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Influence of including *Arthrospira platensis* as a functional ingredient in dairy cow feed☆

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ABSTRACT

The sustainability of dairy production faces challenges due to environmental concerns and the high cost of conventional feed ingredients. *Arthrospira platensis*, a protein-rich cyanobacteria with bioactive compounds, has been proposed as a functional feed ingredient to enhance milk quality while supporting sustainable livestock practices. This study evaluates the effects of Arthrospira supplementation on milk production, nutritional composition, physicochemical properties, and animal health indicators, alongside its economic feasibility. A 35-day feeding trial was conducted on 12 Holstein dairy cows, each having a live body weight of 510–532 kg and 2–4 parities. They were divided into a control group and an Arthrospira-supplemented group (250 g/day, 1 % *w*/w of the feed ration). Milk production was monitored daily while milk composition (protein, fat, lactose, and solids-not-fat), physicochemical properties (cryoscopic point and electrical conductivity), bacterial content, somatic cell count (SCC), and milk urea nitrogen (MUN) were analysed weekly. Economic viability was assessed based on feed cost variations with the different Arthrospira levels included.

The results showed an initial reduction in milk yield due to feed rejection, which stabilized over time. Arthrospira supplementation did not significantly alter key milk components, but bacterial counts decreased at the beginning of the trial, suggesting antimicrobial benefits. The SCC and MUN remained within normal ranges, indicating no adverse effects on udder health or nitrogen metabolism. However, the economic analysis revealed a feed cost increase from €0.30/kg to €0.55/kg when 1 %*w*/w Arthrospira was included, highlighting the need for cost reduction strategies. This preliminary study suggests that *Arthrospira platensis* can serve as a sustainable functional feed ingredient, helping to maintain milk quality while offering antimicrobial benefits - though economic constraints limit large-scale adoption at present.

1. Introduction

The rapid growth of the global population presents significant challenges to food production systems as sustainable solutions are required to meet the increasing demand for high-quality nutrition. According to the United Nations, the world population reached 8.0 billion in mid-November 2022 and is projected to reach 8.5 billion by 2030 [1,2]. This demographic expansion places greater pressure on agricultural systems, particularly the dairy industry, which provides a crucial source of protein, essential micronutrients, and bioactive compounds for human health. However, the intensification of dairy production has raised concerns about its environmental impact, including greenhouse gas emissions, land degradation, water consumption, and feed resource sustainability [3].

Dairy farming significantly contributes to methane emissions, primarily through enteric fermentation and manure decomposition. According to Climate TRACE, in 2022, cattle-related activities directly accounted for approximately 4.5 % of global anthropogenic greenhouse gas emissions [4]. Additionally, the significant water footprint of dairy production, which includes feed crop irrigation, exacerbates concerns about water scarcity in many regions. The industry's reliance on conventional feed ingredients, such as soybean meal and cereal grains, further contributes to environmental degradation due to deforestation, biodiversity loss, and the intensive use of chemical fertilizers. Sustainable strategies are urgently needed to enhance feed efficiency, reduce environmental impact, and maintain milk quality to support the

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growing global demand for dairy products.

The biomass of the cyanobacteria *Arthrospira platensis*, commonly referred to as Arthrospira, and associated with the microalgae group, is a promising protein source in the diets of dairy cows [5,6]. Integrating Arthrospira as a feed ingredient is a potential approach for enhancing the sustainability of dairy production. Arthrospira is a high-protein, nutrient-dense cyanobacteria that serves as an alternative to conventional feed sources, requiring significantly less land and water for cultivation. Especially important is that it can be grown on non-arable land using non-potable water, including brackish water or seawater, thereby minimizing competition with human food crops [7]. Additionally, Arthrospira contains bioactive compounds such as phycocyanin, carotenoids (such as β-carotene), polyphenols, γ-linolenic acid, and sulfated polysaccharides, with antimicrobial and immunostimulatory properties, which may reduce the need for antibiotics, improve bovine health, and enhance milk safety [1]. Its potential for supporting circular economy principles and promoting low-carbon livestock farming positions *Arthrospira platensis* as a viable solution for reducing the environmental footprint of dairy production while maintaining or enhancing milk yield and quality [8]. To the best of our knowledge, only a few studies have evaluated Arthrospira as an ingredient in dairy cow feed.

Although microalgae might be able to replace conventional feed ingredients entirely, this approach is currently limited due to the high production costs of microalgal biomass. Production costs of microalgal biomass are highly variable, typically ranging from 10 to 25 €/kg, depending on production scale, system configuration, and downstream processing efficiency [9]. In contrast, traditional animal feed costs are significantly lower, making full replacement economically unfeasible at present. Consequently, a more viable approach is to incorporate microalgae as a functional feed ingredient at low dietary levels to improve feed quality and enhance livestock performance [8]. Previous research has shown that, even at low inclusion levels, microalgae can significantly enhance the nutritional value of animal diets, leading to improved growth rates, better immune responses, and enhanced product quality in terms of meat, milk, and eggs [1,10].

The inclusion of microalgae as a functional feed ingredient offers several advantages beyond basic nutrition. Microalgae accumulate bioactive compounds such as carotenoids, phycobiliproteins, and polyphenols, which exhibit antimicrobial, anti-inflammatory, antioxidant, and immunostimulatory properties [11,12]. These bioactive components contribute to the overall health and performance of livestock by reducing disease incidence, improving feed conversion efficiency, and enhancing the oxidative stability of meat and dairy products [8]. Furthermore, microalgae can improve the fatty acid profile of animal-derived products by increasing their omega-3 fatty acids content, particularly docosahexaenoic acid (DHA), which is essential for human health [9].

The environmental sustainability of microalgae production further enhances their application as a feed additive. Unlike conventional crops, which require extensive land areas, microalgae can be cultivated on non-arable land and can even be grown in a wastewater-containing medium [13]. Despite their numerous advantages, the widespread adoption of microalgae-based feed additives still faces economic and technical hurdles. The high production cost of microalgal biomass remains a primary barrier, limiting its use to premium livestock and aquaculture markets where nutritional quality is a priority [10]. Advances in large-scale cultivation, harvesting, and processing technologies are essential to reduce production costs and enhance the economic feasibility of microalgae as a mainstream feed ingredient [9].

Given the above-mentioned challenges and opportunities, this study aims to analyze the effect of dietary supplementation with *Arthrospira platensis* on milk properties in a case study conducted at a commercial Holstein dairy cow farm located in Los Pedroches, IFAPA Hinojosa-Córdoba, Spain. By evaluating the impact of *Arthrospira*-based feed on milk composition, this research aims to provide valuable insights into the potential benefits of integrating microalgae into livestock diets as a way to improve both nutritional quality and environmental sustainability. Among various microalgal species, Spirulina (*Arthrospira platensis*) was selected for this study due to its exceptional practical relevance in livestock nutrition. It is currently the most widely cultivated microalga globally, with well-established production systems operating at industrial scale in numerous countries. This widespread production not only ensures a stable and high-volume supply but also translates to lower market costs compared to many other microalgae, whose use remains largely constrained to laboratory or pilot scales.

2. Materials and methods

2.1. Microorganisms and culture conditions

The strain used in this study was obtained from the culture collection of the Department of Chemical Engineering at the University of Almería. Inoculum of *Arthrospira platensis* was maintained at 23 ± 2 °C, pH 10.0 ± 0.1, and 150 μE/m^2·s in batch mode using a modified Arnon medium until a concentration of 1 g/L was achieved. Once the desired concentration was reached, the inoculum was scaled up to a final volume of 12 m^3 under controlled conditions - at pH 10.0 and at a temperature ranging from 18 to 22 °C using freshwater and Mann & Myers medium prepared with fertilizers (0.14 g/L $K(PO_4)_2$, 0.18 g/L $Mg(SO_4)_2$, 0.9 g/L $NaNO_3$) (all of them commercial fetilizers grade from YARA) and 0.03 g/L of Karentol® (Kenogard, Spain), the latter being a commercial solid mixture of micronutrients that includes boron, copper, iron, manganese, molybdenum, and zinc. In addition, $NaHCO_3$ was provided as 8 g/L $NaHCO_3$.

2.2. Raceway reactor design and operating conditions

Microalgae biomass cultivation was carried out using three identical raceway reactors, each operating continuously 24 h a day inside a greenhouse facility. These reactors were part of the pilot-scale production plant at the University of Almería, located at IFAPA, Almería, Spain. Each reactor had a working volume of 12 m^3 and occupied a surface area of 80 m^2. They consisted of two 40-m-long channels, each 1 m wide and 0.40 m high, connected by 180° bends at both ends. A 0.59 m^3 sump (0.65 m long × 0.90 m wide × 1.0 m deep) was positioned 1 m along one of the channels to facilitate circulation and mixing. This design ensured an optimal environment for microalgae growth by providing uniform exposure to light and nutrients while preventing culture sedimentation.

To maintain ideal growth conditions, a programmable logic controller (PLC) system was used, integrating pH, temperature, and dissolved oxygen (DO) probes (5083 T and 5120, Crison, Spain) with a control-transmitter unit (MM44, Crison Instruments, Spain) and LabVIEW data acquisition software (National Instruments, USA). Gas flow rates were precisely regulated using a mass flow meter (PFM 725S-F01-F, SMC, Tokyo, Japan). The pH was kept at 10 via an event-based CO_2 injection system, ensuring adequate carbon supply for microalgae metabolism. Aeration was continuously provided by a blower operating at a 350-mbar overpressure, connected to fine bubble diffusers (AFT2100, ECOTEC, Spain). Air was injected regardless of CO_2 demand to maintain proper circulation and prevent oxygen excess in the culture. Temperature regulation was passive, fluctuating by approximately ±5 °C from the daily mean air temperature, which ranged from 12 °C in winter to 28 °C in summer.

The reactors were initially operated in batch mode for one week, allowing the culture to stabilize before transitioning to semi-continuous mode at a dilution rate of 0.2 day^{-1}. A culture depth of 0.15 m was maintained to optimize light penetration and nutrient uptake. Since evaporation affects the culture volume, daily additions of fresh water were made to compensate for water loss, ensuring stable growth conditions. This operational strategy, combining automated control, efficient gas exchange, and precise nutrient management, enabled a consistent and productive microalgae cultivation system suitable for large-scale applications.

The harvesting of Arthrospira biomass was carried out daily using a vibro-sieving system with a 1.20-m diameter sieve equipped with a 40-μm mesh (Mainca, Spain). The culture was processed at a flow rate of 1 m^3/h, allowing for efficient separation of the biomass from the cultivation medium. This mechanical filtration step enabled the recovery of concentrated sludge while maintaining the integrity of the Arthrospira cells. The sieving process ensured that only the microalgal biomass was retained, while the remaining culture medium, containing fine particulate matter and dissolved nutrients, was further treated to enhance resource recovery and sustainability in the production process.

Following the harvesting process, the concentrated sludge, containing 12 % *w*/w solids, was freeze-dried to preserve the biochemical composition and biomass quality (Cuddon FD100LT Freeze Dryer, New Zealand). The supernatant obtained from the vibro-sieving process was subjected to ultrafiltration using a Pulsion KOCH membrane with a pore size of 0.02 μm (Koch, United States). This filtration system operated at flow rates of 30 L/m^2·h and pressures below 0.5 bar, ensuring efficient removal of any remaining microalgal fragments, bacteria, and suspended particles. A total of 500 kg biomass was produced for later use in the feeding trial. The dry biomass required for the feeding trial was produced over a period of 60 days during late summer (from August to September), under optimal outdoor cultivation conditions in southern Spain. Table 1 shows the chemical composition of the algal biomass.

2.3. Experimental design – cows, diets, feeding and management

Ethical approval. The welfare and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes. To evaluate the impact of *Arthrospira platensis* supplementation on milk production and quality, a controlled feeding experiment was conducted using a total of 12 lactating Holstein cows, each with a live body weight (LBW) of 510–532 kg and with 2–4 parities (4–7 years). The cows were housed at the IFAPA Research Centre of the Andalusian Government (Hinojosa del Duque, Cordoba, Spain, 38°N), and were randomly assigned to one of two groups balanced for live weight and age: a control group ($n = 6$), which received a standard diet without supplementation (a total mixed ration consisting of 74 % hay, 13 % sugar beet pulp, 6 % soybean meal, 4 % wheat flakes and 3 % molasses, on a DM basis) and a treatment group (n = 6), which was fed the standard diet with an additional 250 g of *Arthrospira platensis* per day. The experimental period comprised an adaptation period (thirty days) and a sampling period (35 d), from September to November. During this period, average daytime temperatures ranged from 28 °C in early September to around 15 °C in November, with increasing rainfall and humidity from October onward. These agro-climatic conditions provided a favorable environment for the trial, minimizing heat stress, enhancing animal comfort, and supporting stable digestion and feed intake. A 30-day adaptation period was implemented prior to the trial, during which animals were acclimated to the housing conditions and fed the basal diet without Spirulina supplementation. The supplementation period began immediately afterward and lasted for 35 consecutive days, ensuring that any changes in milk composition could be attributed to the dietary inclusion of *Arthrospira platensis*. Before mixing the complete diet once per day, the sugar beet pulp pellets were soaked in water. The soybean meal and the wheat flakes were mixed with the soaked sugar beet pulp, added to the hay and molasses in a mixing truck and homogenised to avoid feed selection. In the Arthrospira diet, the algal powder was previously mixed with water (1:10) in a unifeed mixer.

Table 1
Chemical composition of the algal biomass (determined using standard analytical methods commonly applied in microalgae biomass characterisation [14]).

	Component content (%dry weight)
Protein	55.81 ± 0.82
Carbohydrates	23.61 ± 0.81
Lipids	7.61 ± 0.92
Ash (minerals)	8.63 ± 0.53
Moisture	3.52 ± 0.52
Dietary fibre	8.22 ± 0.92
Pigments (Chlorophylls+carotenoids)	1.53 ± 0.21

Milking took place at 08:00 and 17:00 h in a Westfalia tandem milking parlour. During the sampling period, samples were collected at each milking and pooled per day according to milk yield. To monitor the biochemical and physical properties of the milk, weekly milk samples (60 mL each) were collected from each cow and stored at −20 °C without preservatives for subsequent laboratory analysis. Duplicate samples were processed to ensure the reliability of the data. The measured parameters included milk production (L/day), fat content (%, *w*/w Milkoscan/ftir), protein content (%, w/w Milkoscan/ftir), somatic cell count (×1000 cells/mL Fossomatic), bacterial count (CFU/mL Bactoscan FC), electrical conductivity (volts), non-fat dry matter (%, *w*/w), urea concentration (mg/L Milkoscan/ftir), and the cryoscopic point (°C Milkoscan/ftir), which provides insight into the milk's freezing characteristics and possible adulteration. All sample analyses were carried out at the Centro de Investigación y Calidad Agroalimentaria del Valle de los Pedroches (CICAP), a specialized research and quality control facility located in Pozoblanco, Spain. The standardized laboratory procedures followed at the CICAP Laboratory ensured precise and reproducible measurements of milk composition and quality, allowing for a comprehensive assessment of how the Arthrospira supplementation influenced dairy performance and milk nutritional properties.

2.4. Statistics

Unless otherwise specified, all reported data represent the mean values derived from three independent measurements, ensuring the reproducibility and statistical robustness of the findings. Data are presented as mean ± standard deviation (SD) to provide a clear representation of variability across replicates. To assess differences on the results statistical analyses were performed using analysis of variance (ANOVA) in JMP 13 software (SAS Institute Inc., USA). This approach allowed for the identification of statistically significant differences in experimental outcomes. Following the ANOVA, a Tukey pairwise comparison test was conducted to determine specific differences between experimental groups, ensuring a comprehensive evaluation of variability within and between treatments. In all cases, a significance threshold of $p < 0.05$ was applied, indicating that differences with a less than 5 % probability of occurring by chance were considered statistically significant.

Additionally, to explore potential relationships between different experimental variables, a bivariate Pearson's correlation analysis was performed. This analysis allowed one to quantify the strength and direction of associations between variables, providing insights into potential dependencies and interactions within the experimental dataset.

3. Results and discussion

This section presents the outcomes of supplementing dairy cow feed with Arthrospira platensis as a sustainable alternative to conventional inputs. Key physicochemical and nutritional parameters of milk were evaluated to assess the impact of Spirulina inclusion on production performance and quality. The findings provide insights into the potential of microalgae-based feed strategies to enhance dairy efficiency while contributing to environmental sustainability in livestock systems.

3.1. Milk production

Fig. 1 shows the evolution of milk production over the 35-day experimental period. At the beginning of the trial, cows supplemented with Arthrospira exhibited a reduction in milk yield, which can be

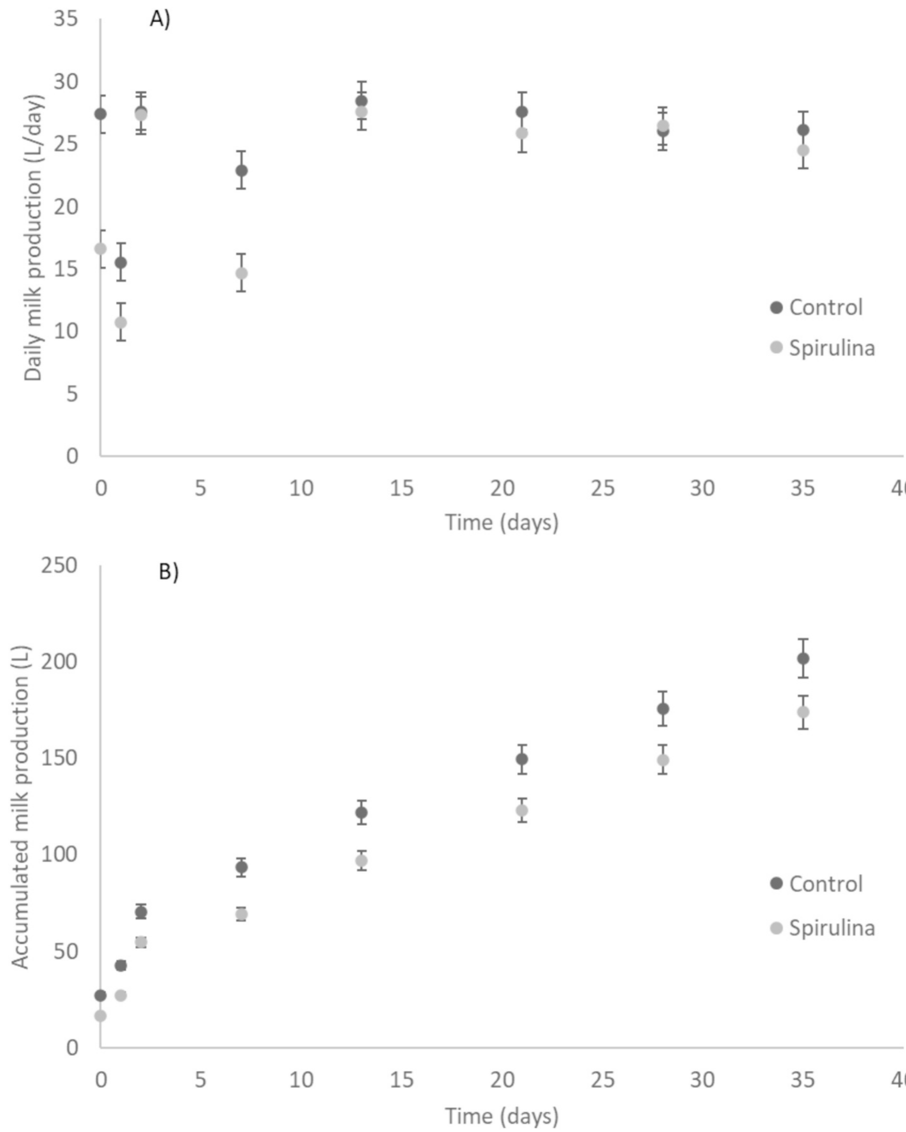

Fig. 1. Analysis of milk production over 35 days of feeding Holstein cows with the control and Arthrospira-supplemented feeds. (A) Daily production for each dietary group, (B) Accumulated milk production during the feeding experiment.

attributed to the initial rejection of the feed due to the characteristic cyanobacterium odour. This initial decrease led to a milk production range between 10.74 and 15.54 L/day in the Arthrospira group, compared to 15.54 to 28.47 L/day in the control group. Lamminen [15] also observed a reduction in DM intake by up to 3.5 % in cows fed with 2.6 %*w*/w Arthrospira included in grass silage-based diets. However, after implementing an improved mixing strategy that blended Arthrospira with cereal, feed acceptance improved, leading to the stabilization of milk production levels. By the end of the experiment, the *Arthrospira*-supplemented group exhibited a recovery in production, reaching values comparable to the control, with no statistically significant differences between treatments ($p > 0.05$).

The mean daily milk production observed in this study aligns with the expected values for dairy cows under similar management and feeding conditions; these typically range from 20 to 30 L/day depending on the breed, diet composition, and lactation stage [16]. These findings suggest that dietary supplementation with *Arthrospira platensis* does not have a detrimental effect on milk yield once the cows have adapted to the new diet. Moreover, the observed fluctuations emphasize the importance of gradual dietary transition when introducing novel feed ingredients to avoid initial feed rejection and potential production losses. Strategies such as including *Arthrospira* biomass in the complete diet to avoid animals selecting or ingesting higher forage proportions might be a convenient strategy for increasing the palatability of microalgae-supplemented diets. Another alternative might be to add liquid molasses, which could enhance the acceptability and improve the palatability of the Arthrospira-supplemented diet, thus resulting in similar total intake levels. Further studies with extended adaptation periods and larger sample sizes could provide deeper insights into the long-term effects of *Arthrospira platensis* supplementation on dairy performance and help ascertain how the algal dosage and feeding system influence feed intake.

3.2. Nutritional value of the milk

Regarding the nutritional value of the milk produced, no variation over time was observed, so only the mean experimental determination values were analysed (Fig. 2). The protein content of the milk from both the control group and the *Arthrospira*-supplemented group averaged between 3.47 % and 3.57 %, in line with typical bovine milk protein levels, which generally range from 3.1 % to 3.8 %, depending on the breed and diet. This consistency suggests that *Arthrospira*

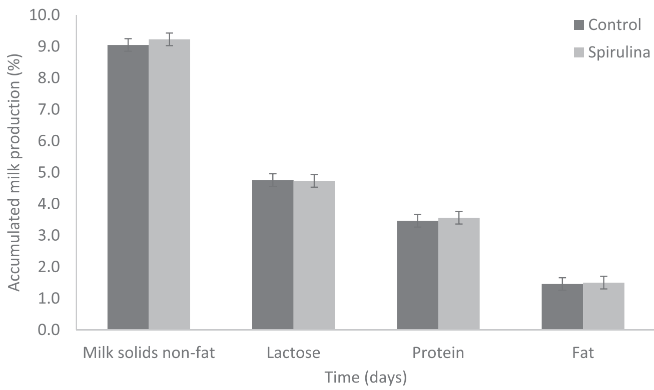

Fig. 2. Analysis of the nutritional quality of the milk produced by Holstein cows fed the control and Arthrospira-supplemented diets. Data correspond to mean values over the entire experiment.

supplementation does not adversely affect the milk protein concentration. In the same way, Manzocchi et al. [6] reported that substituting soybean meal with Arthrospira in the diet of dairy cows did not affect the milk protein content compared to the control group, which received no microalgae. Conversely, some studies have reported increases in the milk protein content with *Arthrospira* supplementation - for instance, a 9.7 % increase in milk protein in cows receiving 2 %*w*/w diets containing *Arthrospira* compared to the controls [17]. Similarly, milk protein levels were enhanced following dietary supplementation levels of 10 g per day per animal of *Arthrospira* [18]. The milk protein concentration increased along with a decreasing milk yield when *Arthrospira* was used to substitute rapeseed meal. These findings align with the well-established observation that milk protein concentration tends to be inversely correlated with milk yield, a relationship that has been confirmed in recent studies using multi-omics approaches to analyze feed efficiency and production traits in ruminants [19]. The above-mentioned discrepancies may result from variations in the *Arthrospira* dosage, diet composition, or differences in experimental conditions. Nonetheless, in the present study, the dose used did not have any influence on this milk parameter.

The fat content in the milk from both groups in our study remained stable, averaging between 1.40 % and 1.43 % (Fig. 2). This is notably lower than the typical fat content in cow's milk, which ranges from 3.7 % in Holstein breeds to 4.9 % in Jersey breeds. Importantly, *Arthrospira* supplementation did not negatively impact the milk fat content. In contrast, some studies have reported increases in milk fat with *Arthrospira* supplementation - an increase in the milk fat content of between 17.6 % and 25.0 % was reported in cows fed diets supplemented with 2 % *Arthrospira* on a dry matter basis, compared to control groups [17]. A similar trend was reported in the milk fat percentages, which increased following *Arthrospira* supplementation [18]. Lamminen [15] also reported increased milk fat concentration when soybean meal was partially replaced with microalgae in dairy cow diets. In their study, *Arthrospira* was included at a rate of 10 % of the concentrate portion of the diet (equivalent to 1.5 % of total dry matter intake). Among the different microalgae tested, *Arthrospira* supplementation resulted in the highest increase in milk fat content, suggesting a specific effect of this strain on lipid metabolism. This supports the potential of this biomass as a functional ingredient capable of enhancing milk fat synthesis when used at nutritionally relevant inclusion levels. The reduced fat percentages observed in the present study could be attributed to factors such as breed differences, lactation stage, or specific dietary formulations. Further studies are required to elucidate how Arthrospira supplementation influences the milk fat content and its fatty acid profile over longer feeding periods.

Changes in the lactose concentration influence milk yield because this disaccharide determines the amount of milk synthesized [20]. Lactose is synthesized in the mammary gland and is dependent on genetic and nutritional factors, among others. The main metabolic substrate for lactose synthesis is propionic acid, which is produced in the rumen during the fermentation process. The lactose levels in the milk from both the control and Arthrospira-supplemented groups in our study were consistent, ranging from 4.76 % to 4.80 % (Fig. 2). These values are within the standard lactose content range for cow's milk, which is typically between 4.6 % and 4.8 %. This finding indicates that the factors responsible for the decreased fat synthesis observed in both dietary groups appear to be dissociated from lactose synthesis. The stable lactose concentrations suggest that Arthrospira supplementation does not influence the carbohydrate composition of the milk. This aligns with previous studies where Arthrospira inclusion in the diet did not affect the milk lactose content. For example, no noticeable differences were reported in the milk lactose content when substituting soybean meal with Arthrospira in dairy cow diets [6]. However, an 11.7 % increase in the milk lactose content was reported with Arthrospira supplementation [17], indicating that responses may vary depending on specific dietary and management conditions.

The solids-not-fat (SNF) content, encompassing proteins, lactose, and minerals, showed no observable differences between the control and Arthrospira-supplemented groups in our study, with values ranging from 9.05 % to 9.18 % (Fig. 2). These figures are consistent with typical SNF levels in cow's milk, which are approximately 9 %. The maintaining of standard SNF levels indicates that Arthrospira supplementation does not alter the overall solid composition of the milk. This accords with studies demonstrating that Arthrospira inclusion does not adversely affect the nutritional quality of milk. For instance, no clear trends in the milk's proximate composition, including SNF, were observed when Arthrospira replaced soybean meal in the diet [6]. However, variations in SNF content have been reported in other studies, suggesting that factors such as Arthrospira dosage, diet composition, and animal health status may influence outcomes.

In summary, this study indicates that the dietary supplementation of dairy cow feed with *Arthrospira platensis* does not significantly impact the protein, fat, lactose, or solids-not-fat content of the milk. The literature suggests that incorporating microalgae into animal feed can enhance the fatty acid profile of milk and other animal-derived products due to their high content of beneficial lipids, such as polyunsaturated fatty acids (PUFAs) [8]. However, in this study, the fatty acid composition was not analysed, and so could be a point to consider in future research. These findings suggest that Arthrospira can be incorporated into dairy rations without having detrimental effects on the milk composition, thus offering a sustainable alternative to conventional feed ingredients. However, variations in responses observed in other studies highlight the need for further research to elucidate the factors influencing the effects of Arthrospira supplementation on milk composition.

3.3. Physicochemical properties of the milk

The cryoscopic point (freezing point) and electrical conductivity of the milk were evaluated to assess the impact of microalgae supplementation on these parameters (Table 2). The cryoscopic point remained constant at −0.53 ± 0.01 °C for both the control and Arthrospira-supplemented groups, indicating that Arthrospira addition does not alter the water balance or solute concentration of the milk as evidenced by the above-mentioned results. This stability is significant, as the freezing point of milk is a critical indicator of its purity and quality, with standard values typically around −0.53 °C. Deviations from this value

Table 2
Physicochemical quality of milk production.

	Control group	Arthrospira group
Cryoscopic point (°C)	−0.53 ± 0.01	−0.53 ± 0.01
Conductivity (mS/cm)	471 ± 84	423 ± 157

can suggest adulteration or contamination.

Electrical conductivity (EC) is another important parameter, reflecting the ionic content of the milk, primarily due to its soluble salt fraction, while components such as lactose do not conduct current, and fat decreases conductivity (Table 1). A slight reduction in EC was observed in the Arthrospira group (423.05 ± 156.59 μS/cm) compared to the control group (470.92 ± 84.71 μS/cm). This decrease may be attributed to modifications in the milk's ionic composition, influenced by the bioactive compounds and minerals present in Arthrospira. Maintaining appropriate electrical conductivity (EC) levels is crucial, as significant deviations can signal mastitis or other health concerns in dairy cows [21].

The inclusion of microalgae into dairy cow diets has been shown to influence various milk properties. For instance, supplementation with DHA-enriched microalgae has been reported to affect the melting and crystallization properties of milk fat, indicating alterations in its physical characteristics. Additionally, studies have demonstrated that microalgae supplementation can modify the fatty acid composition of the milk, enhancing its nutritional profile by increasing the content of beneficial fatty acids [22].

3.4. Effect of Arthrospira platensis on animal health indicators

The bacterial content, milk urea concentration, and somatic cell count (SCC) are critical indicators of animal health, as they provide insight into the physiological status, immune response, and metabolic efficiency of dairy cows. The bacterial content reflects both the hygienic quality of the milk and the potential presence of infections, while the milk urea concentration serves as a key marker of nitrogen utilization efficiency and dietary protein balance, with deviations indicating metabolic imbalances or inefficient feed conversion. SCC is a well-established indicator of udder health, with elevated levels signalling inflammatory responses such as mastitis, which can negatively impact milk yield and quality. For these reasons, the variation in these parameters was studied over time.

The analysis of the milk's bacterial content over the 35-day experiment revealed distinct trends between the control and Arthrospira-supplemented groups (Fig. 3A). In the control group, bacterial counts exhibited an initial increase, rising from 1.0×10^3 CFU/mL on day 1 to a peak of 5.0×10^3 CFU/mL by day 10, before gradually declining. This early proliferation may be attributed to natural bacterial growth dynamics in fresh milk, influenced by environmental factors and animal health conditions. In contrast, the Arthrospira group displayed the opposite trend, with bacterial counts decreasing from 5.1×10^3 CFU/mL on day 1 to 2.3×10^3 CFU/mL by day 10 and then stabilizing at approximately 1.2×10^3 CFU/mL by the end of the trial. This reduction in the bacterial population from the outset in Arthrospira-supplemented milk supports the hypothesis that *Arthrospira platensis* exhibits antimicrobial properties. Previous research has demonstrated that Arthrospira contains bioactive compounds such as phycocyanin, phenolics, and polysaccharides, which exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria [23]. These compounds are known to disrupt bacterial cell membranes and inhibit bacterial enzyme activity, thereby suppressing microbial growth. The consistent decline in bacterial counts in the Arthrospira group aligns with findings from previous studies that have reported similar antibacterial effects after dietary supplementation with microalgae biomass [24,25]. Furthermore, research on Arthrospira-based functional feed ingredients has shown that their antimicrobial effects extend beyond milk quality, contributing to enhanced gut health and reduced pathogen loads in livestock [26]. In ruminants, Arthrospira supplementation has been linked to improved immune responses, which may further contribute to the observed reduction in bacterial content. This suggests that Arthrospira not only acts directly on bacterial populations but also strengthens the host's ability to resist microbial proliferation.

Regarding the somatic cell count (SCC), this exhibited considerable variability over the trial period, with no clear trend differentiating the control and Arthrospira groups (Fig. 3B). Initially, the SCC was slightly higher in the Arthrospira-supplemented cows (230,000 cells/mL) compared to the control group (200,000 cells/mL). However, by the third week, the SCC levels had stabilized in both groups, fluctuating in a range between 180,000 to 220,000 cells/mL. The final SCC values remained within acceptable thresholds for high-quality milk (<300,000 cells/mL), confirming that Arthrospira supplementation did not negatively impact udder health. The variability observed in the SCC over time is not uncommon in dairy studies, as factors such as the lactation stage, seasonal variations, and farm management can all influence SCC dynamics [27]. The lack of a significant increase in the SCC in Arthrospira-treated cows suggests that the supplement did not induce inflammation or predispose the cows to mastitis. In the same way, Manzocchi et al. [6] did not find any change in SCC after feeding two dairy cows a diet including 5 % Arthrospira for 33 days. The results obtained in the present study align with previous research indicating that microalgae supplementation does not compromise udder health and, in some cases, may enhance immune responses due to the microalgae's high content of bioactive compounds. For instance, one study reported a 29.1 % reduction in SCC in cows supplemented with Arthrospira, highlighting its potential to improve milk quality by reducing subclinical mastitis [17]. Additionally, microalgae are rich in bioactive compounds that can enhance the immune system, potentially reducing antibiotic dependence in livestock farming. These bioactive compounds (carotenoids, tocopherols, and polyphenols, among others) may contribute to improved udder health and reduced SCC in dairy cows [28].

Finally, the milk urea concentration values remained relatively stable throughout the experiment, with mean values of 28.5 ± 2.3 mg/dL in the control group and 27.8 ± 2.1 mg/dL in the Arthrospira group (Fig. 3C). However, a slight fluctuation was observed during the first two weeks, where urea levels in the Arthrospira-treated cows momentarily decreased to 24.6 mg/dL before returning to baseline levels. This initial drop may be related to dietary adaptation, as previous studies have suggested that the introduction of microalgae-based feed can temporarily alter nitrogen metabolism before stabilization occurs [29]. The urea concentration in milk indicates the ruminal fermentation process and the nutritional status. The maintenance of optimal urea levels over time suggests that Arthrospira supplementation did not disrupt the protein-energy balance of the cows. This is particularly relevant given that the milk urea concentration is a key indicator of dietary nitrogen utilization, and deviations from the normal range (25–30 mg/dL) can signal either protein excess (high) or deficiency (low) [30]. A long-lasting, high concentration of urea in the milk is a signal that continuing inadequate nutrition might lead to decreased productivity and health issues in the dairy cows. Similar trends have been observed in previous trials where microalgae, such as *Schizochytrium* sp. and *Nannochloropsis* sp., were included in ruminant diets, with the milk urea concentration levels being maintained without having negative impacts on metabolic efficiency [15,30,31].

3.5. Economic aspects of including Arthrospira in animal feed

The incorporation of *Arthrospira platensis* as a functional ingredient in dairy cow feed presents a trade-off between its high nutritional value (high-quality protein with all essential amino acids, bioavailable iron, polyunsaturated fatty acids, vitamins, phycocyanin and carotenoids) and its elevated production costs. The price of microalgae biomass varies widely, from €10 to €300/kg, depending on the production scale and processing method [32]. In contrast, conventional protein sources, such as soybean meal, cost approximately €0.40–€0.50/kg. The *Arthrospira* production cost, estimated to be between €5 and €10/kg under optimized conditions, remains a limiting factor for its widespread adoption in livestock feed [33]. Techno-economic analyses indicate that the cost of microalgae production can be reduced if integrated with CO_2

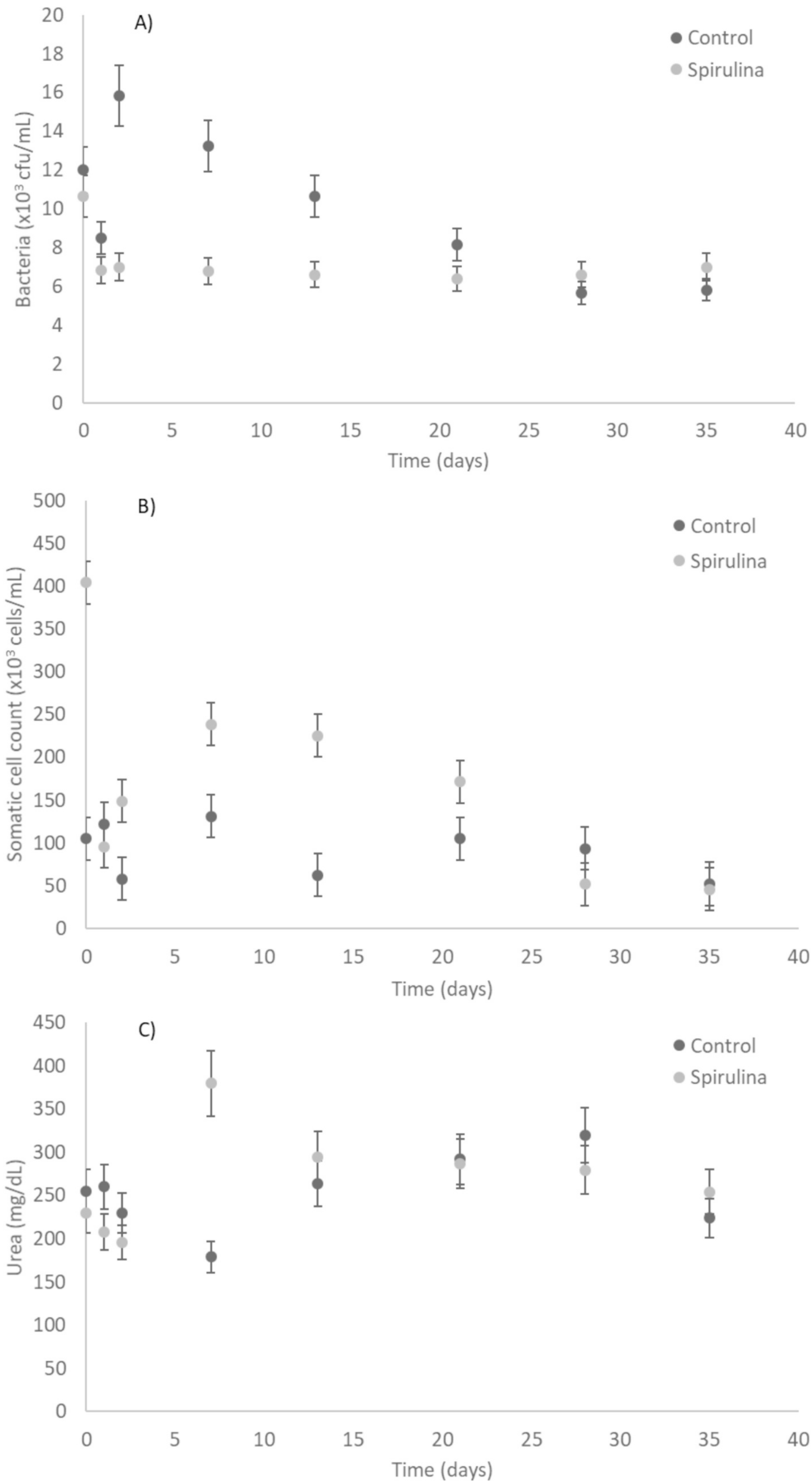

Fig. 3. Variation in animal health indicators over time as a function of the dietary treatment. (A) Bacteria content (B) Somatic cell count, and (C) Urea concentration.

biofixation strategies or if alternative drying technologies, such as solar thermal drying, are utilized, thus reducing drying costs from €2.37/kg (spray drying) to €1.16/kg [34].

Despite its cost, *Arthrospira* supplementation in dairy cow diets can yield economic advantages by enhancing milk composition and improving herd health. Studies indicate that *Arthrospira* can improve milk protein and lipid profiles, potentially leading to premium pricing for dairy products [17,23]. Additionally, Arthrospira's bioactive compounds exhibit antimicrobial properties, contributing to lower veterinary costs associated with mastitis and other infections [14,18]. Economic models suggest that including *Arthrospira* at 1 % inclusion rates increases feed costs from €0.35/kg to €0.55/kg, representing a 16.7 % to 83 % increase compared to standard feed costs of €0.30/kg. However, its potential for reducing healthcare costs and enhancing milk yield efficiency may offset these expenses.

To improve the feasibility of *Arthrospira platensis* as a feed ingredient, further research should focus on cost-reduction strategies, such as integrating *Arthrospira* production with existing agricultural waste streams and optimizing large-scale cultivation systems. The economic viability of *Arthrospira* in dairy production will depend on balancing feed costs with potential benefits in milk quality, animal health, and environmental sustainability, detailed studies in this respect being necessary. Future studies should explore financial incentives, such as carbon credits from CO_2 biofixation, and regulatory policies that support sustainable feed alternatives.

4. Conclusions

This study demonstrates that supplementing dairy cow feed with *Arthrospira platensis* does not negatively impact milk production or its nutritional composition. Although an initial decrease in milk yield was observed due to feed adaptation, production levels stabilized, reaching values comparable to the control group. The inclusion of Arthrospira did not significantly alter key nutritional parameters such as the protein, fat, lactose, or solids-not-fat contents, suggesting that it can be integrated into dairy rations without compromising milk quality. Furthermore, the antimicrobial properties of Arthrospira contributed to a reduction in the milk's bacterial content at the beginning of the trial, unlike in the control group, where bacterial counts initially increased. Additionally, the milk urea concentration and somatic cell count remained within normal ranges, indicating that Arthrospira did not induce metabolic imbalances or inflammatory responses, reinforcing its potential as a functional feed additive.

From an economic perspective, the inclusion of Arthrospira in dairy cow diets presents a trade-off between higher feed costs and potential health and productivity benefits. Although the cost of Arthrospira biomass remains significantly higher than conventional feed ingredients, its potential for enhancing milk quality, reducing veterinary costs, and improving sustainability may justify its inclusion, particularly in high-value dairy markets. Techno-economic analyses suggest that cost reduction strategies, such as integrating Arthrospira production with CO_2 biofixation and alternative drying technologies, could improve its feasibility as a livestock feed. Further large-scale studies and long-term trials are needed to better understand the economic impact and to optimize supplementation strategies that maximize productivity while maintaining financial viability.

CRediT authorship contribution statement

M. Barcelo-Villalobos: Methodology, Investigation. **F. Arrebola:** Methodology, Investigation. **A. Vizcaino:** Validation, Formal analysis, Data curation. **F.J. Alarcón:** Validation, Data curation. **F.G. Acién:** Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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