Supplementary Materials: Supplementary Table S1. The PRISMA 2020 checklist for reporting systematic review; Supplementary Table S2. Study identification characteristics and sample characteristics; Supplementary Table S3. Analyzed factors; Supplementary Table S4. Determination of melatonin in blood/serum/plasma and the condition under which the samples were collected and stored; Supplementary Table S5. Determination of melatonin in milk and the conditions under which the samples were collected and stored; Supplementary Table S6. Results and observations relevant to melatonin concentrations in blood/serum/plasma; Supplementary Table S7. Results and observations relevant to melatonin concentrations in milk; Supplementary Table S8. Risk of bias assessment.

Supplementary Table S1. The PRISMA 2020 checklist for reporting systematic review.

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Pages 5-6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 5, sub-section 2.2. and 2.3.
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Page 4, sub-section 2.1.
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 4, sub-section 2.1.

Section and Topic	Item #	Checklist item	Location where item is reported
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 6, sub-section 2.4.
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Page 5, sub-section 2.4.; and Supplementary Tables S3-S7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Supplementary tables 2
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Pages 5-6; and Supplementary Tables S8
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Supplementary tables 2
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 5, sub-section 2.3.
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Pages 6-7, sub-section 2.6.
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Pages 6-7, sub-section 2.6.
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Pages 6-7, sub-section 2.6.

Section and Topic	Item #	Checklist item	Location where item is reported
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Pages 6-7, sub-section 2.6.
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Pages 6-7, sub-section 2.6.
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Page 6, sub-section 2.5.
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Page 7, sub-section 2.7.
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Page 8, sub-section 3.1.
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Page 8, sub-section 3.1.
Study characteristics	17	Cite each included study and present its characteristics.	Page 8, sub-section 3.1. - Table 1; and Supplementary Tables S2
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supplementary Tables S8
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Page 8, sub-section 3.1. - Table 1; Supplementary Tables S3-S7

Section and Topic	Item #	Checklist item	Location where item is reported
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Page 8, sub-section 3.1. - Table 1; and Supplementary Table 2
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Pages 13-23, sub-section 3.2.; pages 23-31, sub-section 3.3.; and page 31, sub-section 3.4.
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Pages 8-12, sub-section 3.1., and table 2
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Page 11, sub-section 3.1.
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Pages 8-9, sub-section 3.1.
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Pages 8-9, sub-section 3.1.
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Pages 32-38, section 5; and pages 38-43, section 6
	23b	Discuss any limitations of the evidence included in the review.	Pages 31-32, section 4
	23c	Discuss any limitations of the review processes used.	Pages 31-32, section 4

Section and Topic	Item #	Checklist item	Location where item is reported
	23d	Discuss implications of the results for practice, policy, and future research.	Pages 43-44, section 7
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Pages 7-8, sub-section 2.9.
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Pages 7-8, sub-section 2.9.
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Pages 7-8, sub-section 2.9.
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 45
Competing interests	26	Declare any competing interests of review authors.	Page 45
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Page 45

Supplementary Table S2. Study identification characteristics and sample characteristics.

Criterion number	Authors and year	The country	The journal	Type of study	Number of cows	Breed	Lactation (days)	Growing conditions
1.	Kollmann et al., 2008	Germany	Domestic Animal Endocrinology	Controlled experimental study	12 heifers 12 cows	Brown Swiss	Cows: 154 ± 28	Growing up in a shelter
2.	Milagres et al., 2013	Brazil	Journal of the Science of Food and Agriculture	Controlled experimental study	10 cows	Holstein	100-150	Dairy farm
3.	Schaper et al., 2015	Germany	Journal of Food Safety and Food Quality	Comparative observational study	10 cows	Holstein-Friesian	Second lactation	Growing up in a shelter
4.	Asher et al., 2015	Israel	Chronobiology International The Journal of Biological and Medical Rhythm Research	Comparative observational study	E1: 14 cows E2: 14 cows	Holstein	E1: 134.0 ± 8.87 E2: 137.7 ± 9.77	Growing up in a shelter
5.	Romanini et al., 2019	Brazil	Journal of Applied Animal Research	An experimental animal study with a factorial design	30 cows	Holstein	In 276 days' time	Rearing in a free-stall housing system
6.	Choi et al., 2021	South Korea	Journal of Thermal Biology	Controlled experimental study	16 cows	NR	74.3 ± 7.1	Raised in open stables
7.	Murphy et al., 2021	Ireland	Journal of Dairy Sciences	An experimental, randomized controlled trial	E1: 5 cows E2: 40 cows	Holstein-Friesian	E2: Cows with different parities were included in the study	Rotational grazing system from February to November
8.	Lindkvist et al., 2021	Sweden	PloS One	Controlled experimental study	E1: 5 cows E2: 40 cows	Swedish Red	E2: between 117 and 331 days	An environment with controlled conditions in terms of light, food and cleanliness
9.	Lim et al., 2021	South Korea	Journal of Animal Sciences and Technology	Controlled experimental study	24 cows	Holstein	144	Raised in free-range stables

Criterion number	Authors and year	The country	The journal	Type of study	Number of cows	Breed	Lactation (days)	Growing conditions
10.	Teng et al., 2021	China	Journal of Dairy Sciences	Comparative observational study	10 cows	Holstein	150 ± 20	Grown in open, shaded stables
11.	Ma et al., 2022	China	Zoological Sciences	An experimental, randomized controlled trial	36 cows	Holstein	143 ± 11	Raised in semi-enclosed stables
12.	Boztepe et al., 2022	Turkey	Selcuk Journal of Agricultural and Food Sciences	Comparative observational study	27 cows 27 cows	Holstein Jersey	First lactation	Raised in free-range stables
13.	Liu et al., 2024	China	Animal	Controlled experimental study	135 cows	Holstein	The pre- and postnatal periods	Commercial farm
14.	Kim et al., 2024	South Korea	Animal	Controlled experimental study	30 cows	Holstein	226,40 ± 96,83 217,78 ± 83,56 212,80 ± 84,98	Dairy farm
15.	Shen et al., 2026	China	Frontiers in Veterinary Sciences	Controlled experimental study	E1: 196 cows E2: 80 cows	Holstein	E1: N/A E2: 180 ± 40	Raised in semi-open stables
16.	Dall'Olio et al., 2026	Italy	The Thai Journal of Veterinary Medicine	Comparative observational study	40 cows	Holstein	174 ± 140	Raised in compartment-style stables

E1 and E2 - Experiment 1 and Experiment 2 (two animal experiment were conducted within the respective studies); NR - Not reported; N/A - Not applicable.

Supplementary Table S3. Analyzed factors.

Criterion number	Category of factors	Nutritional factors	Technological factors	Milk processing methods
1.	Nutritional factor	Supplementing feed rations with 125 g/cow/day of protected L-tryptophan.	N/A	N/A
2.	Technological factor	N/A	A comparison of melatonin concentrations in cow's milk collected at different times of the day.	N/A
3.	Technological factors and milk processing	N/A	A comparison of melatonin levels in cow's milk collected at two-week intervals.	Analysis of samples of processed dairy products purchased from shops.
4.	Technological factor	N/A	Monitoring of artificial lighting at night.	N/A
5.	Technological factors and milk processing	N/A	To investigate the impact of milking time, season and milk yield in Holstein cows on melatonin concentrations in milk.	Determination of the melatonin content in commercially available milk processed using UHT heat treatment.
6.	Nutritional factor	Supplementing feed rations with 15, 30 and 60 g/cow/day of protected L-tryptophan.	N/A	N/A
7.	Technological factor	N/A	To determine the effect of blue light, directed at a single eye of the animals, on melatonin concentrations in cow's milk.	N/A
8.	Technological factor	N/A	To investigate how the colour and intensity of artificial light affect pupillary response, cow behaviour, feed intake and consumption, milk production and composition, and hormonal parameters (melatonin and IGF-1 levels).	N/A

Criterion number	Category of factors	Nutritional factors	Technological factors	Milk processing methods
9.	Technological factor	N/A	Manipulating the intensity of artificial light and the duration of the photoperiod, with the aim of determining the influence of these technological parameters on milk production and chemical composition, as well as on the cows' hormonal status, blood metabolism, feed intake and activity.	N/A
10.	Technological factor	N/A	To investigate the impact of the circadian rhythm on melatonin concentrations in daytime and night-time milk from Holstein cows, as well as the influence of melatonin on the heat shock proteins HSP70 and HSP90.	N/A
11.	Nutritional factor	Supplementing feed rations with 14 and 28 g/cow/day of protected L-tryptophan.	N/A	N/A
12.	Technological factor	N/A	A comparison of melatonin levels in cow's milk from two different breeds (Holstein and Jersey) collected at different times of the day (in the morning and during the day).	N/A
13.	Nutritional factor	Supplementing feed rations with 50 and 100 g/cow/day of unprotected L-tryptophan, respectively, during the cows' transition period, with the aim of determining the effects of tryptophan on the metabolic changes the animals undergo and on the quality and quantity of milk production recorded immediately after calving.	N/A	N/A

Criterion number	Category of factors	Nutritional factors	Technological factors	Milk processing methods
14.	Nutritional factor	Supplementing feed rations with 20 g/cow/day of protected L-tryptophan and 20 g/cow/day of protected L-ascorbic acid.	N/A	N/A
15.	Technological factor	N/A	To investigate the effect of exposing cows to yellow or red light on milk production, milk quality and the animals' hormonal status.	N/A
16.	Technological factor	N/A	To investigate the differences in melatonin content between day and night milk collected during winter, and to establish a correlation between the reduction in somatic cell count and the presence of higher melatonin concentrations in cows' milk.	N/A

N/A - Not applicable; IGF-1 - Insulin-like Growth Factor 1; HSP70 - Heat Shock Protein 70; HSP90 - Heat Shock Protein 90.

Supplementary Table S4. Determination of melatonin in blood/serum/plasma and the condition under which the samples were collected and stored.

Criterion number	Sample type (serum/plasma)	Time and date of harvest (day/night)	Method for determining melatonin	Unit of measurement	Comments
1.	Plasma	Every three hours between 8:00 a.m. and 5:00 a.m.	ELISA (CV: lower limit 7.5%, upper limit 11.3%)	pg/mL (AUC)	The ELISA kit may have limitations in terms of the specificity of the results.
2.	N/A	N/A	N/A	N/A	N/A
3.	N/A	N/A	N/A	N/A	N/A
4.	N/A	N/A	N/A	N/A	N/A
5.	N/A	N/A	N/A	N/A	N/A
6.	Plasma	2:00 a.m.	ELISA	ng/L	The ELISA kit may have limitations in terms of the specificity of the results.
7.	Plasma	4:00 p.m.; 5:00 p.m.; 6:00 p.m.; 8:00 p.m.; 10:00 p.m.; 11:00 p.m.; 4:00 a.m.	RIA	pg/mL (sensitivity ranging from 0.3 to 0.9 pg/mL)	The antibodies used to measure melatonin can also bind to the metabolites of this hormone, which increases the risk of obtaining unrealistic results.
8.	Plasma	8:30 a.m.; 4:00 p.m.; 10:30 p.m.; 00:00 p.m.	ELISA	pg/mL (sensitivity 1.6 pg/mL; intra-assay CV 5.3%; inter-assay CV 15%)	The ELISA kit may have limitations in terms of the specificity of the results.
9.	Serum	2:00 a.m.	ELISA	pg/mL	The ELISA kit may have limitations in terms of the specificity of the results.
10.	N/A	N/A	N/A	N/A	N/A
11.	Serum	10:00 a.m.; 10:00 p.m.	RIA	pg/mL	The antibodies used to measure melatonin can also bind to the metabolites of this hormone, which increases the risk of obtaining unrealistic results.
12.	N/A	N/A	N/A	N/A	N/A
13.	Serum	3:00 p.m.	LC-MS/MS	ng/mL	The possibility of matrix errors occurring if samples are not prepared in accordance with the procedure.
14.	N/A	N/A	N/A	N/A	N/A
15.	Serum	5:30 a.m.	ELISA	pg/mL	The ELISA kit may have limitations in terms of the specificity of the results.
16.	N/A	N/A	N/A	N/A	N/A

ELISA - Enzyme-Linked Immunosorbent Assay; CV - Coefficient of Variation; pg/mL - Picograms per milliliter; AUC - Area Under the Curve; N/A - Not applicable; ng/L - Nanograms per liter; RIA - Radioimmunoassay; LC-MS/MS - Liquid Chromatography-Tandem Mass Spectrometry; ng/mL - Nanograms per milliliter.

Supplementary Table S5. Determination of melatonin in milk and the conditions under which the samples were collected and stored.

Criterion number	Type of sample (raw/processed milk)	Time and date of harvest (day/night)	Method for determining melatonin	Unit of measurement	Comments
1.	Raw milk	4:15 a.m. 3:45 p.m.	ELISA (LOD 3 pg/mL; intra-assay CV 7.5%, inter-assay CV 11.3%)	pg/mL	The comparability of results is influenced by the method of analysis and the degree to which the method is standardised.
2.	Raw milk	2:00 a.m. 3:00 p.m.	ELISA	pg mL^{-1}	
3.	Raw milk and processed milk	3:30 a.m.	ELISA	pg/mL	
4.	Raw milk	4:30 a.m. 12:30 p.m.	ELISA	pg/mL	
5.	Raw milk and processed milk	5:00 a.m. - 6:00 a.m.	ELISA	pg/mL	
6.	N/A	N/A	N/A	N/A	N/A
7.	N/A	N/A	N/A	N/A	N/A
8.	N/A	N/A	N/A	N/A	N/A
9.	Raw milk	Milk samples were taken from each milking parlour on the farm.	ELISA	pg/mL	The comparability of results is influenced by the method of analysis and the degree to which the method is standardised.
10.	Raw milk	5:00 a.m. 3:00 p.m.	ELISA	pg/mL	
11.	N/A	N/A	N/A	N/A	N/A
12.	Skimmed milk	3:00 a.m. - 5:00 p.m. 3:00 a.m. - 5:00 p.m.	ELISA	pg/mL	The comparability of results is influenced by the method of analysis and the degree to which the method is standardised.
13.	Raw milk	2:00 p.m.	LC-MS/MS	ng/mL	
14.	Raw milk	6:00 a.m. 6:00 p.m.	ELISA	pg/mL	
15.	Raw milk	5:00 p.m.	ELISA	pg/mL	
16.	Skimmed milk	4:00 a.m. 4:00 p.m.	ELISA	pg/mL	

ELISA - Enzyme-Linked Immunosorbent Assay; LOD - Limit of Detection; CV - Coefficient of Variation; pg/mL - Picograms per milliliter; N/A - Not applicable; LC-MS/MS - Liquid Chromatography-Tandem Mass Spectrometry.

Supplementary Table S6. Results and observations relevant to melatonin concentrations in blood/serum/plasma.

Criterion number	Melatonin content	The significant difference	The effect	Comments
1.	Plasma AUC between 3 and 150 pg/mL	A significant difference in blood melatonin levels was reported depending on the time at which the blood samples were taken.	Blood melatonin levels increased as a result of supplementation with protected tryptophan.	Other effects reported by the authors included the impact of supplementation with protected tryptophan on plasma concentrations of tryptophan, serotonin, prolactin and oxytocin.
2.	N/A	N/A	N/A	N/A
3.	N/A	N/A	N/A	N/A
4.	N/A	N/A	N/A	N/A
5.	N/A	N/A	N/A	N/A
6.	~ 100 ng/L → C ~ 200 ng/L → S1 ~ 250 ng/L → S2 ~ 150 ng/L → S3	The highest levels of melatonin were found in the blood of cows whose feed rations had been supplemented with 30 g per cow per day.	Supplementing cattle feed rations with rumen-protected tryptophan improves the rate of pineal melatonin production.	Under certain conditions and at certain doses, tryptophan has the potential to cause certain undesirable reactions in cows, such as a loss of appetite.
7.	42.75 - 50.50 pg/mL	A blue light intensity of 225 lx directed at a single eye of the cows was the factor that suppressed melatonin production to the extent that melatonin concentrations in the animals' blood were found to be similar to those typical of the daytime.	A dose-dependent effect was reported: as the intensity of the blue light was increased, the concentration of melatonin in the blood decreased.	In cows exposed to blue light directed at a single eye, an increase in milk production of approximately 2 kg was reported, as well as an increase in IGF-1 concentrations.
8.	8.2 - 28.6 pg/mL	Plasma melatonin follows a well-defined circadian rhythm, and the concentration of melatonin produced at night by the pineal gland can be influenced by the colour of the light to which animals are exposed during the day.	Plasma melatonin concentration increases in response to the perception of low light intensities. Red and yellow light treatments had the least inhibitory effect on pineal melatonin synthesis, whilst white and blue light had a significantly greater inhibitory effect.	There were no significant differences in peak melatonin levels between the artificial light treatments, suggesting that the intensity and duration of the body's exposure to light are factors that have a greater influence on the synthesis of pineal melatonin.
9.	25.55 pg/mL → C 23.72 pg/mL → S1 17.44 pg/mL → S2 17.03 pg/mL → S3	Blood melatonin concentrations follow light signals from the natural environment, such that serum melatonin levels decreased as light intensity and the duration of the light photoperiod increased.	Light intensity and the duration of the body's exposure to a longer photoperiod inhibit the synthesis of pineal melatonin.	The differences in melatonin levels in the animals' blood were statistically significant, thereby demonstrating the circadian rhythm of pineal melatonin synthesis.

Criterion number	Melatonin content	The significant difference	The effect	Comments
10.	N/A	N/A	N/A	N/A
11.	42.89 pg/mL → C 48.73 pg/mL → S1 50.20 pg/mL → S2	Serum melatonin levels increased in proportion to the dose of supplemental tryptophan, and melatonin produced by the pineal gland acts as a mediator between tryptophan and lactation hormones such as prolactin and IGF-1.	The increase in serum melatonin levels occurred gradually as the experiment progressed and stabilised on day 16 of administration of protected L-tryptophan.	The authors reported that there were no significant differences between the serum melatonin concentrations measured in samples taken at different times of the day. This finding suggests that tryptophan supplementation may stabilise daytime serum melatonin levels.
12.	N/A	N/A	N/A	N/A
13.	~ 1.0 pg/mL → C ~ 4.0 pg/mL → S1 ~ 6.0 pg/mL → S2	The administration of 100 g/cow/day of unprotected L-tryptophan resulted in the greatest increase in serum melatonin concentration during the pre-partum period.	After calving, serum melatonin levels fell progressively in all three groups (the experimental group, and those receiving supplements of 50 and 100 g per cow per day, respectively).	The amount of tryptophan administered must be determined with care, as this amino acid has different effects depending on the animals' physiological state; once the transition period is over, the dose should be reduced.
14.	N/A	N/A	N/A	N/A
15.	36.30 - 59.48 pg/mL	Exposing the animals to yellow light during the dark photoperiod stimulated the synthesis of pineal melatonin.	The yellow light also proved to be beneficial because the animals adapted very quickly to the new housing conditions, as an increase in plasma melatonin concentrations was reported as early as the fourth day of light treatment.	Red light did not significantly affect serum melatonin concentrations, a finding which suggests that not only the intensity but also the wavelength of light directly influences pineal melatonin synthesis.
16.	N/A	N/A	N/A	N/A

pg/mL - Picograms per milliliter; N/A - Not applicable; ng/L - Nanograms per liter; lx - Lux; IGF-1 - Insulin-like Growth Factor 1; C - Control group; S1, S2, S3 - Supplementation 1, 2, 3.

Supplementary Table S7. Results and observations relevant to melatonin concentrations in milk.

Criterion number	Melatonin content	The significant difference	Effects	Comments
1.	8.3 - 10.5 pg/mL in the morning 3.1 - 4.0 pg/mL in the evening	A significant difference in the melatonin content of milk was reported depending on the time at which the samples were collected.	The concentration of melatonin in milk increased, but there was a clear difference between the individuals studied.	Other effects reported by the authors included the impact of supplementation with protected tryptophan on the concentrations of tryptophan, serotonin, prolactin and oxytocin.
2.	39.43 pg/mL at night 4.03 pg/mL during the day	The time at which milk is collected has a direct and significant influence on the concentration of melatonin in cow's milk.	During the night, the pineal gland in cattle produces melatonin, which it can release into the milk via the bloodstream.	Night-time milk with a high melatonin content can be used in human nutrition as a functional food, consumed to help treat conditions related to the circadian rhythm and difficulties in achieving good-quality sleep.
3.	1.3 - 25.0 pg/mL in the morning	Each cow maintained a fairly constant level of melatonin in its milk over the two weeks, although significant differences were reported between animals in terms of melatonin concentrations in milk.	Cows maintain a relatively constant level of melatonin in their milk provided that their feeding patterns and/or rearing and husbandry conditions remain unchanged.	There may be significant individual variations in the melatonin content of cow's milk amongst animals from the same batch.
4.	30.70 ± 1.79 pg/mL → S1 in the morning 5.36 ± 0.33 pg/mL → S1 at midday 17.81 ± 0.33 pg/mL → S2 in the morning 3.30 ± 0.18 pg/mL → S2 at midday	Exposing cows to artificial light at night also affected melatonin concentrations in their daytime milk.	Melatonin concentrations were higher in milk from animals that had been exposed to natural darkness.	The authors reported that exposing cows to artificial light at night also increased the somatic cell count in their milk.
5.	6.98 pg/mL (average in cow's milk) 4.71 pg/mL (average in pooled milk) 5.62 (average in UHT milk)	The season, the time of milking and the amount of milk produced by each individual animal have a significant influence on the concentration of melatonin in raw milk.	The highest concentration of melatonin was reported in night-time milk, collected in the early hours of the morning, from low-yielding cows and during the winter.	The concentration of melatonin in milk decreases as the milk is diluted. Consequently, the more milk the cows produce, the more the same amount of melatonin produced by the body and released into the milk is diluted in a larger volume of milk.
6.	N/A	N/A	N/A	N/A
7.	N/A	N/A	N/A	N/A

Criterion number	Melatonin content	The significant difference	Effects	Comments
8.	N/A	N/A	N/A	N/A
9.	17.17 - 25.64 pg/mL → C 17.85 - 22.11 pg/mL → S1 21.79 - 26.95 pg/mL → S2 25.22 - 31.86 pg/mL → S3	The highest concentration of melatonin in milk was reported in the group of cows that were exposed to the highest intensity of artificial light.	Melatonin levels in the animals' milk increased in line with the treatment administered. The higher the light intensity, the more melatonin the cows' milk contained.	The results presented by the authors of this study indicate a significant difference between the rate of pineal melatonin synthesis, as determined from serum samples, and the concentration of melatonin present in milk. These differences are due to the experimental protocol on which the research was based.
10.	90.21 pg/mL → Daytime milk 120.07 pg/mL → Night-time milk	The circadian rhythm directly influences the chemical composition of milk, both in terms of its melatonin content and other major components such as proteins, fats and carbohydrates.	Milking at two different times of the day makes it possible to produce a functional food (night-time milk) characterised by a distinct chemical composition that reflects the cows' night-time metabolism.	The authors reported low concentrations of MDA and elevated levels of interferon-gamma in night-time milk, suggesting that night-time milk may be a functional food designed to reduce inflammation and support the immune system.
11.	N/A	N/A	N/A	N/A
12.	2.912 pg/mL - Daytime milk (Holstein) 11.314 pg/mL - Night-time milk (Holstein) 2.924 pg/mL - Daytime milk (Jersey) 6.954 pg/mL - Night-time milk (Jersey)	The melatonin content in cow's milk varies depending on the breed.	Night-time milk contains more melatonin than day-time milk, regardless of the breed of the farm animals.	The concentration of melatonin in the milk of Holstein-Freiesa cows ranged from 1.168 to 5.887 pg mL^{-1} in daytime milk and from 3.520 to 21.510 pg mL^{-1} in night-time milk. In Jersey cows, the melatonin concentration ranged from 1.085 to 4.577 pg mL^{-1} for daytime milk and from 3.422 to 15.722 pg mL^{-1} for night-time milk.
13.	~ 8,0 pg/mL → C ~ 10,0 pg/mL → S1 ~ 9,0 pg/mL → S2	Immediately after calving, the concentration of melatonin in the milk was relatively higher compared with the results reported in other studies in which the cows were not in the transition period.	Melatonin concentrations in cows' milk decreased after calving in line with the progression of experience over time, reaching as low as 1 pg/mL for all three groups studied.	The results of this study have shown that, during the transition period, protected tryptophan is not utilised in large quantities to produce melatonin, but is instead redirected towards certain metabolic and immune pathways that take priority during this period.

Criterion number	Melatonin content	The significant difference	Effects	Comments
14.	3.63 - 6.60 pg/mL → C 5.28 - 13.53 pg/mL → RP-Trp 3.92 - 6.81 pg/mL → Vitamin C	Supplementing cattle feed rations with 20 g/cow/day of rumen-protected L-tryptophan resulted in a twofold increase in the melatonin content of the animals' milk, compared with the control group.	Tryptophan has been shown to be much more effective than L-ascorbic acid in producing milk with a higher melatonin content.	The lowest melatonin concentrations in the treatment groups were recorded in August, most likely due to the heat stress to which the animals were subjected. In September, the highest concentrations of melatonin were reported in milk collected from animals in all three groups, most likely due to the longer nights and lower temperatures.
15.	20,49 - 29,22 pg/mL	Exposing animals to yellow light results in increased concentrations of melatonin in their milk.	Stimulating melatonin synthesis in the pineal gland will directly increase the amount of melatonin released by cows into their milk.	Milk partly reflects the concentration of melatonin in the blood, as melatonin levels in milk are lower than those in serum.
16.	6.80 ± 0.75 pg/mL → Daytime milk 15.63 ± 1.90 pg/mL → Night-time milk	The higher concentrations of melatonin found in night-time milk are due to the circadian rhythm of pineal melatonin synthesis.	Higher concentrations of melatonin in milk are associated with a reduction in somatic cell count, an observation which suggests that melatonin plays a protective role in the udder.	Collecting cow's milk at night may be a strategy for enhancing the milk's antioxidant and anti-inflammatory properties by increasing the concentration of melatonin in the final product.

pg/mL - Picograms per milliliter; C - Control group; S1, S2, S3 - Supplementation 1, 2, 3; N/A - Not applicable; MDA - Malondialdehyde; RP-Trp - Ruminal Protected L-Tryptophan.

Supplementary Table S8. Risk of bias assessment.

Criterion number	Melatonin in the blood	Melatonin in milk	Controlled factors	Risk of bias	Comments
1.	Plasma AUC between 3 and 150 pg/mL	3.0 - 10.5 pg/mL	Monitored photoperiod; standardised circadian harvesting; standardised diet; controlled study design (control group vs. Trp group).	Moderate	High variability at the individual level.
2.	N/A	4.03 - 39.43 pg mL^{-1}	Standardised circadian sampling.	Moderate	The lack of details regarding the animals' husbandry conditions increases the likelihood that variations in the melatonin content measured in Holstein cows' milk may also be influenced by other factors.
3.	N/A	1.3 - 25.0 pg/mL	Circadian milk collection at a single time of day; standardised diet; controlled study design (raw milk was collected at two-week intervals).	Moderate	The photoperiod was not monitored, and milk was not collected at two different times of the day.
4.	N/A	3.30 - 30.70 pg/mL	Monitored photoperiod; standardised circadian harvesting; standardised diet; controlled study design (control group vs. ALAN).	Moderate	The existence of other factors that may influence the melatonin content in milk, such as farm management and the animals' stress levels.
5.	N/A	2.69 - 41.94 pg/mL	Monitored photoperiod; standardised seasonal and circadian milking; standardised diet; controlled study design (low-yield group vs. high-yield group; milk collected from individual cows vs. milk collected from temporary storage tanks; raw milk vs. UHT milk).	Moderate	The samples of milk heat-treated using the UHT pasteurisation process did not comply with the traceability chain for raw materials, as they were purchased from retailers. This does not fully guarantee that heat treatment does not affect the melatonin content of milk.
6.	100 - 250 ng/L	N/A	Experimental design (control group versus three experimental groups).	Moderate	The lack of circadian blood sampling prevents a full assessment of the circadian profile of melatonin production, which limits the ability to correlate the administration of protected tryptophan and plasma melatonin concentrations with the photoperiod and the time of day.

Criterion number	Melatonin in the blood	Melatonin in milk	Controlled factors	Risk of bias	Comments
7.	1.7 - 9.4 pg/mL	N/A	Light intensity; controlled design (E1: design controlled by crossover and controlled environment; E2: control group vs. treatment group).	E1: Moderate→high E2: Low→moderate	A blue light intensity of 225 lx directed at just one eye of the cows was sufficient to almost completely suppress pineal melatonin production; however, further studies are needed to investigate the effect of blue light directed at the entire animal housing area.
8.	8.2 - 28.6 pg/mL	N/A	Environment, type and intensity of light; study design (experimental design with randomised groups and blocks).	E1: Moderate→high E2: Moderate	The high degree of variation in the ELISA kit used to measure melatonin may have led to some measurement errors. The absence of a control group that had not received light treatment meant that the results were compared across the different light colours to which the animals were exposed; this meant that the results could not be compared with a situation where no light was present.
9.	17.03 - 25.55 pg/mL	17.17 - 31.86 pg/mL	Monitored photoperiod; standardised circadian harvesting; standardised diet; controlled study design (control group versus three experimental groups).	Low	The findings presented by the authors of this study make a significant contribution to our understanding of how melatonin is released into the bloodstream of cows during milk production. However, there are also a number of limitations to the research methodology, due to the lack of a standardised circadian schedule for the collection of blood and milk samples.
10.	N/A	90.21 - 120.07 pg/mL	Standardised circadian sampling; standardised diet.	Low	The melatonin content in the blood and milk of animals can be influenced by a number of factors, not just photoperiod, and a lack of control over nutrition and environmental conditions that may cause stress to cows are situations that can disrupt pineal melatonin synthesis.
11.	42.75 - 50.20 pg/mL	N/A	Monitored photoperiod; standardised circadian harvesting; standardised diet; controlled study design (control group vs. Trp group).	Moderate	The study has certain limitations due to the method of administering tryptophan (mixed into flour) and the lack of control over technological factors.

Criterion number	Melatonin in the blood	Melatonin in milk	Controlled factors	Risk of bias	Comments
12.	N/A	1.168 - 21.510 pg ml^{-1} Holstein 1.085 - 15.722 pg ml^{-1} Jersey	Standardised circadian harvesting; a comparative experimental design across species.	Moderate	The lack of information regarding animal nutrition and diet increases the likelihood that the melatonin levels in the milk of the two cattle breeds may also be influenced by their feed.
13.	~ 1.0 - 6.0 pg/mL	~ 1.0 - 10.0 pg/mL	Experimental design (control group versus two experimental groups).	Moderate	The lack of circadian sampling of blood and milk limits our ability to determine the potential effects that tryptophan may have on the bovine organism during the night; it is possible that the low levels of melatonin detected in cow's milk are due to the photoperiod rather than the transition period.
14.	N/A	3.63 - 13.53 pg/mL	Experimental design (control group versus two experimental groups).	Moderate	The authors did not report separate results for the melatonin content in daytime and night-time milk, which means it is not possible to determine the impact of the photoperiod in this experiment.
15.	36.30 - 59.48 pg/mL	20.49 - 29.22 pg/mL	Monitored and controlled photoperiod; standardised circadian harvesting; standardised diet; controlled study design (control group versus two treatment groups).	Moderate	Melatonin concentrations in milk were determined from pooled milk samples, which meant that melatonin concentrations could not be measured for individual samples.
16.	N/A	6.80 - 15.63 pg/mL	Monitored photoperiod; standardised circadian harvesting; standardised diet.	Moderate	High inter-individual variability may affect the reproducibility of results due to natural variations in the chemical composition of milk.

AUC - Area under of curve; pg/mL - Picograms per milliliter; Trp - Tryptophan; N/A - Not applicable; ALAN - Artificial Light At Night; UHT - Ultra-High Temperature; E1 and E2 - Experiment 1 and Experiment 2 (two animal experiment were conducted within the respective studies); ELISA - Enzymatic-Linked Immunosorbent Assay; ng/mL - Nanograms per milliliter.