of chronic cardiovascular disease, and had no food allergies. The physical activity level of the participants was evaluated through the short version of the International Physical Activity Questionnaire (IPAQ) before the study. All the subjects were informed about all the procedures performed and signed a consent form. The protocol was evaluated and approved by the ethics committee of the Centro de Investigación en Alimentación y Desarrollo, A.C. (Approval No. CE/010/2018).

Sample size and randomization

Convenience sampling was performed for the current study. The randomization process was stratified with a randomized block design within each stratum. Three imbalance blocks (block 1, $n = 8$; block 2, $n = 15$; block 3, $n = 9$) were used in the total randomized process. Age, sex, and VO_2max variables were considered for randomization. Randomization was performed by a researcher with no relationship to the study.

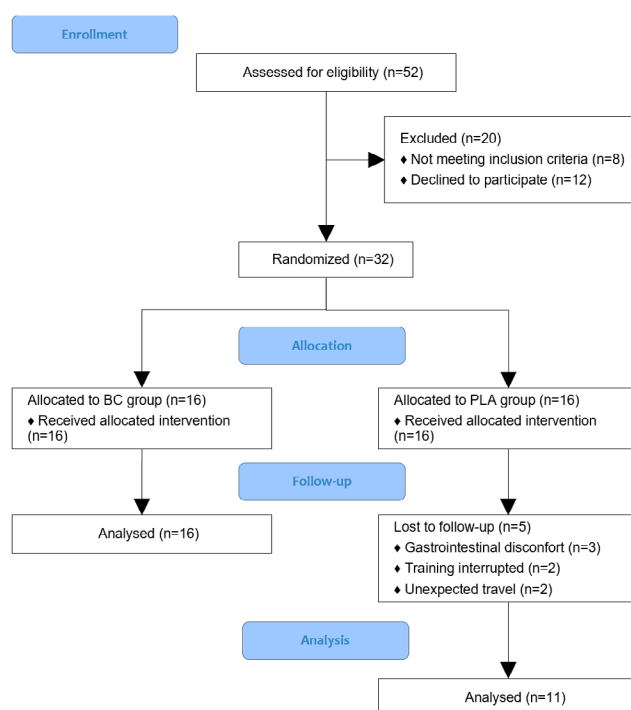


Figure 1. Participants flow chart.

Figura 1. Diagrama de flujo de participantes.

Intervention protocol

Participants visited the laboratory twice, previous intervention (PRE) and post intervention (POST). They avoided vigorous exercise and alcohol consumption for 24 h and reported no respiratory symptoms during the prior two weeks. After 12 h of fasting, they provided 18 mL of whole-blood and underwent body composition assessments. A coworker, uninvolved in the study, prepared weekly BC or PLA powder doses in hermetic containers. Participants took them daily, diluted in water on an empty stomach. The 4-week intervention used BC (Colostrum-LD, defatted colostrum with liposomal delivery) or PLA (milk powder), providing 100 kcal and 20 g protein per dose.

Anthropometry and body composition

Body mass was measured via Seca Model 874 (Hamburg, GER), and height was measured via Seca Model 984 (Hamburg, GER). A qualified technician evaluated body composition via dual-energy X-ray absorptiometry through Hologic Model ASY-05119 (Massachusetts, USA).

Dietary and training load report

Dietary intake was assessed with two nonconsecutive 24-hour recalls at baseline, 15 days, and four weeks. Vigorous activity was verified via IPAQ Short version. Participants recorded daily running distance during supplementation, following training programs designed by their team trainers.

VO_2max assessment

VO_2max was estimated via a physical performance test at PRE and POST (Hansen *et al.*, 2015). Heart rate was measured via a Wahoo TICKR heart rate monitor (Atlanta, USA).

Blood and immunological analysis

Blood was collected in EDTA-treated tubes, leukocytes were obtained by centrifuging a 16 mL blood sample at 1200 rpm for 15 min, and erythrocytes were lysed using Santa Cruz Biotechnology® RBC lysis buffer X10 (California, USA).

A phagocytic assay was performed by adding 2 µL of FluoSpheres® (Invitrogen, USA) to 2×10^6 cells suspended in 300 µL of RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). The samples were incubated for one hour at 37 °C. The reaction was stopped by placing the samples on ice for 10 min. The remaining spheres were removed by washing with PBS. The samples were analyzed using FACSCanto II instrument (BD, USA). The results represent the percentage of positive fluorescent cells.

Oxidative burst activity was evaluated via a reactive oxygen species (ROS) assay at 150 nm (Invitrogen, USA). A total of 2×10^6 cells were preincubated in 125 µL of RPMI 1640 medium supplemented with 10% FBS. The samples were incubated with 100 µL of the ROS Assay 150 nm solution for one hour at 37 °C; then, 25 µL of 1 mg/mL lipopolysaccharide (LPS) was added, and the samples incubated for one hour at 37 °C to stimulate ROS production. The samples were analyzed via a FACSCanto II (BD, USA). The results are presented as a percentage of positive cells to fluorescent signals and the mean fluorescence intensity (MFI).

Upper tract respiratory symptoms

Respiratory symptoms were recorded using the Wisconsin Upper Respiratory Symptoms Survey 11 items (WURSS-11) (Obasi *et al.*, 2014). The participants completed the survey daily during the intervention. The variables are presented as URTS days, the total number of days the participants reported any symptoms, the symptom score, or the global score on a numeric cumulative scale.

Statistical analysis

Numerical variables are presented as the means $\pm$ standard deviations. Paired data were analyzed using Student's *t* test or the Wilcoxon signed-rank test, and independent data were

analyzed via independent *t* test or Mann–Whitney U test according to normality via the Shapiro–Wilk test. Changes in variables were represented as PRE and POST differences (Δ). Missing cell function data were managed with multiple imputations (five imputations per missing value), and mean group imputation was used for missing URTS data. Analyses were conducted with $\alpha = 0.05$ using SPSS version 22.0 (IBM Corporation, New York, USA) for statistics and GraphPad Prism version 8.0.2 (California, USA) for figures. Post hoc effect size (ES) and power estimations were performed with G*Power version 3.1.9.4 (Kiel, GER).

RESULTS AND DISCUSSION

Participant characteristics

Fifty-two recreational athletes were recruited (August–September 2018). Thirty-two met the inclusion criteria and were randomly assigned to the BC or PLA group. Five PLA participants were lost to follow-up due to gastrointestinal discomfort or travel. The final groups were BC ($n = 16$) and PLA ($n = 11$) (Figure 1). One BC blood sample was lost during the phagocytosis assay and excluded from the analysis.

Eight male and eight female athletes were in the BC (39.1 ± 11.2 years), and six male and ten female athletes were in the PLA group (41.5 ± 9.10 years). Nonsignificant differences in body characteristics were found between the groups (Table 1). The characteristics of the participants, including loss of follow-up, are presented in the supplementary Table 1.

Dietary intake and training load

Energy, carbohydrates, fat, and protein intake were similar between BC and PLA during the intervention (Supplementary Table 2). Total and weekly training loads also showed no significant differences between groups (Supplementary Table 3).

Circulating leukocyte count

Leukocyte populations were similar at PRE (Supplementary Table 4). Paired *t*-tests showed no significant changes in neutrophil, lymphocyte, or monocyte concentrations in either group, nor differences in Δ between groups.

Table 1. General characteristics of the participants.

Tabla 1. Características generales de los participantes

	BC			PLA			<i>p</i> value*
	Males ($n=8$)	Females ($n=8$)	Total ($n=16$)	Males ($n=4$)	Females ($n=7$)	Total ($n=11$)	
Age (yrs)	41.6 ± 11.5	36.6 ± 11.0	39.1 ± 11.2	48.3 ± 5.0	37.6 ± 10.2	41.5 ± 10.0	0.58
Height (m)	1.7 ± 0.1	1.6 ± 0.0	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.0	1.7 ± 0.1	0.56
Body mass (kg)	70.1 ± 13.1	59.7 ± 8.0	64.9 ± 11.8	79.3 ± 10.9	63.4 ± 10.2	69.2 ± 12.8	0.38
BMI (kg/m ²)	24.0 ± 4.6	22.9 ± 2.7	23.5 ± 3.7	25.7 ± 0.8	23.6 ± 2.9	24.4 ± 2.5	0.51
BF (%)	21.7 ± 5.5	30.8 ± 8.1	26.2 ± 8.2	25.7 ± 1.5	32.4 ± 5.1	30.0 ± 5.2	0.19
FFM (kg)	78.3 ± 5.5	69.2 ± 8.1	73.8 ± 8.2	74.3 ± 1.5	67.6 ± 5.1	70.0 ± 5.2	0.19
Hip (cm)	94.7 ± 8.2	94.3 ± 8.2	94.5 ± 7.9	99.9 ± 2.5	99.7 ± 8.2	99.8 ± 6.5	0.08
Waist (cm)	83.2 ± 11.4	72.5 ± 12.1	77.8 ± 12.6	87.5 ± 4.9	77.1 ± 10.2	80.9 ± 9.8	0.51

Data presented as mean $\pm$ standard deviation. BMI, body mass index. BF, body fat. FFM, fat-free mass. * Independent student's *t*-test *p*-value at 0.05.

Datos presentados como media $\pm$ desviación estándar. BMI, índice de masa corporal. BF, grasa corporal. FFM, masa corporal magra. * Prueba *t* de Student independiente, valor *p* = 0,05.

Table 2. Upper tract respiratory symptoms during the intervention.**Tabla 2.** Síntomas del tracto respiratorio superior durante la intervención.

	Mean $\pm$ SD	<i>p</i> value*
<i>URTS Days</i>		
BC	5.06 $\pm$ 7.56	<i>p</i> = 0.42
PLA	3.82 $\pm$ 5.46	
<i>Symptoms Score</i>		
BC	19.2 $\pm$ 35.6	<i>p</i> = 0.58
PLA	17.1 $\pm$ 25.4	
<i>Global Score</i>		
BC	9.06 $\pm$ 15.2	<i>p</i> = 0.65
PLA	7.45 $\pm$ 9.71	

SD, standard deviation. URTS, upper respiratory tract symptoms. BC, bovine colostrum. PLA, placebo. * U-Mann Whitney test *p* value.

SD, desviación estándar. URTS, síntomas del tracto respiratorio superior. BC, calostro bovino. PLA, placebo. * Valor *p* de la prueba U de Mann-Whitney.

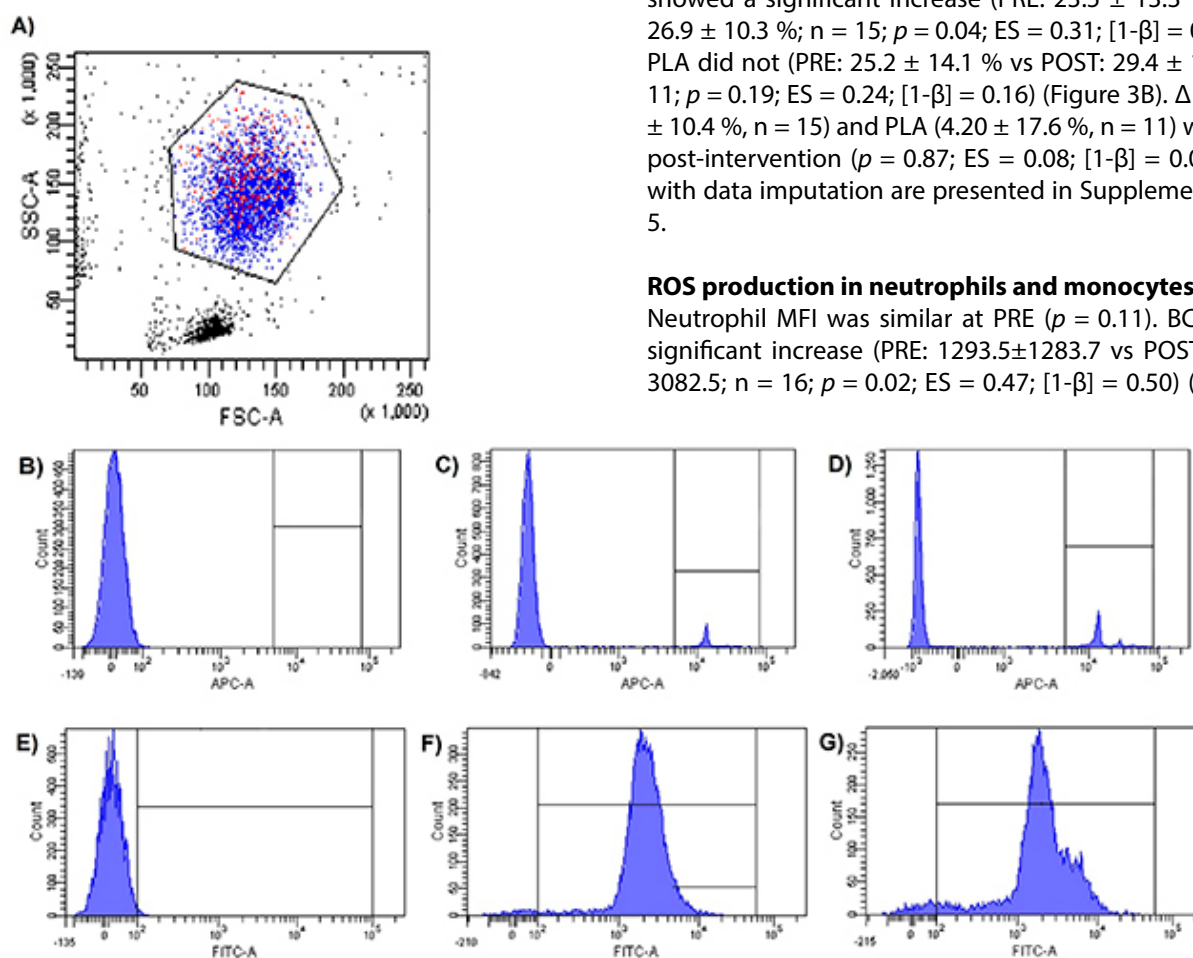


Figure 2. Representative flow cytometry analysis. A) Cell populations analyzed using FSC-A and SSC-A. B) Cells with no treatment. No signal was detected on the APC channel. C) Fluorescence signal emitted by cells incubated with fluorophores at 4 °C for negative control. D) Fluorescence signal emitted by cells incubated with fluorophores at 37 °C to evaluate cell phagocytic function. E) Cells without treatment show no signal detected on the FITC channel. F) Fluorescence signal emitted by cells incubated with ROS assay kit with no LPS stimulation for negative control. G) Signal emitted by LPS stimulated cells incubated with ROS assay kit to evaluate ROS production.

Figura 2. Análisis representativo de citometría de flujo. A) Poblaciones celulares analizadas utilizando FSC-A y SSC-A. B) Células sin tratamiento. No se detectó ninguna señal en el canal APC. C) Señal de fluorescencia emitida por células incubadas con fluoróforos a 4 °C como control negativo. D) Señal de fluorescencia emitida por células incubadas con fluoróforos a 37 °C para evaluar la función fagocítica celular. E) Las células sin tratamiento no muestran ninguna señal detectada en el canal FITC. F) Señal de fluorescencia emitida por células incubadas con el kit de ensayo ROS sin estimulación con LPS como control negativo. G) Señal emitida por células estimuladas con LPS incubadas con el kit de ensayo ROS para evaluar la producción de ROS.

Phagocytosis assay

The phagocytic function was assessed via flow cytometry. Cells were identified by forward/side scatter. After detecting autofluorescence in untreated cells, phagocytosis was evaluated at 4 °C (control) and 37 °C (Figure 2). PRE values were similar between groups for neutrophils and monocytes (*p* = 0.57).

Paired analysis showed a significant increase in neutrophil phagocytosis in BC (PRE: 35.1 $\pm$ 25.9 % vs POST: 42.8 $\pm$ 19.6 %; *n* = 15; *p* = 0.02; ES = 0.41; [1- β] = 0.40) (Figure 3A). In contrast, PLA showed no significant change (PRE: 35.4 $\pm$ 22.9 % vs POST: 41.1 $\pm$ 20.5 %; *n* = 11; *p* = 0.19; ES = 0.27; [1- β] = 0.19). The independent *t*-test showed similar Δ between groups: BC = 10.3 $\pm$ 17.6 %, PLA = 5.8 $\pm$ 28.9 % (*p* = 0.62; ES = 0.20; [1- β] = 0.15).

Monocyte phagocytosis was similar at PRE (*p* = 0.73). BC showed a significant increase (PRE: 23.5 $\pm$ 13.3 % vs POST: 26.9 $\pm$ 10.3 %; *n* = 15; *p* = 0.04; ES = 0.31; [1- β] = 0.27), while PLA did not (PRE: 25.2 $\pm$ 14.1 % vs POST: 29.4 $\pm$ 12.7 %; *n* = 11; *p* = 0.19; ES = 0.24; [1- β] = 0.16) (Figure 3B). Δ in BC (5.18 $\pm$ 10.4 %, *n* = 15) and PLA (4.20 $\pm$ 17.6 %, *n* = 11) were similar post-intervention (*p* = 0.87; ES = 0.08; [1- β] = 0.06). Results with data imputation are presented in Supplementary Table 5.

ROS production in neutrophils and monocytes

Neutrophil MFI was similar at PRE (*p* = 0.11). BC showed a significant increase (PRE: 1293.5 $\pm$ 1283.7 vs POST: 2927.5 $\pm$ 3082.5; *n* = 16; *p* = 0.02; ES = 0.47; [1- β] = 0.50) (Figure 4A),

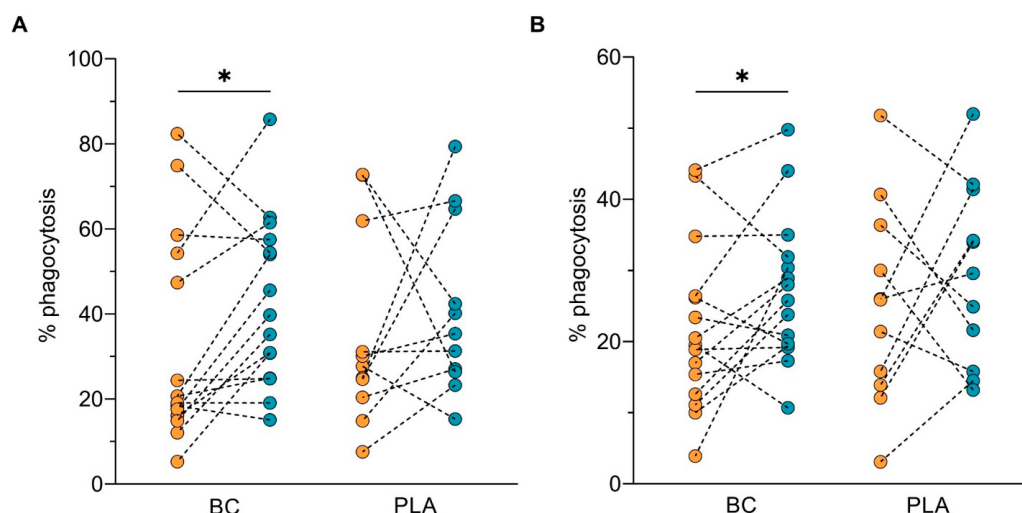


Figure 3. Effect of bovine colostrum in neutrophils and monocyte phagocytic activity. BC, bovine colostrum. PLA, placebo. Orange dots represent baseline values. Blue dots represent post-supplementation values. *Statistically significant $p \leq 0.05$. A) neutrophils ($p = 0.02$), while no significant changes were observed in the PLA group. B) monocytes; significant increase in monocyte phagocytosis post 4 weeks of supplementation ($p = 0.04$).

Figura 3. Efecto del calostro bovino en la actividad fagocítica de los neutrófilos y los monocitos. BC, calostro bovino. PLA, placebo. Los puntos naranjas representan los valores iniciales. Los puntos azules representan los valores posteriores a la suplementación. *Estadísticamente significativo $p \leq 0,05$. A) neutrófilos; se observó un aumento significativo de la actividad fagocítica tras 4 semanas de suplementación con BC ($p = 0.02$), mientras que no se observaron cambios significativos en el grupo PLA. B) monocitos; se observaron cambios estadísticos en la fagocitosis de los monocitos tras 4 semanas de suplementación ($p = 0.04$).

while PLA did not (PRE: 2519.1 ± 1974.4 vs POST: 6199.6 ± 8755.0 ; $n = 11$; $p = 0.07$; ES = 0.46; $[1-\beta] = 0.38$). Δ between groups was not significant (BC: 1634.0 ± 3459.1 , PLA: 3680.6 ± 7555.1 ; $p = 0.88$; ES = 0.35; $[1-\beta] = 0.20$).

Monocyte MFI (Figure 4B) was similar at PRE (BC: 1011.1 ± 1170.2 , PLA: 2166.7 ± 1825.2 ; $p = 0.11$). BC increased significantly (PRE: 1011.2 ± 1170.2 vs POST: 2071.4 ± 2215.3 ; $n = 16$; $p = 0.04$; ES = 0.46; $[1-\beta] = 0.50$), while PLA did not (PRE: 2166.7 ± 1825.2 vs POST: 3354.0 ± 3771.3 ; $n = 11$; $p = 0.08$; ES = 0.39; $[1-\beta] = 0.28$). Δ between groups was similar (BC: 1060.2 ± 2280.4 , PLA: 1187.3 ± 2564.0 ; $p = 0.89$; ES = 0.45; $[1-\beta] = 0.06$).

Fluorescence-positive cells indicated ROS-producing activated cells. Neutrophil activation was similar at PRE ($p = 0.82$) (Figure 4C). Significant increases occurred in BC (PRE: $81.0 \pm 32.6\%$ vs POST: $94.7 \pm 8.0\%$; $n = 16$; $p = 0.04$; ES = 0.45; $[1-\beta] = 0.47$) and PLA (PRE: $90.2 \pm 16.1\%$ vs POST: $97.9 \pm 6.4\%$; $n = 11$; $p = 0.008$; ES = 0.43; $[1-\beta] = 0.32$). Monocyte activation was also similar at PRE ($p = 0.27$) (Figure 4D), increasing in BC (PRE: $71.5 \pm 30.2\%$ vs POST: $86.1 \pm 27.5\%$; $p = 0.007$; ES = 0.80; $[1-\beta] = 0.91$) and PLA ($79.9 \pm 26.5\%$ vs $93.9 \pm 15.9\%$; $p = 0.008$; ES = 0.83; $[1-\beta] = 0.78$). Δ between groups was similar for neutrophils ($p = 0.57$; ES = 0.26; $[1-\beta] = 0.14$) and monocytes ($p = 0.52$; ES = 0.03; $[1-\beta] = 0.05$). Results with data imputation are presented in Supplementary Table 5.

Upper respiratory tract symptoms

The total URTS days in the BC and PLA are presented in Table 2. There were no significant differences between the groups

in total URTS days ($p = 0.42$), nor in the symptom score ($p = 0.58$) or global score ($p = 0.65$). Results with data imputation are presented in Supplementary Table 6.

The current study aimed to evaluate the effects of BC supplementation on phagocytosis and oxidative burst activity in neutrophils and monocytes from endurance athletes. Previous studies have shown improvements in immune function variables in athletes from different sports, such as swimmers and runners (Cieřlicka *et al.*, 2023; Crooks *et al.*, 2010; Jones *et al.*, 2015; Shing *et al.*, 2007). However, research on phagocytosis and oxidative burst activity in response to BC supplementation is scarce and limited to nonathletic subjects.

This is the first study to examine the effects of BC supplementation on neutrophil and monocyte phagocytic functions in athletes. Our findings revealed increased activity 24 h after the last dose, suggesting that the effect persisted for at least this duration. These results differ from those of previous studies, possibly because of the higher dosage used in the present study. Greater dosages and durations may be needed to stimulate antibacterial function. Some authors have reported increased polymorphonuclear leukocytes and monocyte activity after an acute BC dose of 125 mg, whereas others reported no changes after six weeks with a 1.2 g/d BC supplementation (Wolters *et al.*, 2006).

The effect of BC supplementation on neutrophil oxidative function has been evaluated in previous studies. Even though Jones *et al.* (2013) reported no benefits from 12 weeks of BC supplementation, the same authors reported

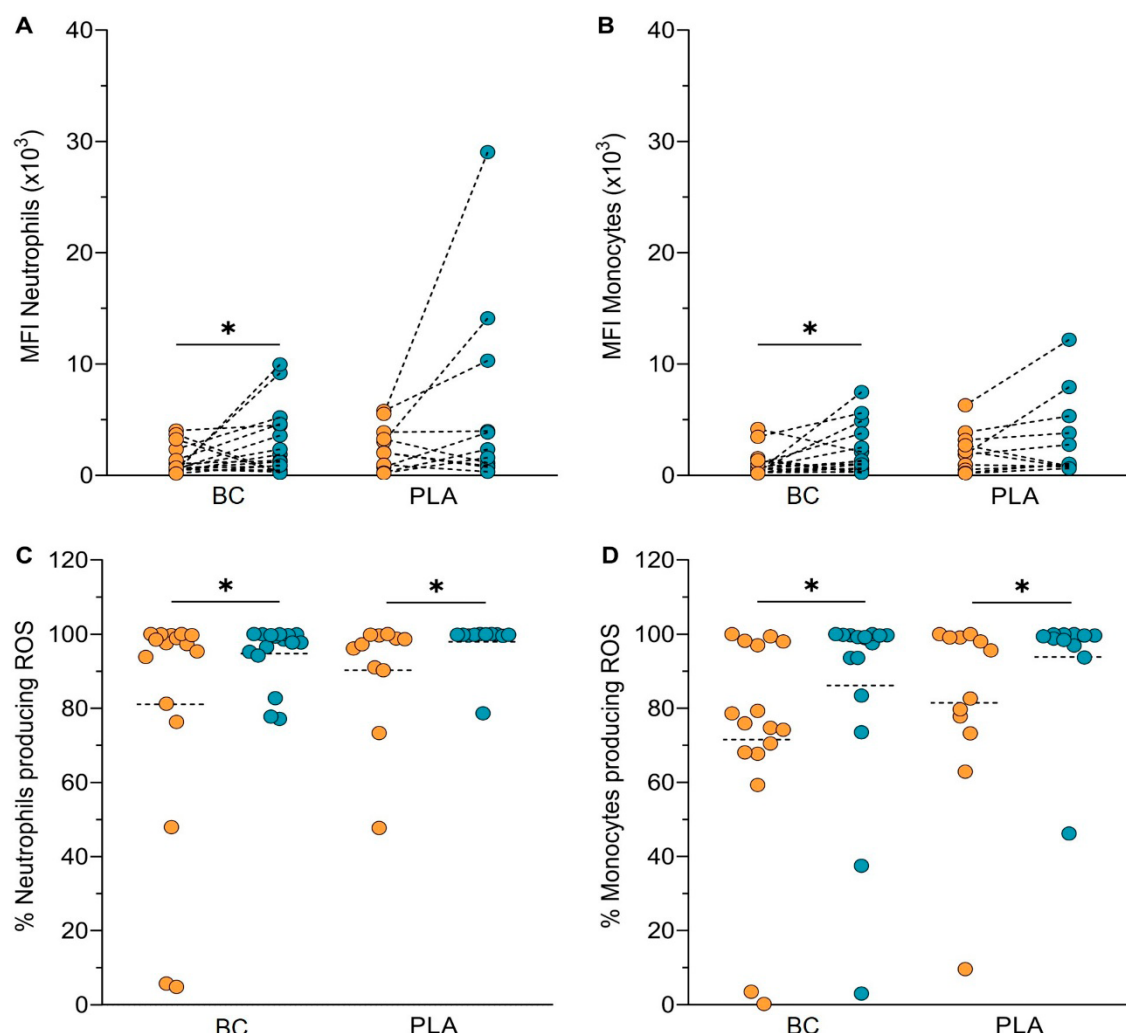


Figure 4. Effect of bovine colostrum in oxidative function of neutrophils and monocytes. BC, bovine colostrum. PLA, placebo. Orange dots represent baseline values. Blue dots represent post supplementation values. Horizontal bars in MFI figures represent group means. Percentage cells producing ROS represent mean and S.D.*: p value level by Wilcoxon signed-rank test; statistically significant $p \leq 0.05$. A) There was a significant increase in MFI neutrophils in the BC group ($p = 0.02$). B) A significant increase were observed in MFI monocyte from baseline ($p = 0.04$). C) The PLA group presented a significant increase in %Neutrophils producing ROS at the end of the study. D). Both groups presented a significant increase in the percentage of monocytes producing ROS at the end of the intervention.

Figura 4. Efecto del calostro bovino en la función oxidativa de los neutrófilos y monocitos. BC, calostro bovino. PLA, placebo. Los puntos naranjas representan los valores basales. Los puntos azules representan los valores posteriores a la suplementación. Las barras horizontales en las figuras MFI representan las medias de los grupos. El porcentaje de células que producen ROS representa la media y la desviación estándar.*: nivel de valor p según la prueba de rangos con signo de Wilcoxon; estadísticamente significativo $p \leq 0.05$. A) Se observó un aumento significativo de los neutrófilos MFI en el grupo BC ($p = 0.02$). B) Se observaron cambios en los monocitos MFI con respecto al valor inicial ($p = 0.04$). C) El grupo PLA presentó un aumento significativo del % de neutrófilos productores de ROS al final del estudio. D). Ambos grupos presentaron un aumento significativo del porcentaje de monocitos productores de ROS al final de la intervención.

greater neutrophil oxidative burst after four weeks of BC supplementation (Jones *et al.*, 2015). In the present study, BC supplementation increased neutrophil oxidative function at post-supplementation. Although the blood collection protocol used in the present study was different from that used by Jones *et al.* (2015), the benefits of BC supplementation on neutrophil oxidative burst may be influenced by time, as short periods could be more effective.

To assess ROS production, the percentage of fluorescence-emitting cells was measured, reflecting activation rather than ROS intensity. In BC, neutrophils and monocytes

producing ROS increased post-intervention, as in PLA. This suggests training enhances activation capacity. Further studies in sedentary individuals are needed to confirm "normal" neutrophil oxidative burst. BC showed higher neutrophil MFI than PLA group, indicating increased oxidative function per cell rather than more ROS-producing cells.

In this study, the URTS incidence and severity were similar between the groups. Some studies suggest that BC supplementation for 5–10 weeks may reduce URTS (Crooks *et al.*, 2010; Shing *et al.*, 2007). Jones *et al.* (2013) reported fewer URTS episodes in healthy subjects after 8 weeks of 20 g

BC supplementation, although the effect was not sustained in subsequent weeks. Similarly, fewer URTS episodes were reported in athletes after 8 weeks with 60 g BC supplementation (Brinkworth and Buckley, 2003). These findings suggest that athletes may need at least 8 weeks of BC supplementation to reduce URTS, but the benefits may not persist beyond this period.

BC supplementation has been shown to have different immunomodulatory effects in other populations. Differences in the immunomodulatory components of BC may be present in the diverse products available, which suggests that some products may be more effective than others (Playford *et al.*, 2020). In these terms, discrepancies in the effect of BC supplementation on the variety of products may be affected by this factor; further research is needed to clarify whether different BC collection times or BC qualities affect the immunomodulatory effect of BC.

Immunomodulatory components in BC include cytokines, immunoglobulins, growth factors, and microRNA (Bermon *et al.*, 2017). The combination of these components may explain the potential immunomodulatory effects this supplements (Karakulah *et al.*, 2025). Additionally, although it has been reported that some components may resist the digestion process (Burton *et al.*, 2020), the product reported in the present study is a liposomal delivery vehicle used in nutritional products to improve transportation and absorption across the gastrointestinal tract and increase bioavailability (Shade, 2016).

Even though BC has been proposed to increase phagocytosis due to its high content of IgG, which serves as a primer for phagocytosis due to its opsonizing capacity, changes in cell membrane structure or another type of change, have also been reported. Shing *et al.* (2007) reported increases in membrane receptors in polymorphonuclear leukocytes through BC supplementation, such as CD89 which is related to phagocytosis; however, owing to the many immunomodulatory compounds presented in BC, we believe that BC compounds may act as an external stimulus and enhance cell function, as BC supplementation has been shown to increase the production of several cytokines and other molecules (Duan *et al.*, 2024; Durkalec-Michalski *et al.*, 2024; Ooi *et al.*, 2023; Rusu *et al.*, 2010); however, contradictory results have been reported (Skarpańska-Stejnborn *et al.*, 2021). On the other hand, it seems that cells could adapt to BC stimuli and decrease or even lose responsiveness after long supplementation periods, as we observed improvements in neutrophil function after four weeks of BC consumption. In contrast, other reports showed no effects after six or even more weeks of supplementation. It has been proposed that the potential immunomodulatory effect of BC may not be attributable to one mechanism (Główka *et al.*, 2020), but rather to various priming factors for cell function. Additionally, it seems that subjects should have some physical stress, such as regular training, to obtain sustained benefits in phagocytic activity through BC supplementation.

The main limitations of the present study are sample size, and the number of dropouts presented during the intervention. This limitation may have affected the statistical analysis power, as paired comparisons were significant for some variables, whereas independent tests were not statistically significant.

CONCLUSIONS

Four weeks of bovine colostrum supplementation improved the phagocytic and oxidative functions of neutrophils and monocytes in endurance recreational athletes. Despite the beneficial effects on neutrophil function, four weeks of supplementation did not improve the incidence or symptomatology of upper tract respiratory symptoms.

ACKNOWLEDGMENTS

We are grateful for the support of Bertha Isabel Pacheco Moreno and Sovereign Laboratories for providing the bovine colostrum for this experimental project.

AUTHOR CONTRIBUTIONS

Conceptualization, M.A.V.-B. and J.H.; methodology, M.A.V.-B., A.E.R.-S.; C.E.V.-G., M.R.-S.; M.J.N.-I.; formal analysis, M.A.V.-B.; validation, M.A.V.-B.; G.C.-J., J.H.; resources, J.H.; data curation, M.A.V.-B.; original draft preparation, M.A.V.-B.; writing, review, and editing, M.A.V.-B. and J.H.; funding acquisition, J.H. All the authors have read and agreed to the version of this manuscript submitted for publication.

FUNDING

This research received no external funding.

INFORMED CONSENT STATEMENT

The study was approved by the CIAD ethical committee (Approval No. CE/010/2018).

DATA AVAILABILITY STATEMENT

Data are contained within the article or supplementary material.

CONFLICTS OF INTEREST

"The authors declare no conflicts of interest."

REFERENCES

- Balogh, L., Szklenár, E., Diós, Á., Arany, A.C., Pucsok, J.M., Bács, Z.M., Rátgéber, L., Csiki, Z., Gyetvai, Á. and Papp, G. 2026. The acute effects of high-intensity interval training on oxidative stress markers and phagocyte oxidative burst activity in young professional athletes and non-athlete university students. *Life*. 16(1): 84. <https://doi.org/10.3390/life16010084>
- Bermon, S., Castell, L.M., Calder, P.C., Bishop, N.C., Blomstrand, E., Mooren, F.C., Krüger, K., Kavazis, A.N., Quindry, J.C., Senchina, D.S., Nieman, D.C., Gleeson, M., Pyne, D.B., Kitic, C.M., Close, G.L., Larson-Meyer, D.E., Marcos, A., Meydani, S.N., Wu, D., ...

- Nagatomi, R. 2017. Consensus statement immunonutrition and exercise. *Exercise Immunology Review*. 23: 8–50.
- Brinkworth, G.D. and Buckley, J.D. 2003. Concentrated bovine colostrum protein supplementation reduces the incidence of self-reported symptoms of upper respiratory tract infection in adult males. *European Journal of Nutrition*. 42(4): 228–232. <https://doi.org/10.1007/s00394-003-0410-x>
- Burton, R.E., Kim, S., Patel, R., Hartman, D.S., Tracey, D.E., Fox, B.S., Avaxia, F. and Circle, B. 2020. Structural features of bovine colostrum immunoglobulin that confer proteolytic stability in a simulated intestinal fluid. *Journal of Biological Chemistry*. 295(34): 12317–12327. <https://doi.org/10.1074/jbc.RA120.014327>
- Cieślicka, M., Stankiewicz, B., Muszkieta, R., Tafil-Klawe, M., Klawe, J., Skarpańska-Stejnborn, A. and Ostapiuk-Karolczuk, J. 2023. Long-term bovine colostrum supplementation in football players. *Nutrients*. 15(22): 4779. <https://doi.org/10.3390/nu15224779>
- Crooks, C., Cross, M.L., Wall, C. and Ali, A. 2010. Effect of bovine colostrum supplementation on respiratory tract mucosal defenses in swimmers. *International Journal of Sport Nutrition and Exercise Metabolism*. 20: 224–235. <https://doi.org/10.1123/ijsnem.20.3.224>
- Crooks, C.V., Wall, C.R., Cross, M.L. and Rutherford-Markwick, K.J. 2006. The effect of bovine colostrum supplementation on salivary IgA in distance runners. *International Journal of Sport Nutrition and Exercise Metabolism*. 16(1): 47–64. <https://doi.org/10.1123/ijsnem.16.1.47>
- Davison, G. 2021. The use of bovine colostrum in sport and exercise. *Nutrients*. 13(6): 1789. <https://doi.org/10.3390/nu13061789>
- Davison, G. and Diment, B.C. 2010. Bovine colostrum supplementation attenuates the decrease of salivary lysozyme and enhances the recovery of neutrophil function after prolonged exercise. *British Journal of Nutrition*. 103(10): 1425–1432. <https://doi.org/10.1017/S0007114509993503>
- Duan, H., Sun, Q., Chen, C., Wang, R. and Yan, W. 2024. A review: The effect of bovine colostrum on immunity in people of all ages. *Nutrients*. 16(13): 1–22. <https://doi.org/10.3390/nu16132007>
- Durkalec-Michalski, K., Głowska, N., Podgórski, T., Woźniewicz, M. and Nowaczyk, P.M. 2024. The effect of 12-week high-dose colostrum bovinum supplementation on immunological, hematological and biochemical markers in endurance athletes: a randomized crossover placebo-controlled study. *Frontiers in Immunology*. 15: 1425785. <https://doi.org/10.3389/fimmu.2024.1425785>
- Głowska, N., Durkalec-Michalski, K. and Wozniewicz, M. 2020. Immunological outcomes of bovine colostrum supplementation in trained and physically active. *Nutrients*. 12(1023): 1–24.
- Hansen, D., Jacobs, N., Thijs, H., Dendale, P. and Claes, N. 2015. Validation of a single-stage fixed-rate step test for the prediction of maximal oxygen uptake in healthy adults. *Clinical Physiology and Functional Imaging*. 36(5): 401–406. <https://doi.org/10.1111/cpf.12243>
- Jaeger, E., Maurer, D.J., Wallimann, A., Kistler, W. and Villiger, B. 2025. Immunity, inflammation and airway dysfunction in elite cross-country skiers and ice hockey players: A systematic review. *Scandinavian Journal of Medicina & Science in Sports*. 35: e70046. <https://doi.org/10.1111/sms.70046>
- Jones, A.W., Cameron, S.J.S., Thatcher, R., Beecroft, M.S., Mur, L.A.J. and Davison, G. 2013. Effects of bovine colostrum supplementation on upper respiratory illness in active males. *Brain, Behavior, and Immunity*. 39: 194–203. <https://doi.org/10.1016/j.bbi.2013.10.032>
- Jones, A.W., Thatcher, R., March, D.S. and Davison, G. 2015. Influence of 4 weeks of bovine colostrum supplementation on neutrophil and mucosal immune responses to prolonged cycling. *Scandinavian Journal of Medicine & Science in Sports*. 25(6): 788–796. <https://doi.org/10.1111/sms.12433>
- Karakulah, Y.S., Yalçintaş, Y.M., Bechelany, M. and Karav, S. 2025. Clinical applications of bovine colostrum in gastrointestinal disorders: Mechanisms, evidence, and therapeutic potential. *International Journal of Molecular Sciences*. 26(21): 1–39. <https://doi.org/10.3390/ijms262110673>
- Obasi, C.N., Brown, R.L. and Barrett, B.P. 2014. Item reduction of the Wisconsin Upper Respiratory Symptom Survey (WURSS-21) leads to the WURSS-11. *Quality of Life Research*. 23(4): 1293–1298. <https://doi.org/10.1007/s11136-013-0561-z>Item
- Ooi, T.C., Ahmad, A., Rajab, N.F. and Sharif, R. 2023. The effects of 12 weeks colostrum milk supplementation on the expression levels of pro-inflammatory mediators and metabolic changes among older adults: Findings from the biomarkers and untargeted metabolomic analysis. *Nutrients*. 15(14): <https://doi.org/10.3390/nu15143184>
- Playford, R.J., Cattell, M. and Marchbank, T. 2020. Marked variability in bioactivity between commercially available bovine colostrum for human use; implications for clinical trials. *PLoS ONE*. 15(6): 1–16. <https://doi.org/10.1371/journal.pone.0234719>
- Rathe, M., Müller, K., Sangild, P.T. and Husby, S. 2014. Clinical applications of bovine colostrum therapy: A systematic review. *Nutrition Reviews*. 72(4): 237–254. <https://doi.org/10.1111/nure.12089>
- Rusu, D., Drouin, R., Pouliot, Y., Gauthier, S. and Poubelle, P.E. 2010. A bovine whey protein extract stimulates human neutrophils to generate bioactive IL-1Ra through a NF-κB- and MAPK-dependent mechanism. *Journal of Nutrition*. 140(2): 382–391. <https://doi.org/10.3945/jn.109.109645>
- Shi, X., Hu, L., Nieman, D.C., Li, F., Chen, P., Shi, H. and Shi, Y. 2025. Exercise workload: a key determinant of immune health – a narrative review. In *Frontiers in Immunology*. 16: 1–15. <https://doi.org/10.3389/fimmu.2025.1617261>
- Shing, C.M., Peake, J., Suzuki, K., Okutsu, M., Pereira, R., Stevenson, L., Jenkins, D.G. and Coombes, J.S. 2007. Effects of bovine colostrum supplementation on immune variables in highly trained cyclists. *Journal of Applied Physiology*. 102(3): 1113–1122. <https://doi.org/10.1152/jappphysiol.00553.2006>
- Skarpańska-Stejnborn, A., Mirosława, C., Dziewiecka, H., Kujawski, S., Marcinkiewicz, A., Trzeciak, J., Basta, P., Maciejewski, D. and Latour, E. 2021. Effects of long-term supplementation of bovine colostrum on the immune system in young female basketball players. *Randomized Trial*. *Nutrients*. 13(118): 1–16. <https://doi.org/https://doi.org/10.3390/nu13010118>
- Soligard, T., Steffen, K., Palmer, D., Alonso, J.M., Bahr, R., Lopes, A.D., Dvorak, J., Grant, M.E., Meeuwisse, W., Mountjoy, M., Pena Costa, L.O., Salmina, N., Budgett, R. and Engebretsen, L. 2017. Sports injury and illness incidence in the Rio de Janeiro 2016 Olympic Summer Games: A prospective study of 11274 athletes from 207 countries. *British Journal of Sports*

- Medicine. 51(17): 1265–1271. <https://doi.org/10.1136/bjsports-2017-097956>
- Walsh, N.P. 2019. Nutrition and athlete immune health: New perspectives on an old paradigm. Sports Medicine. 49: 153–168. <https://doi.org/10.1007/s40279-019-01160-3>
- Williams, N.C., Killer, S.C., Svendsen, I.S., Jones, A.W., Williams, N.C., Killer, S.C., Svendsen, I.S., Jones, A.W. and Jones, W.Y.N. 2018. Immune nutrition and exercise : Narrative review and practical recommendations recommendations. European Journal of Sport Science. 0(0): 1–13. <https://doi.org/10.1080/17461391.2018.1490458>
- Wolvers, D.A.W., Van Herpen-Broekmans, W.M.R., Logman, M.H.G.M., Van Der Wielen, R.P. J. and Albers, R. 2006. Effect of a mixture of micronutrients, but not of bovine colostrum concentrate, on immune function parameters in healthy volunteers: A randomized placebo-controlled study. Nutrition Journal. 5(1): 1–11. <https://doi.org/10.1186/1475-2891-5-28>