

## Article

# Does Colostrum Bovinum Supplementation Affect Swimming Performance in Endurance-Trained Males? A Randomized Placebo-Controlled Crossover Study

Krzysztof Durkalec-Michalski <sup>1,\*</sup>, Natalia Głowska <sup>1</sup>, Tomasz Podgórski <sup>2</sup>, Mikołaj Szymocha <sup>1</sup>,  
Błażej Przybylik <sup>1</sup>, Krystian Wochna <sup>3</sup>, Małgorzata Woźniewicz <sup>4</sup> and Paulina M. Nowaczyk <sup>1</sup>

<sup>1</sup> Department of Sports Dietetics, Poznan University of Physical Education, 61-871 Poznań, Poland; glowka@awf.poznan.pl (N.G.); szymocha@awf.poznan.pl (M.S.); blazejprzybylik@gmail.com (B.P.); nowaczyk@awf.poznan.pl (P.M.N.)

<sup>2</sup> Department of Physiology and Biochemistry, Poznan University of Physical Education, 61-871 Poznań, Poland; podgorski@awf.poznan.pl

<sup>3</sup> Department of Swimming and Water Lifesaving, Poznan University of Physical Education, 61-871 Poznań, Poland; kwochna@awf.poznan.pl

<sup>4</sup> Department of Human Nutrition and Dietetics, Poznań University of Life Sciences, 60-624 Poznań, Poland; malgorzata.wozniewicz@up.poznan.pl

\* Correspondence: durkalec-michalski@awf.poznan.pl

**Abstract: Background/Objectives:** *Colostrum Bovinum* (COL) is recognized for its unique composition and potential ergogenic and immunological benefits. Unlike mature milk, COL is rich in immunoglobulins, lactoferrin, and various growth factors, making it one of the most potent natural immune stimulants. The purpose of this study was to evaluate the effects of 12-weeks of COL supplementation on swimming-specific performance (SSP) and exercise adaptations in endurance-trained male athletes. **Methods:** Twenty-eight male triathletes and swimmers (age:  $31.1 \pm 10.2$  years; body mass:  $81.9 \pm 9.0$  kg; height:  $1.82 \pm 0.06$  m) participated in a randomized, double-blind, placebo (PLA)-controlled crossover study and received  $25 \text{ g} \cdot \text{day}^{-1}$  of COL or PLA for 12 weeks. The study assessed the effects of COL on SSP ( $8 \times 100$  m performed at various intensities) and exercise adaptations [heart rate (HR) and blood lactate concentrations ( $[La^-]$ )]. Four main study visits were conducted—before and after COL ( $COL_{PRE}$  and  $COL_{POST}$ ) and PLA ( $PLA_{PRE}$  and  $PLA_{POST}$ ) supplementation. **Results:** COL had no significant effect on SSP. Still, the total time of the SSP test was about  $\sim 3.04$  s shorter after COL supplementation, and  $\sim 7.13$  s longer after PLA supplementation. Neither COL nor PLA supplementation affected HR during the SSP test. Post-exercise blood  $[La^-]$  was significantly reduced after both COL and PLA supplementation. The analysis of SSP results in the consecutive study visits revealed possible existence of the practice effect. **Conclusions:** *Colostrum Bovinum* and high-quality milk protein (PLA) seem to be comparably effective in evoking exercise adaptation in endurance-trained male athletes. Long-term crossover supplementation protocols in athletes must consider the impact of possible practice effect when interpreting the outcomes related to exercise performance, but not biochemical or physiological markers of exercise adaptation.

**Keywords:** supplementation; sport nutrition; triathlon; swimming; ergogenic support



**Citation:** Durkalec-Michalski, K.; Głowska, N.; Podgórski, T.; Szymocha, M.; Przybylik, B.; Wochna, K.; Woźniewicz, M.; Nowaczyk, P.M. Does *Colostrum Bovinum* Supplementation Affect Swimming Performance in Endurance-Trained Males? A Randomized Placebo-Controlled Crossover Study. *Nutrients* **2024**, *16*, 3204. <https://doi.org/10.3390/nu16183204>

Academic Editor: David C. Nieman

Received: 3 September 2024

Revised: 17 September 2024

Accepted: 19 September 2024

Published: 22 September 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

For over 20 years, *Colostrum Bovinum* (COL) has been in the field of interest in sports nutrition research. Although its impact on physical capacity and recovery has been studied, and some research has shown its protective effects against decline in exercise capabilities due to intense training, there remains limited evidence regarding its influence on exercise performance [1].

COL is a substance secreted by the mammary glands of mammals during the first few days after giving birth (up to 48–72 h after calving). COL is characterized by a

higher concentration of macronutrients (carbohydrates, proteins, and fats) [2,3] as well as biologically active substances [3] than human colostrum. The compounds contained in COL can be divided into three groups: nutrients, immune factors, and growth factors. The high concentration of bioactive components has led scientists to investigate the benefits of COL supplementation for exercise performance. However, potential effects on performance and recovery may be achieved through improved health outcomes, maintenance of immune functions, and protection of the intestines, as well as improved training adaptations [1].

Recent research on the impact of COL on physical performance has centered on three key areas: body composition and strength, high-intensity intermittent exercise, and endurance. Given that COL contains a higher concentration of micronutrients compared to whey, it may significantly contribute to adaptations during intense training [1]. Some studies [4,5] have shown that when combined with resistance training, COL supplementation may lead to increased muscle strength, muscle mass, and fat loss compared to a placebo. However, other research has found no significant differences [6,7]. These inconsistent results may be due to earlier studies using maltodextrin as a placebo, which does not have the same protein content as COL.

One of the aspects that COL studies focus on is the improvement in endurance performance, which may be achieved through enhanced recovery, buffering capacity and training adaptations [1]. In one such study, Kotsis et al. [8] showed that a low dose of COL ( $3.2 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$ ) over 6 weeks of supplementation reduced muscle damage (C-reactive protein, creatine kinase) and inflammatory (interleukin 6) markers after an exercise task simulating the physiological demands of soccer practice. This suggests COL may support performance adaptations. Moreover, Buckley et al. [9] found that a high dose ( $60 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$ ) of COL improved running performance, based on an incremental running test, after 8 weeks compared to a placebo (PLA), with greater improvements in distance covered. Additionally, Shing et al. [10] demonstrated that  $10 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  for 6 weeks prevented a decline in time-trial (TT) performance following a 5-day intensified training period and increased TT intensity (percentage of maximal oxygen uptake [ $\text{VO}_{2\text{MAX}}$ ]) while maintaining the ventilatory threshold. Furthermore, Coombes et al. [11] found that both 20 and  $60 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  for 8 weeks improved endurance performance in cyclists, as measured by cycling work capacity (a work-based TT following a 2-h cycle at 65%  $\text{VO}_{2\text{MAX}}$ ).

Additionally, it is worth emphasizing that COL contains several growth factors, such as proteins, insulin-like growth factor-1 (IGF-1), or transforming growth factor-beta (TGF- $\beta$ 1), which may also indirectly affect performance through the stimulation of protein synthesis, the modulation of muscle growth and recovery, as well as the enhancement of immune function, gut health, and anti-inflammatory effects. A study by Buckley et al. [12] showed that athletes who took COL for 8 weeks experienced shorter recovery time and less muscle soreness compared to those who did not. The COL group also showed enhanced performance in sprint tests after recovery. Moreover, prolonged or intense exercise can suppress the immune system, leaving athletes more susceptible to illness. Meanwhile, COL contains immunoglobulins (IgG, IgA) and lactoferrin, which help support immune health by neutralizing pathogens and boosting immune responses. Jones et al. [13] found that athletes using COL had a lower incidence of upper respiratory tract infections, which is a common issue among endurance athletes, allowing them to train more consistently. Intense endurance exercises can lead to gastrointestinal permeability, which hampers nutrient absorption and leads to inflammation. COL contains growth factors (i.e., IGF-1, TGF- $\beta$ 1) that help repair and strengthen the gut lining. Shing et al. [14] found that COL supplementation in athletes helped maintain intestinal integrity during endurance events, which might prevent the performance drop linked to gut dysfunctions and ensure better nutrient absorption for energy demands during prolonged exercise. COL's anti-inflammatory properties are linked to compounds like lactoferrin and certain cytokines. These bioactive compounds reduce muscle and joint inflammation caused by exercise, which aids in faster recovery and less discomfort. Inflammation is a natural part of the recovery process, but excessive or

prolonged inflammation can slow down muscle repair. COL helps moderate inflammation to ensure it is controlled without hampering recovery [15].

While some studies have examined the effectiveness of COL supplementation on exercise performance, additional research is necessary. Future studies should incorporate well-matched PLA and control groups to thoroughly assess the impact of COL on endurance performance during athletic training.

Therefore, the objective of this study was to assess the impact of a 12-week regimen of 25 g<sub>COL</sub>·day<sup>-1</sup> of COL supplementation in a group of healthy, moderately endurance-trained males engaged in triathlon and swimming practice. We assessed the impact of COL supplementation on exercise performance and adaptations in the body to exercise based on selected physiological (heart rate; HR) and biochemical markers. We hypothesized that COL supplementation would improve results in the swimming-specific performance (SSP) test (8 × 100 m of swimming performed at various intensities)—namely, shortened times for particular distances and the total times for SSP test overall (primary outcomes). We also hypothesized that COL supplementation would trigger favorable changes in exercise adaptation, evaluated based on HR and blood lactate concentrations ([La<sup>-</sup>]; secondary outcomes).

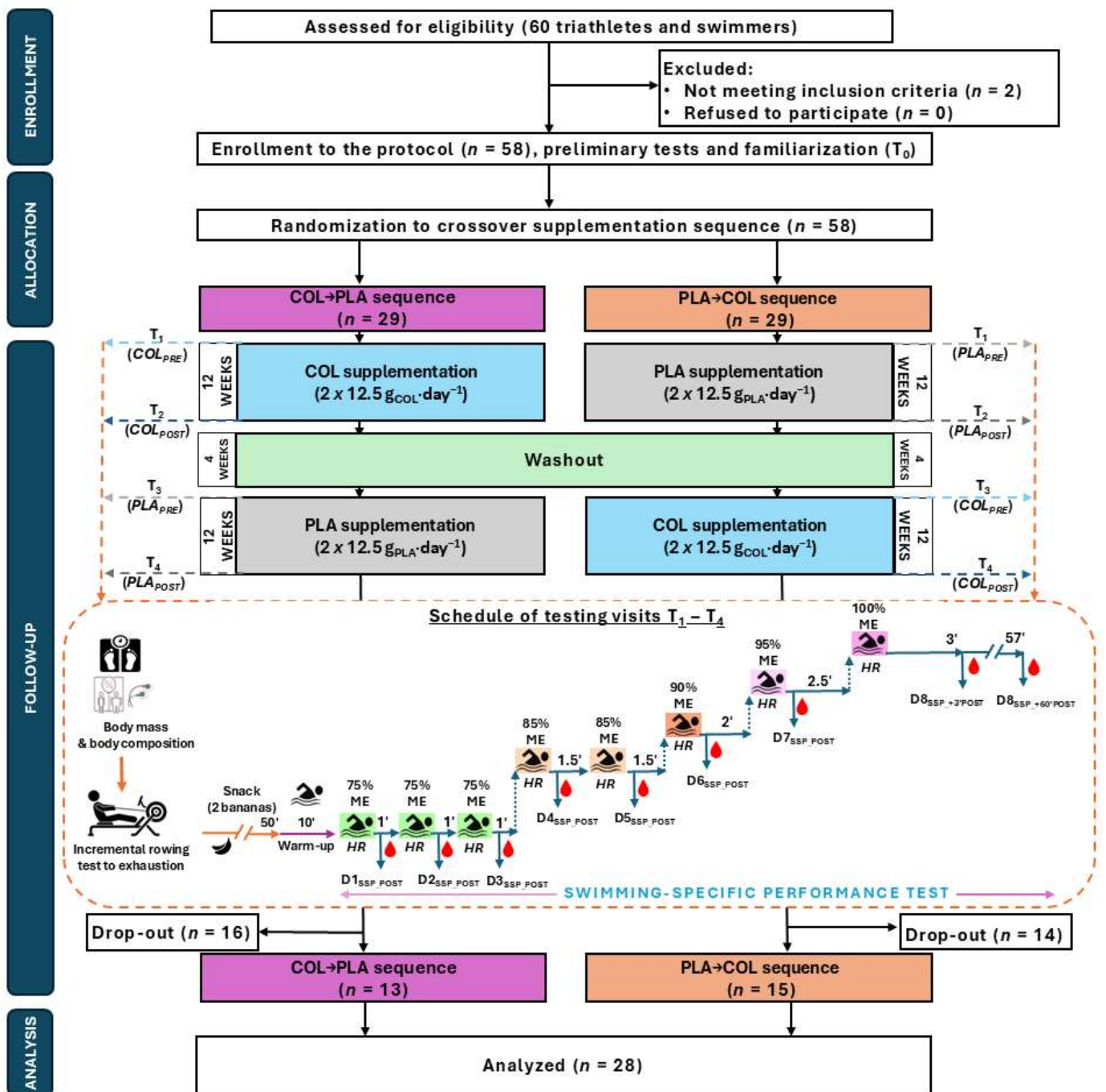
## 2. Materials and Methods

### 2.1. Study Participants

The study initially enrolled 58 male participants involved in moderate endurance training (at least 3–5 times per week). However, 30 participants dropped out due to various reasons: injuries ( $n = 8$ ), antibiotic therapy ( $n = 4$ ), business trips ( $n = 3$ ), family reasons ( $n = 3$ ), and withdrawing without providing a reason during the washout period ( $n = 12$ ). Ultimately, 28 athletes ( $31.1 \pm 10.2$  years;  $81.9 \pm 9.0$  kg body mass (BM);  $1.82 \pm 0.06$  m height;  $68.5 \pm 6.2$  kg fat-free mass [FFM];  $13.3 \pm 5.5$  kg fat mass [FM]), including 17 triathletes and 11 swimmers, completed the entire study protocol (see Figure 1). The participants were recruited primarily from sports clubs in Poland (mainly Poznań, Szczecin, and Wrocław).

Eligibility criteria required participants to be healthy, to not have chronic and/or autoimmune diseases, and to have a valid medical certificate to prove their fitness for sports. Alongside these criteria, participants needed to have been engaged in triathlon or swimming for at least 5 years and to have regularly participated in national competitions (at least 2–3 times per year). The participants in our study could be categorized as being at least Tier 2 (trained/developmental) according to the latest training status classification framework by McKay et al. [16]. Exclusion criteria included being allergic to cow's milk proteins, being lactose intolerant, showing symptoms of infection or use of any drugs within the last month prior to the study. All athletes claimed that they did not change their way of life, training process, eating habits, or supplementation throughout the research.

The Bioethics Committee at the Poznan University of Medical Sciences (identifier: 486/19) approved the research protocol and the study was registered at ClinicalTrials.gov (NCT06390670). This study followed CONSORT guidelines and is in line with the ethical aspects of the Declarations of Helsinki 2013. With the use of G\*Power software version 3.1.9.4 (Universität Düsseldorf, Germany), sample size calculations were done to ensure approximately an 80% power ( $\alpha = 0.05$ ) and partial eta squared large effect size 0.14 under repeated measures with the ANOVA framework. The analysis revealed that a convenient sample size for this study was 26 participants.



**Figure 1.** Flowchart of the study protocol. Abbreviations: COL, *Colostrum Bovinum*; D<sub>SSP</sub>, the distance number in swimming-specific performance test; HR, heart rate; ME, maximal effort; PLA, placebo; SSP, swimming-specific performance test.

### 2.2. Study Design and Visits

A randomized double-blind crossover design was utilized in this study, which followed a 12-week supplementation protocol with either COL or PLA. The protocol included an initial familiarization session (T<sub>0</sub>) and four main laboratory visits (T<sub>1</sub>–T<sub>4</sub>) carried out before and after the supplementation periods (COL<sub>PRE</sub>, COL<sub>POST</sub>, PLA<sub>PRE</sub>, and PLA<sub>POST</sub>). Visits T<sub>1</sub> and T<sub>3</sub> served as baseline assessments before supplementation (see Figure 1). The research was conducted in several waves between November 2021 and May 2023 at the Poznan University of Physical Education in Poland. Each wave commenced either during

autumn or winter and finished before spring or summer to balance the risks associated with upper respiratory tract infection occurrences.

After familiarization, participants were randomly assigned into supplementation sequences groups (COL→PLA or PLA→COL) via stratified randomization based on body composition results, handled by an impartial biostatistician. A 4-week washout period was included between the treatment phases. Each visit (T<sub>1</sub>–T<sub>4</sub>) involved evaluations of BM and body composition, with nine blood samples taken in total: during the SSP test and 3 and 60 min post-exercise (see Figure 1).

Testing was consistently scheduled in the morning to ensure standardization of study conditions and to minimize the effects of diurnal physiological fluctuations in the obtained results. Participants consumed a standardized meal three hours before each visit [17–19].

### 2.2.1. Supplementation

In the experimental procedure, each athlete was given a daily dose of 25 g of either COL or PLA over 12 weeks, following a randomized crossover sequence. The COL supplement, derived from the first post-delivery milking, contained a high immunoglobulin G content (30%; certified *Colostrum Bovinum*; Agrapak, Poznań, Poland). The PLA was an isoenergetic and isomacronutrient product made from high-quality milk protein (Agrapak, Poznań, Poland). Both supplements were in powder form and taken twice daily (12.5 g in the morning and 12.5 g in the afternoon), dissolved in 250 mL of plain water.

To ensure blinding, unique codes marked all containers that distributed the supplements. A researcher who was not directly involved in conducting research procedures and evaluating results was responsible for the preparation of the supplements (COL and PLA) beforehand. Thus double-blind settings were maintained, and neither participants nor researchers knew whether the participant was receiving COL or PLA at the particular supplementation phase, according to procedures of the highest standards of good practices in conducting clinical trials [20]. Until the final completion of this study, randomization details were anonymized. Out of 28 participants who completed the entire study protocol, as many as 12 athletes declared that they were sure of what supplement they had taken during each phase; but only 2 participants were in fact right about the sequence of supplementation. This fact confirms a full effectiveness of the implemented double-blinding procedure.

### 2.2.2. Body Mass and Body Composition Evaluation

Participants were asked to refrain from engaging in any form of exercise 24 h prior to any visit. At the beginning of each visit, the anthropometric measurements were taken to keep the test conditions as equal as possible. The BM and height were measured with a calibrated scale with a stadiometer (WPT 60/150 OW, Radwag, Radom, Poland). Body composition was analyzed using electrical bioimpedance with a BIA-101ASE device (Akern, Pontassieve, Italy), following all recommended procedures for measurement conditions as previously described [21]. This analysis determined total body water (TBW; L and %), FFM (kg and %), and FM (kg and %).

### 2.2.3. Exercise Protocol

During the SSP tests in the indoor 25 m lap swimming pool, all recommended conditions for such assessments were kept constant and aligned with the recommendations set out by World Aquatics (previously known as the *Fédération Internationale de Natation* [FINA]) [22,23]. The ambient air temperature was maintained between 30–34 °C, whereas the water temperature was not less than 28 °C, with a relative humidity of around 60–70%. These tests were performed at the same time of day for all the participants (in the morning). Screening was done at the time of enrolment (T<sub>0</sub>) where the participant was tested on the procedures before going through the main procedure (T<sub>1</sub>–T<sub>4</sub>) as described in the protocol.

The test was performed after a 10-min warm-up in the swimming pool. The SSP test comprised of performing eight 100-m distances split into ramp parts with increasing intensity. Ramps one to three (D<sub>1SSP</sub>, D<sub>2SSP</sub>, D<sub>3SSP</sub>) were swum at 75% of the individual's

maximal effort (ME; assessed one week before each study visit); ramps four (D4<sub>SSP</sub>) and five (D5<sub>SSP</sub>) at 85% ME; ramp six (D6<sub>SSP</sub>) at 90% ME; ramp seven (D7<sub>SSP</sub>) at 95% ME; and ramp eight (D8<sub>SSP</sub>) at 100% ME. Recovery periods between each ramp ranged between 1 and 2.5 min, as shown in Figure 1. The step test we implemented is a modified version of a previously validated swimming lactate threshold protocol [24,25]. During the SSP test, the time for each 100-m section, as well as the total time for the whole SSP test, was measured using a stopwatch (Garmin Fenix 5X, Garmin, Olathe, KS, USA); for accuracy, the time for each test was measured to a hundredth of a second.

Moreover, HR was constantly monitored using a telemetric system (Garmin Fenix 5X and HRM-Swim™; Garmin, Olathe, KS, USA) during the SSP test. From the collected data, HR<sub>MEAN</sub> and HR<sub>MAX</sub> were determined.

#### 2.2.4. Blood Collection and Sample Analysis

Fingertip capillary blood samples were collected at nine different time points during each study visit: seven times during exercise protocol (immediately after each set of 100-m section, during the recovery time after each section—D1<sub>SSP\_POST</sub>, D2<sub>SSP\_POST</sub>, D3<sub>SSP\_POST</sub>, D4<sub>SSP\_POST</sub>, D5<sub>SSP\_POST</sub>, D6<sub>SSP\_POST</sub>, and D7<sub>SSP\_POST</sub>), as well as 3 (D8<sub>SSP\_+3'POST</sub>), and 60 min (D8<sub>SSP\_+60'POST</sub>) after completion of D8<sub>SSP</sub> (see Figure 1). Each blood sample (50 µL) was promptly placed into microtubes containing 250 µL of 0.6 M perchloric acid. An [La<sup>-</sup>] measurement was performed according to the method described previously by Maughan [26], and successfully implemented in our earlier studies [17,27].

#### 2.3. Statistical Analysis

Variables were checked for a normal distribution with the Shapiro–Wilk test. Furthermore, kurtosis, skewness [28], and a graphical evaluation of the distribution of each variable data were performed. Data was analyzed with analysis of variance with repeated measurements (RM ANOVA), with the effect size (ES) expressed as partial eta-squared ( $\eta^2_p$ ). A Huynh–Feldt adjustment was made when sphericity was violated (as indicated by Mauchly’s test). Post-hoc comparisons were performed using the Bonferroni test for multiple comparisons. The ES was interpreted as follows:  $\eta^2_p$ : <0.010 *no* effect; from 0.010 to 0.059 *small* effect; from 0.060 to 0.139 *moderate* effect; and  $\geq 0.140$  *large* effect. To verify the possible practice effect, the comparisons in time of particular SSP distances and total SSP time between consecutive study visits (T<sub>1</sub>–T<sub>4</sub>) were performed using RM ANOVA. Moreover, differences in percentage changes in the times of the SSP tests between COL and PLA supplementation were analyzed using a *t*-test for dependent variables. An alpha of <0.05 was taken as a statistically significant value. The data were analyzed using the STATISTICA 13.3 software (StatSoft Polska Sp. z o.o., 2024, Zestaw Plus version 5.0.96, Poland).

### 3. Results

#### 3.1. Swimming-Specific Performance

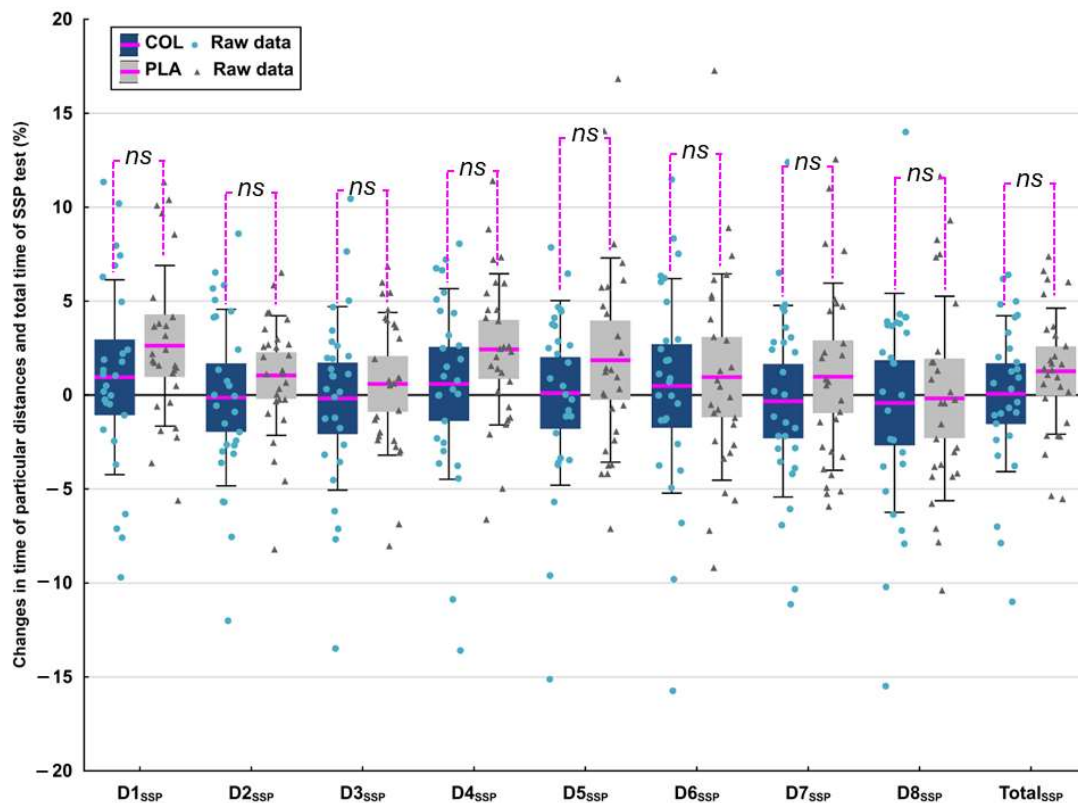
Neither COL nor PLA supplementation affected the total time of the SSP test or times at particular distances during the SSP test (D1<sub>SSP</sub>–D8<sub>SSP</sub>; Table 1). Nevertheless, the total time of the SSP test after COL supplementation was ~3.04 s shorter, while after PLA supplementation it was ~7.13 s longer compared to pre-supplementation measurements (Table 1).

There were no significant differences in the percentage changes in time for particular distances or the total time of the SSP test between COL and PLA supplementation (see Figure 2).

**Table 1.** Time (min:s) in the swimming-specific performance test.

Distance	COL <sub>PRE</sub>	COL <sub>POST</sub>	PLA <sub>PRE</sub>	PLA <sub>POST</sub>	[p]; $\eta^2_p$
D1 <sub>SSP</sub> (75% ME)	1:30.51 ± 18.30 (1:23.42–1:37.61)	1:31.01 ± 17.02 (1:24.41–1:37.61)	1:30.18 ± 18.50 (1:23.01–1:37.35)	1:32.09 ± 16.80 (1:25.58–1:38.61)	[0.147]; 0.064
D2 <sub>SSP</sub> (75% ME)	1:32.06 ± 20.30 (1:24.19–1:39.93)	1:31.39 ± 17.79 (1:24.50–1:38.29)	1:31.53 ± 19.24 (1:24.07–1:38.99)	1:32.19 ± 18.07 (1:25.18–1:39.20)	[0.705]; 0.017
D3 <sub>SSP</sub> (75% ME)	1:32.38 ± 21.49 (1:24.05–1:40.72)	1:31.67 ± 18.92 (1:24.34–1:39.01)	1:32.36 ± 20.25 (1:24.51–1:40.21)	1:32.61 ± 19.10 (1:25.20–1:40.01)	[0.763]; 0.014
D4 <sub>SSP</sub> (85% ME)	1:29.42 ± 21.88 (1:20.93–1:37.91)	1:29.38 ± 19.46 (1:21.84–1:36.93)	1:28.77 ± 19.58 (1:21.18–1:36.36)	1:30.82 ± 19.93 (1:23.09–1:38.55)	[0.185]; 0.057
D5 <sub>SSP</sub> (85% ME)	1:27.55 ± 22.07 (1:18.99–1:36.11)	1:28.60 ± 19.41 (1:21.07–1:36.13)	1:28.39 ± 19.81 (1:20.70–1:36.07)	1:29.61 ± 19.52 (1:22.14–1:37.27)	[0.565]; 0.047
D6 <sub>SSP</sub> (90% ME)	1:27.55 ± 22.07 (1:18.99–1:36.11)	1:27.49 ± 20.15 (1:19.68–1:35.31)	1:26.95 ± 20.25 (1:19.09–1:34.80)	1:27.60 ± 20.26 (1:19.75–1:35.46)	[0.930]; 0.005
D7 <sub>SSP</sub> (95% ME)	1:26.18 ± 21.39 (1:17.88–1:34.47)	1:25.37 ± 19.17 (1:17.94–1:32.81)	1:25.62 ± 19.52 (1:18.05–1:33.19)	1:26.38–19.99 (1:18.63–1:34.13)	[0.678]; 0.018
D8 <sub>SSP</sub> (100% ME)	1:24.13 ± 21.18 (1:15.92–1:32.34)	1:23.27 ± 19.14 (1:15.85–1:30.70)	1:24.08–19.88 (1:16.38–1:31.79)	1:23.60 ± 18.85 (1:16.30–1:30.91)	[0.789]; 0.013
Total time of the SSP test	11:51.24 ± 2:45.69 (10:46.99–12:55.49)	11:48.20 ± 2:29.44 (10:50.26–12:46.15)	11:47.87 ± 2:35.89 (10:47.43–12:48.32)	11:55.01 ± 2:30.87 (10:56.51–12:53.51)	[0.586]; 0.023

The results are expressed as the mean ± standard deviation and 95% confidence interval (in parentheses). Abbreviations: D<sub>SSP</sub>, the distance number in swimming-specific performance test; ME, maximal effort; SSP, swimming-specific performance test. Data were analyzed with ANOVA with repeated measurements (RM ANOVA); the effect size expressed as partial eta square ( $\eta^2_p$ ).



**Figure 2.** Changes in time (%) of particular distances in the swimming-specific performance (SSP) test and in total time of the SSP test. The data are expressed as the mean (line), 95% confidence interval (box), 95% CI + one standard deviation (whisker), and raw data of individuals. The data were analyzed with *t*-test for dependent variables. Abbreviations: COL, *Colostrum Bovinum*; D<sub>SSP</sub>, the distance number in swimming-specific performance test; *ns*, statistically not significant; PLA, placebo; SSP, swimming-specific performance test.

### 3.2. Heart Rate during the SSP Test

COL and PLA supplementation had no effect on the  $HR_{MEAN}$ . However, the  $HR_{MEAN}$  was significantly lower at  $PLA_{POST}$  compared to  $COL_{PRE}$ , with no differences between the remaining measuring time points ( $p = 0.011$ ,  $\eta^2_p = 0.127$ ;  $COL_{PRE}$  vs.  $PLA_{POST}$ :  $p = 0.006$ ; see Table 2). COL and PLA supplementation had no effect on  $HR_{MAX}$  during the SSP test (see Table 2).

**Table 2.** Heart rate (bpm) during swimming-specific performance test.

Measured Indices	$COL_{PRE}$	$COL_{POST}$	$PLA_{PRE}$	$PLA_{POST}$	[ $p$ ]; $\eta^2_p$
$HR_{MEAN}$	143 ± 13 <sup>†</sup> (138–148)	139 ± 11 (135–143)	139 ± 12 (135–144)	135 ± 11 (131–139)	<b>[0.011]; 0.127</b>
$HR_{MAX}$	175 ± 12 (170–180)	174 ± 13 (169–179)	173 ± 13 (168–179)	172 ± 11 (167–176)	[0.412]; 0.035

The results are expressed as the mean ± standard deviation and 95% confidence interval (in parentheses). Abbreviations:  $HR_{MEAN}$ , mean heart rate during the swimming-specific test;  $HR_{MAX}$ , maximal heart rate during the swimming-specific test. Data were analyzed with ANOVA with repeated measurements (RM ANOVA); the effect size expressed as partial eta square ( $\eta^2_p$ ). Results in bold refer to statistically significant differences. <sup>†</sup>  $COL_{PRE}$  vs.  $PLA_{POST}$ :  $p = 0.006$ .

### 3.3. Blood Lactate Concentration

From  $D1_{SSP\_POST}$  to  $D4_{SSP\_POST}$  there were no differences in blood  $[La^-]$  between measuring time points (see Table 3). At  $D5_{SSP\_POST}$  ( $p < 0.001$ ,  $\eta^2_p = 0.236$ ;  $COL_{PRE}$  vs.  $PLA_{POST}$ :  $p = 0.001$ ;  $PLA_{PRE}$  vs.  $PLA_{POST}$ :  $p < 0.001$ ; Table 3), and  $D6_{SSP\_POST}$  ( $p = 0.003$ ,  $\eta^2_p = 0.176$ ;  $COL_{PRE}$  vs.  $PLA_{POST}$ :  $p = 0.013$ ;  $PLA_{PRE}$  vs.  $PLA_{POST}$ :  $p = 0.003$ ) blood  $[La^-]$  was significantly lower at  $PLA_{POST}$  compared to  $COL_{PRE}$  and  $PLA_{PRE}$ , but with no differences between  $COL_{POST}$  and the other measuring time points. At  $D7_{SSP\_POST}$  ( $p = 0.001$ ,  $\eta^2_p = 0.188$ ;  $COL_{POST}$  vs.  $PLA_{PRE}$ :  $p = 0.029$ ;  $PLA_{POST}$  vs.  $PLA_{PRE}$ :  $p = 0.001$ ; Table 3), blood  $[La^-]$  was significantly lower at  $COL_{POST}$  and  $PLA_{POST}$  compared to  $PLA_{PRE}$ , with no differences between  $COL_{POST}$  and  $PLA_{POST}$ , as well as between  $COL_{PRE}$  and remaining measuring time points. At  $D8_{SSP\_+3'POST}$  ( $p < 0.001$ ,  $\eta^2_p = 0.279$ ;  $COL_{PRE}$  vs.  $COL_{POST}$ :  $p = 0.003$ ;  $PLA_{PRE}$  vs.  $PLA_{POST}$ :  $p < 0.001$ ;  $PLA_{PRE}$  vs.  $COL_{POST}$ :  $p < 0.001$ ;  $COL_{PRE}$  vs.  $PLA_{POST}$ :  $p = 0.004$ ; Table 3), blood  $[La^-]$  was significantly lower at  $COL_{POST}$  and  $PLA_{POST}$  compared to  $COL_{PRE}$  and  $PLA_{PRE}$ , respectively (with no differences between  $COL_{POST}$  vs.  $PLA_{POST}$ ). Moreover,  $[La^-]$  was lower at  $COL_{POST}$  vs.  $PLA_{PRE}$ , and at  $PLA_{POST}$  vs.  $COL_{PRE}$ . At  $D8_{SSP+60'POST}$ , no differences in blood  $[La^-]$  between any measuring time points were found (see Table 3).

**Table 3.** Blood lactate concentration (mmol·L<sup>-1</sup>).

Measuring Time Point	$COL_{PRE}$	$COL_{POST}$	$PLA_{PRE}$	$PLA_{POST}$	[ $p$ ]; $\eta^2_p$
$D1_{SSP\_POST}$	5.0 ± 1.8 (4.4–5.8)	4.7 ± 1.9 (4.0–5.4)	5.3 ± 1.8 (4.6–6.0)	5.1 ± 1.8 (4.4–5.8)	[0.372]; 0.038
$D2_{SSP\_POST}$	6.1 ± 1.9 (5.3–6.9)	5.8 ± 2.5 (4.9–6.8)	6.3 ± 2.1 (5.5–7.1)	5.7 ± 1.7 (5.0–6.3)	[0.420]; 0.034
$D3_{SSP\_POST}$	6.9 ± 2.2 (6.0–7.7)	6.7 ± 2.6 (5.7–7.7)	6.8 ± 2.1 (6.0–7.6)	6.4 ± 2.0 (5.7–7.2)	[0.650]; 0.020
$D4_{SSP\_POST}$	7.8 ± 2.6 (6.8–8.8)	7.5 ± 2.5 (6.6–8.5)	7.9 ± 2.2 (7.0–8.7)	7.0 ± 2.0 (6.2–7.8)	[0.180]; 0.060



**Table 3.** Cont.

Measuring Time Point	COL <sub>PRE</sub>	COL <sub>POST</sub>	PLA <sub>PRE</sub>	PLA <sub>POST</sub>	[p]; η <sup>2</sup> <sub>p</sub>
D5 <sub>SSP_POST</sub>	8.7 ± 2.7 <sup>†</sup> (7.7–9.8)	7.8 ± 2.4 (6.8–8.7)	8.9 ± 2.2 <sup>‡</sup> (8.0–9.7)	6.8 ± 1.5 (6.2–7.4)	<b>[&lt;0.001]; 0.236</b>
D6 <sub>SSP_POST</sub>	9.7 ± 2.9 <sup>§</sup> (8.6–10.8)	8.6 ± 2.3 (7.7–9.5)	9.9 ± 2.0 <sup>*</sup> (9.2–10.7)	8.0 ± 1.6 (7.4–8.6)	<b>[0.003]; 0.176</b>
D7 <sub>SSP_POST</sub>	10.5 ± 2.7 (9.5–11.6)	9.7 ± 2.8 <sup>††</sup> (8.6–10.7)	11.4 ± 2.3 (10.5–12.3)	9.0 ± 2.0 <sup>‡‡</sup> (8.2–9.8)	<b>[0.001]; 0.188</b>
D8 <sub>SSP_+3'POST</sub>	13.0 ± 3.2 <sup>§§</sup> (11.7–14.2)	10.6 ± 3.5 <sup>~</sup> (9.2–11.9)	13.4 ± 3.5 <sup>**</sup> (12.1–14.8)	10.6 ± 3.4 <sup>£</sup> (9.3–11.9)	<b>[&lt;0.001]; 0.279</b>
D8 <sub>SSP_+60'POST</sub>	2.2 ± 0.9 (1.9–2.6)	2.5 ± 1.3 (1.9–3.0)	2.6 ± 1.3 (2.1–3.1)	2.0 ± 0.7 (1.8–2.3)	[0.119]; 0.069

The results are expressed as the mean ± standard deviation and 95% confidence interval (in parentheses). Data were analyzed with ANOVA with repeated measurements (RM ANOVA); the effect size expressed as partial eta square (η<sup>2</sup><sub>p</sub>). Results in bold refer to statistically significant differences. D5<sub>SSP\_POST</sub><sup>†</sup> COL<sub>PRE</sub> vs. PLA<sub>POST</sub>: p = 0.001; <sup>‡</sup> PLA<sub>PRE</sub> vs. PLA<sub>POST</sub>: p < 0.001. D6<sub>SSP\_POST</sub><sup>§</sup> COL<sub>PRE</sub> vs. PLA<sub>POST</sub>: p = 0.013; \* PLA<sub>PRE</sub> vs. PLA<sub>POST</sub>: p = 0.003. D7<sub>SSP\_POST</sub><sup>††</sup> COL<sub>POST</sub> vs. PLA<sub>PRE</sub>: p = 0.029; <sup>‡‡</sup> PLA<sub>POST</sub> vs. PLA<sub>PRE</sub>: p = 0.001. D8<sub>SSP\_+3'POST</sub><sup>§§</sup> COL<sub>PRE</sub> vs. COL<sub>POST</sub>: p = 0.003; \*\* PLA<sub>PRE</sub> vs. PLA<sub>POST</sub>: p < 0.001; <sup>~</sup> COL<sub>POST</sub> vs. PLA<sub>PRE</sub>: p < 0.001; <sup>£</sup> PLA<sub>POST</sub> vs. COL<sub>PRE</sub>: p = 0.004.

### 3.4. Practice Effect

The D1<sub>SSP</sub> time (p = 0.005, η<sup>2</sup><sub>p</sub> = 0.145; T<sub>1</sub> vs. T<sub>4</sub>: p = 0.003; Table 4) was significantly shorter at the T<sub>1</sub> compared to the T<sub>4</sub>, with no differences between the remaining study visits. The D8<sub>SSP</sub> time (p = 0.009, η<sup>2</sup><sub>p</sub> = 0.132; T<sub>1</sub> vs. T<sub>3</sub>: p = 0.007; Table 4) was significantly shorter at the T<sub>3</sub> compared to the T<sub>1</sub>, with no differences between the remaining study visits. There were no significant differences in the time for remaining distances, including the total time of the SSP test, between consecutive study visits (see Table 4).

**Table 4.** Time (min:s) in the swimming-specific performance test in the consecutive study visits.

Distance	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	[p]; η <sup>2</sup> <sub>p</sub>
D1 <sub>SSP</sub> (75% ME)	1:29.30 ± 17.66 <sup>†</sup> (1:22.45–1:36.15)	1:30.81 ± 17.52 (1:24.01–1:37.60)	1:31.39 ± 19.06 (1:24.00–1:38.78)	1:32.30 ± 16.26 (1:26.00–1:38.61)	<b>[0.005]; 0.145</b>
D2 <sub>SSP</sub> (75% ME)	1:31.73 ± 19.56 (1:24.14–1:39.32)	1:31.32 ± 18.29 (1:24.23–1:38.42)	1:31.86 ± 19.98 (1:24.11–1:39.61)	1:32.26 ± 17.56 (1:25.45–1:39.07)	[0.714]; 0.017
D3 <sub>SSP</sub> (75% ME)	1:32.78 ± 21.46 (1:24.46–1:40.10)	1:31.46 ± 19.53 (1:23.88–1:39.03)	1:21.96 ± 20.27 (1:24.10–1:39.82)	1:32.82 ± 18.46 (1:25.67–1:39.98)	[0.370]; 0.038
D4 <sub>SSP</sub> (85% ME)	1:29.68 ± 21.92 (1:21.18–1:38.18)	1:29.62 ± 20.32 (1:21.74–1:37.50)	1:28.51 ± 19.52 (1:20.94–1:36.80)	1:30.58 ± 19.07 (1:23.19–1:37.98)	[0.202]; 0.055
D5 <sub>SSP</sub> (85% ME)	1:29.05 ± 21.77 (1:20.61–1:37.49)	1:28.92 ± 20.11 (1:21.12–1:36.72)	1:28.34 ± 19.84 (1:20.65–1:36.03)	1:29.38 ± 18.82 (1:22.09–1:36.68)	[0.770]; 0.014
D6 <sub>SSP</sub> (90% ME)	1:28.45 ± 22.49 (1:19.73–1:37.17)	1:27.76 ± 20.83 (1:19.69–1:35.84)	1:26.05 ± 19.71 (1:18.41–1:33.70)	1:27.33 ± 19.55 (1:19.75–1:34.92)	[0.169]; 0.060
D7 <sub>SSP</sub> (95% ME)	1:27.09 ± 21.17 (1:18.89–1:35.30)	1:26.48 ± 20.36 (1:18.58–1:34.37)	1:24.70 ± 19.70 (1:17.06–1:32.34)	1:25.27 ± 18.77 (1:17.99–1:32.55)	<b>[0.035]; 0.100</b>
D8 <sub>SSP</sub> (100% ME)	1:25.64 ± 21.07 <sup>‡</sup> (1:17.47–1:33.81)	1:23.48 ± 19.07 (1:16.09–1:30.88)	1:22.57 ± 19.88 (1:14.87–1:30.28)	1:23.39 ± 18.92 (1:16.06–1:30.73)	<b>[0.009]; 0.132</b>
Total time of DSP test	11:53.72 ± 2:44.84 (10:49.80–12:57.64)	11:49.86 ± 2:34.51 (10:49.94–12:49.77)	11:45.39 ± 2:36.69 (10:44.64–12:46.15)	11:53.35 ± 2:25.74 (10:56.84–12:49.87)	[0.434]; 0.032

The results are expressed as the mean ± standard deviation and 95% confidence interval (in parentheses). Data were analyzed with ANOVA with repeated measurements (RM ANOVA); the effect size expressed as partial eta square (η<sup>2</sup><sub>p</sub>). Results in bold refer to statistically significant differences. <sup>†</sup> T<sub>1</sub> vs. T<sub>4</sub>: p = 0.003. <sup>‡</sup> T<sub>1</sub> vs. T<sub>3</sub>: p = 0.007.

#### 4. Discussion

The present double-blind randomized placebo-controlled crossover study aimed to investigate the ability of a 12-week  $25 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  supplementation to enhance the swimming performance of endurance-trained male athletes. The outcomes of the study demonstrated that COL supplementation may not improve swimming-specific performance in triathletes and swimmers more than PLA. Moreover, similar effects of COL and PLA supplementation on adaptations in the body to exercise based on selected physiological (HR) or biochemical ( $[\text{La}^-]$ ) markers were observed.

To the best of our knowledge, this is the first study conducted on swimmers and triathletes in terms of the effect of COL supplementation on swimming performance. Only one previous study [29] examined COL effectiveness in swimmers, but no exercise protocols were implemented and so there was no assessment of COL's influence on performance. In our current study, exercise performance measured by the SSP test showed no differences between COL and PLA in the total time of the SSP test, or the time for the particular distances during the SSP test performed at various intensities. Nevertheless, after COL supplementation, the participants finished the test faster than after PLA. In terms of endurance performance, there is evidence suggesting that COL supplementation may enhance recovery from various types of strenuous exercise and improve subsequent performance [1]. Additionally, COL has been demonstrated to enhance recovery from repeated exercise sessions [12], to improve TT performance after extended submaximal exercise [11], and to sustain exercise performance after a period of high-intensity training (HIT) [10]. Buckley et al. [12] conducted a study where 39 male participants were given either COL ( $60 \text{ g} \cdot \text{day}^{-1}$ ) or PLA over an 8-week period. During this time, their training included three 45-min running sessions per week. The participants underwent two incremental treadmill tests, separated by a 20-min passive recovery, at the start of the study, and again after 4 and 8 weeks of supplementation. After 4 weeks, no significant changes in running performance were observed. However, after 8 weeks, those in the COL group covered a significantly greater distance and completed more work in the second treadmill test compared to the PLA group (a 4.6% increase;  $p < 0.04$ ). For these reasons, the remarkable enhancement of running performance observed could not be explained by any increases in respiratory exchange ratio, lactate threshold, or even insulin-like growth factor (IGF-1) concentrations. Also, on COL supplementation, no effect on  $\text{VO}_{2\text{MAX}}$  was observed, though there was improved endurance performance [11,12]. In a double-blind, placebo-controlled study, performed by Coombes et al. [11], 42 cyclists underwent a cycle TT of  $2.8 \text{ kJ} \cdot \text{kg}^{-1}$  after a 2-h endurance ride, both before and after an 8-week supplementation period. Participants were divided into groups receiving either 20 or  $60 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  or a PLA (whey protein powder). Results revealed a significant improvement in TT performance for those who took COL compared to PLA, with time decreases of 158 s in the  $20 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  group and 134 s in the  $60 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  group (both  $p < 0.05$ ). The comparable performance gains in both COL groups suggest that increasing COL dose beyond a certain point may not yield additional performance benefits [1,30]. Although, improvements in endurance performance after COL supplementation do not seem to be linked to increased circulating IGF-1 levels, a study by Shing et al. [10] demonstrated that COL maintained ventilatory threshold and enhanced economy during periods of HIT. A dose of  $10 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  improved 40 km TT (TT40) performance following a 5-day HIT regimen, but not during regular training, compared to a whey protein PLA [10]. It appears that although the effects of COL on performance remain unclear, COL supplementation may be particularly advantageous for endurance performance during intense training phases or periods of overload, which might lead to fatigue and a reduced ventilatory threshold [1,30].

Adaptation of the body to exercise can be measured based on selected physiological (HR) and biochemical ( $[\text{La}^-]$ ) markers. Training adaptations based on these markers refer to the physiological changes that occur as a result of consistent exercise. In sports practice, HR and  $[\text{La}^-]$  should be indicated as the most important and clinically relevant markers for monitoring the intensity and specificity of energy processes during exercise (aerobic

vs. anaerobic). In our study, there were no differences in  $HR_{MEAN}$  or  $HR_{MAX}$  during the SSP test between COL and PLA. HR, reflecting the body's ability to supply oxygen to working muscles during prolonged exercise, serves as an important indicator of endurance performance. A lower resting HR and quicker recovery after exercise also signal improved cardiovascular endurance. HR monitoring enables athletes to regulate the intensity of their training to achieve the desired improvement of their endurance-related capabilities as well as performance [31]. The majority of studies [12,32–37] that have examined HR during exercise, similarly to our results, showed no differences in HR (peak or mean) between COL and PLA treatments. Nevertheless, in one of the previously described studies, Shing et al. [10] showed that at the end of the HIT period, compared to PLA, COL maintained TT40 HR ( $2.5 \pm 3.7\%$ ; a possible benefit to enhance TT40 intensity and prevent a decrease in submaximal HR during high-intensity training).

In the current study, there were no differences between COL and PLA in  $[La^-]$  during/after the SSP and after 60 min of recovery. The lactate threshold refers to the point during exercise at which lactate—a byproduct of anaerobic metabolism—begins to accumulate in the bloodstream at a faster rate than it can be removed. This threshold is an important marker for endurance athletes, as it represents the intensity of exercise at which the body transitions from primarily aerobic energy production to increased reliance on anaerobic pathways [38]. In terms of high-intensity and intermittent exercise performance, some research [6,8,39] has suggested that COL might improve this type of performance by either increasing the intracellular buffer capacity to neutralize hydrogen ions ( $H^+$ ) or boosting the extracellular buffer capacity to allow greater  $H^+$  efflux from the muscles. Similarly to our results, no differences in  $[La^-]$  were found in other studies [12,34,35,37,39,40]. Interestingly, Brinkworth et al. [39] proposed that COL supplementation might reduce the rate of intramuscular acidosis during intense exercise. The impact of COL on blood buffering capacity was examined during a 9-week training program involving 13 elite female rowers, who were given either  $60 \text{ g} \cdot \text{day}^{-1}$  of COL or  $60 \text{ g} \cdot \text{day}^{-1}$  of whey protein. The study utilized two incremental rowing tests (each consisting of four 3-min stages and separated by 15 min) to assess performance before and after the supplementation period. After 9 weeks of COL supplementation blood buffering capacity was increased based on the evaluation of blood  $[La^-]$  and pH at the end of each workload during the tests. Despite the increased blood buffering capacity, no significant differences in exercise performance were found between the groups. In a follow-up study, Brinkworth and Buckley [6] reanalyzed the data from previous work [39] to determine which component of blood buffering capacity was enhanced by COL. The analysis revealed no significant differences in resting hemoglobin levels, plasma bicarbonate concentrations, or plasma buffering capacity (all systemic buffers) between the groups. The observed increase in blood buffering capacity may have resulted from either an increase in intracellular phosphate levels [30] or an improvement in hemoglobin's ability to buffer  $H^+$ . In a different investigation, Kotsis et al. [8] investigated how recovery performance following an intermittent exercise program intended to replicate the physiological demands of soccer was affected by a 6-week course of low dose ( $3.2 \text{ g}_{COL} \cdot \text{day}^{-1}$ ) COL supplementation. These results showed that COL accelerated the recovery of explosive power, as assessed by squat jump performance, and decreased markers of inflammation and muscle damage brought on by the workout regimen. These impacts may have a significant influence on training for intermittent sports adaptations and long-term performance gains. Further research is needed to determine if COL supplementation increases muscle buffering capacity, which would require direct measurement of muscle pH and  $[La^-]$ . On the other hand, it must be emphasized that in the current study, COL supplementation resulted in significantly decreased  $[La^-]$  (post- vs. pre-supplementation) at  $D8_{SSP\_+3}POST$ , while PLA supplementation significantly decreased  $[La^-]$  (post- vs. pre-supplementation) at  $D5_{SSP\_POST}$ ,  $D6_{SSP\_POST}$ ,  $D7_{SSP\_POST}$ , and  $D8_{SSP\_+3}POST$ . These observations suggest improved bodies adaptation to exercise after COL and PLA (high-quality milk protein) supplementation. In this respect, lower  $[La^-]$  (during- and post-exercise) after COL and PLA interventions and similar/improved exer-

cise performance capabilities (a clinically significant shorter total time for the SSP test after COL) may be evidence of improved aerobic contribution to physical effort, better energetic economy, lower physiological load on the body, and therefore better exercise adaptation.

To summarize, COL has been marketed as a potential supplement for improving performance in endurance sports due to its high concentration of growth factors, immunoglobulins, and bioactive compounds. However, scientific evidence does not consistently support its effectiveness for enhancing endurance performance, which was shown in our current study. Endurance sport disciplines rely heavily on aerobic fitness and capacity, which is measured by  $VO_{2max}$ . Studies have shown that COL supplementation does not lead to significant improvements in  $VO_{2max}$ , which is a key factor in endurance performance. A study by Buckley et al. [12] demonstrated that although COL increased lean body mass, it had no significant effect on  $VO_{2max}$  or time to exhaustion in trained endurance athletes. Moreover, while COL is known to contain immunoglobulins that could potentially aid immune function, its impact on athletes' immunity during prolonged exercise remains uncertain. Endurance athletes often suffer from immune suppression after intense training or competition, leading to illnesses that can hinder performance. Some studies showed mild improvements in immune markers, but these effects are not consistent across all research. A study by Jones et al. [41] found that COL might help maintain immune function, but these benefits do not translate into direct performance gains in endurance sports. Furthermore, for endurance athletes, maintaining efficient energy metabolism is also critical. COL does not appear to significantly enhance carbohydrate or fat oxidation, which are crucial for sustained endurance performance. Research published by Shing et al. [42] indicated that while COL might enhance recovery, its impact on energy metabolism during prolonged exercise remains unclear, limiting its usefulness for boosting endurance. In general, the results of studies examining COL supplementation and endurance performance are inconsistent. Some studies report minor improvements in recovery time or reduction in gut permeability (which can be beneficial during long-duration events), but these findings are equivocal. Still, the variation in study outcomes may be attributed to differences in study design, dosage, and duration of supplementation, making it difficult to draw definitive conclusions about the effectiveness of COL for endurance performance.

This study stands out for taking a novel strategy, especially as it uses a crossover design and supplementation with  $25 \text{ gCOL} \cdot \text{day}^{-1}$  for 12 weeks. One of the crossover strategy's main advantages is that it lessens the impact of variables and inter-individual variability in group comparisons. Future studies should assess the long-term effects of COL supplementation on athletes within regular training cycles, possibly by comparing results across consecutive seasons or by using a parallel group design, given the difficulty of accounting for changes in performance over an extended period of time. A carefully planned 4-week washout time in between treatments further helped our study since it addressed the intricacies of COL's pharmacokinetics, which include bioactive components with variable elimination half-lives. A balanced comparison between seasons was ensured by having all participants start the study during the autumn/winter season. By keeping each participant's visit timings consistent, the study protocol was further reinforced, which improved the reliability of the findings.

The study results could have been impacted by a number of limitations. Although a crossover design has its benefits, there are drawbacks as well, namely the possibility of carryover effects and the impact of treatment order on results. These issues are especially pertinent to research involving athletes, whose training loads change based on the unique cycles of their capabilities. Athletes are a demanding group to investigate, particularly when the additional supplementation intervention needs to avoid interfering with their training schedules. A further obstacle in the current study was the high dropout rate, which was mainly caused by participants quitting during the washout period ( $n = 12$ ). This suggests that a parallel group design, which does not require a washout period, would have been a more appropriate and economical course of action. Injuries were the second most common reason for dropout ( $n = 8$ ), underscoring the high risk of injury and illness among

athletes, which can temporarily prevent them from training and competing. Despite these issues, the final sample size of 28 participants exceeded the a priori calculated requirement of 26, allowing the study to maintain its validity. Another possible limitation may have been the choice of the swimming protocol. Although we modified the original version of the  $8 \times 100$  m SSP test to adjust it as well as possible to the capabilities of triathletes and swimmers, there is a possibility that a different swimming test would have shown different results. The chosen SSP test may have been too intense and too difficult for triathletes, possibly affecting their performance. Nevertheless, the main goal of this test was to induce as much fatigue as possible, and to evoke subsequent  $La^-$  production as a response to high-intensity effort, which was clearly achieved. Based on changes in  $[La^-]$  we were able to evaluate COL/PLA capabilities to provide exercise-induced adaptation.

## 5. Conclusions

In conclusion, 12 weeks of supplementation with  $25 \text{ g}_{\text{COL}} \cdot \text{day}^{-1}$  in moderately-trained male triathletes and swimmers had no significant effect on swimming performance. Still, the total time of the swimming-specific performance test was about  $\sim 3.04$  s shorter after COL supplementation, and  $\sim 7.13$  s longer after PLA supplementation. Neither COL nor PLA supplementation affected heart rate during the specific exercise test. However, post-exercise blood lactate was significantly reduced after both COL and PLA supplementation. Long-term crossover supplementation protocols in athletes must consider the impact of a possible practice effect when interpreting the outcomes related to exercise performance, but not biochemical or physiological markers of exercise adaptation. *Colostrum Bovinum* and high-quality milk protein seem to be comparably effective in evoking exercise adaptation.

**Author Contributions:** Conceptualization, K.D.-M. and N.G.; methodology, K.D.-M., N.G. and T.P.; software, K.D.-M. and P.M.N.; validation, K.D.-M.; formal analysis, K.D.-M. and P.M.N.; investigation, K.D.-M., N.G., T.P., M.S., B.P., K.W., M.W. and P.M.N.; resources, K.D.-M.; data curation, K.D.-M., N.G. and P.M.N.; writing—original draft preparation, K.D.-M., N.G., M.S. and P.M.N.; writing—review and editing, K.D.-M., N.G., T.P., K.W., M.W. and P.M.N.; visualization, K.D.-M. and P.M.N.; supervision, K.D.-M.; project administration, K.D.-M.; funding acquisition, K.D.-M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was funded by the Nutricia Foundation (Fundacja Nutricia) grant, project no. RG 3/2019. Furthermore, K.D.-M. has participated in the Exchange Programmes for Scientists as part of bilateral cooperation financed by The Polish National Agency for Academic Exchange (NAWA: BPN/BIL/2021/1/00108/U/00001 and PPN/WYM/2019/1/00267/U/01). K.D.-M. and N.G. have participated in the PROM Programme (NAWA: PPI/PRO/2019/1/00045/U/00001; financed by The Polish National Agency for Academic Exchange).

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Bioethics Committee of the Poznan University of Medical Sciences (protocol code 486/19 approved on 11 April 2019).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The deidentified data are available from the corresponding author upon reasonable request.

**Acknowledgments:** We thank the athletes for their participation in the study. The authors wish to thank the Agrapak Sp. z o.o., (Poland) for the donation of the evaluated high-quality preparations (*Colostrum Bovinum* and high-quality milk protein).

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Davison, G. The Use of Bovine Colostrum in Sport and Exercise. *Nutrients* **2021**, *13*, 1789. [[CrossRef](#)] [[PubMed](#)]
2. Godhia, M.; Patel, N. Colostrum—Its Composition, Benefits As A Nutraceutical: A Review. *Curr. Res. Nutr. Food Sci.* **2013**, *1*, 37–47. [[CrossRef](#)]

3. Bagwe, S.; Tharappel, L.J.P.; Kaur, G.; Buttar, H.S. Bovine Colostrum: An Emerging Nutraceutical. *J. Complement. Integr. Med.* **2015**, *12*, 175–185. [CrossRef]
4. Antonio, J.; Sanders, M.S.; Van Gammeren, D. The Effects of Bovine Colostrum Supplementation on Body Composition and Exercise Performance in Active Men and Women. *Nutrition* **2001**, *17*, 243–247. [CrossRef]
5. Kerksick, C.; Kreider, R.; Rasmussen, C.; Lancaster, L.; Starks, M.; Greenwood, M.; Milnor, P.; Almada, A.; Earnest, C. Effects of Bovine Colostrum Supplementation on Training Adaptations II: Performance. *FASEB J.* **2001**, *15*, LB315.
6. Brinkworth, G.D.; Buckley, J.D.; Slavotinek, J.P.; Kurmis, A.P. Effect of Bovine Colostrum Supplementation on the Composition of Resistance Trained and Untrained Limbs in Healthy Young Men. *Eur. J. Appl. Physiol.* **2004**, *91*, 53–60. [CrossRef] [PubMed]
7. Buckley, J.D.; Brinkworth, G.D.; Abbott, M.J. Effect of Bovine Colostrum on Anaerobic Exercise Performance and Plasma Insulin-like Growth Factor I. *J. Sports Sci.* **2003**, *21*, 577–588. [CrossRef]
8. Kotsis, Y.; Mikellidi, A.; Aresti, C.; Persia, E.; Sotiropoulos, A.; Panagiotakos, D.B.; Antonopoulou, S.; Nomikos, T. A Low-Dose, 6-Week Bovine Colostrum Supplementation Maintains Performance and Attenuates Inflammatory Indices Following a Loughborough Intermittent Shuttle Test in Soccer Players. *Eur. J. Nutr.* **2018**, *57*, 1181–1195. [CrossRef] [PubMed]
9. Buckley, J.D. Bovine Colostrum: Does It Improve Athletic Performance? *Nutrition* **2002**, *18*, 776–777. [CrossRef]
10. Shing, C.M.; Jenkins, D.G.; Stevenson, L.; Coombes, J.S. The Influence of Bovine Colostrum Supplementation on Exercise Performance in Highly Trained Cyclists. *Br. J. Sports Med.* **2006**, *40*, 797–801. [CrossRef]
11. Coombes, J.S.; Conacher, M.; Austen, S.K.; Marshall, P.A. Dose Effects of Oral Bovine Colostrum on Physical Work Capacity in Cyclists. *Med. Sci. Sports Exerc.* **2002**, *34*, 1184–1188. [CrossRef] [PubMed]
12. Buckley, J.; Abbott, M.; Brinkworth, G.; Whyte, P. Bovine Colostrum Supplementation during Endurance Running Training Improves Recovery, but Not Performance. *J. Sci. Med. Sport* **2002**, *5*, 65–79. [CrossRef] [PubMed]
13. Jones, A.W.; Cameron, S.J.S.; Thatcher, R.; Beecroft, M.S.; Mur, L.A.J.; Davison, G. Effects of Bovine Colostrum Supplementation on Upper Respiratory Illness in Active Males. *Brain Behav. Immun.* **2014**, *39*, 194–203. [CrossRef] [PubMed]
14. Shing, C.M.; Peake, J.; Suzuki, K.; Okutsu, M.; Pereira, R.; Stevenson, L.; Jenkins, D.; Coombes, J. Effects of Bovine Colostrum Supplementation on Immune Variables in Highly Trained Cyclists. *J. Appl. Physiol.* **2007**, *102*, 1113–1122. [CrossRef] [PubMed]
15. Playford, R.J.; Macdonald, C.E.; Johnson, W.S. Colostrum and Milk-Derived Peptide Growth Factors for the Treatment of Gastrointestinal Disorders. *Am. J. Clin. Nutr.* **2000**, *72*, 5–14. [CrossRef] [PubMed]
16. McKay, A.K.A.; Stellingwerff, T.; Smith, E.S.; Martin, D.T.; Mujika, I.; Goosey-Tolfrey, V.L.; Sheppard, J.; Burke, L.M. Defining Training and Performance Caliber: A Participant Classification Framework. *Int. J. Sports Physiol. Perform.* **2022**, *17*, 317–331. [CrossRef]
17. Głównka, N.; Malik, J.; Podgórski, T.; Stemplewski, R.; Maciaszek, J.; Ciałczyńska, J.; Zawieja, E.E.; Chmurzynska, A.; Nowaczyk, P.M.; Durkalec-Michalski, K. The Dose-Dependent Effect of Caffeine Supplementation on Performance, Reaction Time and Postural Stability in CrossFit—A Randomized Placebo-Controlled Crossover Trial. *J. Int. Soc. Sports Nutr.* **2024**, *21*, 2301384. [CrossRef]
18. Durkalec-Michalski, K.; Kamińska, J.; Saunders, B.; Pokrywka, A.; Łoniewski, I.; Steffl, M.; Podgórski, T. Does Sodium Bicarbonate Based Extra-Cellular Buffering Support Reduce High Intensity Exercise-Induced Fatigue and Enhance Short-Term Recovery Assessed by Selected Blood Biochemical Indices? *Biol. Sport* **2024**, *41*, 17–27. [CrossRef]
19. Durkalec-Michalski, K.; Nowaczyk, P.M.; Siedzik, K. Effect of a Four-Week Ketogenic Diet on Exercise Metabolism in CrossFit-Trained Athletes. *J. Int. Soc. Sports Nutr.* **2019**, *16*, 16. [CrossRef]
20. Hróbjartsson, A.; Boutron, I. Blinding in Randomized Clinical Trials: Imposed Impartiality. *Clin. Pharmacol. Ther.* **2011**, *90*, 732–736. [CrossRef]
21. Durkalec-Michalski, K.; Zawieja, E.E.; Zawieja, B.E.; Podgórski, T. Evaluation of the Repeatability and Reliability of the Cross-Training Specific Fight Gone Bad Workout and Its Relation to Aerobic Fitness. *Sci. Rep.* **2021**, *11*, 7263. [CrossRef] [PubMed]
22. FINA Rules | Fina.Org—Official FINA Website. Available online: <http://www.fina.org/content/fina-rules> (accessed on 29 November 2020).
23. Competition Regulations. Available online: <https://www.worldaquatics.com/rules/competition-regulations> (accessed on 11 September 2024).
24. Sportowy Trening Plywacki—Maciej Rakowski. Available online: <https://ebooks.com.pl/sportowy-trening-plywacki-maciej-rakowski.html> (accessed on 14 November 2020).
25. Swimming Even Faster by Maglischo, Ernest W. Available online: <https://www.biblio.com/swimming-even-faster-by-maglischo-ernest-w/work/383295> (accessed on 29 November 2020).
26. Maughan, R.J. A Simple, Rapid Method for the Determination of Glucose, Lactate, Pyruvate, Alanine, 3-Hydroxybutyrate and Acetoacetate on a Single 20-Mul Blood Sample. *Clin. Chim. Acta* **1982**, *122*, 231–240. [CrossRef]
27. Durkalec-Michalski, K.; Zawieja, E.E.; Podgórski, T.; Łoniewski, I.; Zawieja, B.E.; Warzybok, M.; Jeszka, J. The Effect of Chronic Progressive-Dose Sodium Bicarbonate Ingestion on CrossFit-like Performance: A Double-Blind, Randomized Cross-over Trial. *PLoS ONE* **2018**, *13*, e0197480. [CrossRef] [PubMed]
28. Blanca, M.J.; Alarcón, R.; Arnau, J. Non-Normal Data: Is ANOVA Still a Valid Option? *Psicothema* **2017**, *29*, 552–557. [CrossRef]
29. Crooks, C.; Cross, M.L.; Wall, C.; Ali, A. Effect of Bovine Colostrum Supplementation on Respiratory Tract Mucosal Defenses in Swimmers. *Int. J. Sport Nutr. Exerc. Metab.* **2010**, *20*, 224–235. [CrossRef]

30. Shing, C.M.; Hunter, D.; Stevenson, L. Bovine Colostrum Supplementation and Exercise Performance: Potential Mechanisms. *Sports Med.* **2009**, *39*, 1033–1054. [[CrossRef](#)]
31. Achten, J.; Jeukendrup, A.E. Heart Rate Monitoring. *Sports Med.* **2003**, *33*, 517–538. [[CrossRef](#)] [[PubMed](#)]
32. Buckley, J.D.; Butler, R.N.; Southcott, E.; Brinkworth, G.D. Bovine Colostrum Supplementation during Running Training Increases Intestinal Permeability. *Nutrients* **2009**, *1*, 224–234. [[CrossRef](#)]
33. McKenna, Z.; Berkemeier, Q.; Gorini, F.; Kuennen, M.; Naylor, A.; Kleint, A.; Gillum, T. Effects of Exercise in Hot and Humid Conditions and Bovine Colostrum on Salivary Immune Markers. *J. Therm. Biol.* **2020**, *93*, 102717. [[CrossRef](#)]
34. Davison, G.; Diment, B.C. Bovine Colostrum Supplementation Attenuates the Decrease of Salivary Lysozyme and Enhances the Recovery of Neutrophil Function after Prolonged Exercise. *Br. J. Nutr.* **2010**, *103*, 1425–1432. [[CrossRef](#)]
35. Jones, A.W.; Thatcher, R.; March, D.S.; Davison, G. Influence of 4 Weeks of Bovine Colostrum Supplementation on Neutrophil and Mucosal Immune Responses to Prolonged Cycling. *Scand. J. Med. Sci. Sports* **2015**, *25*, 788–796. [[CrossRef](#)]
36. Cieślicka, M.; Ostapiuk-Karolczuk, J.; Buttar, H.S.; Dziewiecka, H.; Kasperska, A.; Skarpańska-Stejnborn, A. Effects of Long-Term Supplementation of Bovine Colostrum on Iron Homeostasis, Oxidative Stress, and Inflammation in Female Athletes: A Placebo-Controlled Clinical Trial. *Nutrients* **2022**, *15*, 186. [[CrossRef](#)] [[PubMed](#)]
37. March, D.S.; Jones, A.W.; Thatcher, R.; Davison, G. The Effect of Bovine Colostrum Supplementation on Intestinal Injury and Circulating Intestinal Bacterial DNA Following Exercise in the Heat. *Eur. J. Nutr.* **2019**, *58*, 1441–1451. [[CrossRef](#)] [[PubMed](#)]
38. Brooks, G.A. The Science and Translation of Lactate Shuttle Theory. *Cell Metab.* **2018**, *27*, 757–785. [[CrossRef](#)]
39. Brinkworth, G.; Buckley, J.; Bourdon, P.; Gulbin, J.; David, A. Oral Bovine Colostrum Supplementation Enhances Buffer Capacity but Not Rowing Performance in Elite Female Rowers. *Int. J. Sport Nutr. Exerc. Metab.* **2002**, *12*, 349–365. [[CrossRef](#)] [[PubMed](#)]
40. Marchbank, T.; Davison, G.; Oakes, J.R.; Ghatei, M.A.; Patterson, M.; Moyer, M.P.; Playford, R.J. The Nutraceutical Bovine Colostrum Truncates the Increase in Gut Permeability Caused by Heavy Exercise in Athletes. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2011**, *300*, G477–G484. [[CrossRef](#)]
41. Jones, A.W.; March, D.S.; Thatcher, R.; Diment, B.; Walsh, N.P.; Davison, G. The Effects of Bovine Colostrum Supplementation on in Vivo Immunity Following Prolonged Exercise: A Randomised Controlled Trial. *Eur. J. Nutr.* **2019**, *58*, 335–344. [[CrossRef](#)]
42. Shing, C.M.; Peake, J.M.; Suzuki, K.; Jenkins, D.G.; Coombes, J.S. Bovine Colostrum Modulates Cytokine Production in Human Peripheral Blood Mononuclear Cells Stimulated with Lipopolysaccharide and Phytohemagglutinin. *J. Interferon Cytokine Res.* **2009**, *29*, 37–44. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.