Systematic Review

Nutritional and Technological Factors Influencing Melatonin Contents in Cow's Milk

Administrative information
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1. Introduction

Melatonin (the sleep hormone) is a bioactive molecule that has attracted the attention of the scientific community ever since its discovery in 1958, owing to the many roles and functions it performs in living organisms. The synthesis of pineal melatonin only in the absence of light, the release of this molecule into the bloodstream, and its amphiphilic nature are the characteristics that formed the basis for the development of the concept of "night milk": a 100% natural food product with a more complex chemical composition than milk collected during the day, due to the fact that milk collected in the early hours of the morning contains a higher amount of melatonin in its chemical composition. Essentially, milk is a dynamic biological fluid, the chemical composition of which varies considerably depending on when it is collected. In this context, the hypothesis was put forward that night-time milk containing melatonin could be used as a chronofunctional food, designed to improve sleep quality and support the general health of the human body in those who consume it. However, the production of night-time milk containing melatonin cannot be limited solely to the management of the photoperiod or to standardised circadian milking, as melatonin synthesis is influenced by several factors, including the vertebrate organism's bioavailability of tryptophan (the main precursor involved in melatonin synthesis) and the absence of stress (under stressful conditions, the animal's body produces cortisol; and as the main markers of the circadian rhythm, melatonin and cortisol exhibit a mutually antagonistic relationship with regard to their synthesis processes).

There are several studies in the specialist literature which have examined the effects of various factors on the melatonin content in the blood and milk of cows. However, the authors of those studies either assessed only the impact of a single category of factors on serum and milk melatonin concentrations in cows, or investigated the effects of certain nutritional and technological factors on other physiological and metabolic parameters in dairy cows, and the determination of melatonin content in the animals' blood and milk was a secondary objective of the research, which has not been studied in sufficient detail.

The main aim of this systematic review is to provide answers to the following three questions:

- *What are the main nutritional and technological factors that influence the melatonin content in cow's milk?*
- *Which of the identified nutritional and technological factors are associated with achieving the highest concentrations of melatonin in cow's milk?*
- *What is the relative importance of the factors identified in determining the melatonin content of milk and, to the extent that the available evidence allows for such an assessment, in what order do these factors limit the accumulation of melatonin in milk?*

The secondary objective of this study is to assess the potential impact of milk processing and transformation on the melatonin content of finished dairy products, as well as to identify the technological processes associated with changes in melatonin content compared with raw milk.

2. Methodology

Inclusion criteria

This systematic review will only include practical studies that have investigated the influence of one or more nutritional and/or technological factors on the melatonin content of cow's milk. Research in which the melatonin content in the blood of animals was determined will also be included. There is no time limit imposed on the systematic search for scientific papers.

Exclusion criteria

This systematic review will exclude review articles, conference papers for which no final manuscript is available, research articles in which only in vitro experiments were carried out, research articles in which practical experiments were carried out on animal species other than dairy cows, research articles in which melatonin concentrations in the blood or milk of cows were not determined, and papers that are not available in English.

3. Sources of Information

The systematic search will be carried out in three databases, namely: PubMed/MEDLINE, Web of Science and ScienceDirect. An additional search will be carried out on Google Scholar with the aim of identifying any papers that meet the inclusion criteria but have not been indexed in any of the three databases consulted.

4. Search Strategy

The search strategy will be developed on the basis of the key concepts underlying the research questions and will combine controlled terms, where available, with free-text terms and synonyms. Combinations of keywords relating to melatonin, cow's milk, dairy cows, nutritional factors and technological factors will be used.

The search terms will be combined using the Boolean operators AND and OR, and truncation will only be used where appropriate. The search strategy will be tailored tu each database, its controlled vocabulary and specific syntax.

Search strategy for PubMed/MEDLINE and ScienceDirect

Nutritional factors: melatonin AND (cow OR cows OR cattle OR bovin OR "dairy cow" OR "dairy cows") AND (tryptophan OR L-triptophan OR supplementation OR nutrition OR diet OR feeding OR feed OR forage.

Technological factors: melatonin AND (cow OR cows OR cattle OR bovin OR "dairy cow" OR "dairy cows") AND (milk OR milking) AND ("milking time" OR "night milking" OR "nocturnal milking" OR photoperiod OR "light exposure" OR light OR "light-dark cycle" OR "circadian rhythm" OR circadian OR season OR seasonal OR seasonality.

Processing factors: melatonin AND (milk OR "dairy product" OR "dairy products") AND (processing OR pasteurization OR pasteurisation OR UHT OR "head treatment" OR "thermal treatment" OR storage OR refrigeration OR "shelf life".

Search strategy in Web of Science

Nutritional factors: melatonin AND (cow milk OR bovin milk OR ruminal protected L-triptophan OR unprotected L-tryptophan).

Technological factors: melatonin AND (bovin milk OR night milk).

Processing factors: melatonin AND processing milk.

Search strategy for Google Scholar

Nutritional factors: "melatonin, cow milk, tryptophan"; "melatonin, dairy cow, tryptophan"; "melatonin, cow milk, supplementation"; "melatonin, dairy cow, nutrition"; "melatonin, dairy cow, diet".

Technological factors: "melatonin, cow milk, night milking"; "melatonin, dairy cow, milking time"; "melatonin, cow milk, photoperiod"; "melatonin, dairy cow, light exposure"; "melatonin, dairy cow, circadian rhythm"; "melatonin, cow milk, season variation".

Processing factors: "melatonin, cow milk, processing"; "melatonin, cow milk, pasteurization"; "melatonin, cow milk, UHT"; "melatonin, cow milk, heat treatment"; "melatonin, cow milk, storage"; "melatonin, dairy product, melatonin processing".

5. The Selection Process

The studies identified through the search strategy will be imported into a reference management software programme, and duplicates will be removed. The selection of studies will take place in two stages: (1) screening of titles and abstracts, and (2) evaluation of the full text of potentially eligible studies. Eligibility will be determined on the basis of the inclusion and exclusion criteria set out in the protocol. The selection process will be presented using the PRISMA diagram, and the reasons for excluding studies at the manuscript assessment stage will be documented.

6. Data Extraction

The data will be extracted using a set of standardised forms, which will be attached to the review in the "Supplementary Materials" section in the form of tables. Information will be collected on the identifying characteristics of the studies and the animals, the factors under investigation, the methods used to determine and measure melatonin, and the reported levels of melatonin in the animals' blood and milk.

7. Assessment of Quality and Risk of Bias

Methodological quality and the risk of bias will be assessed using tools appropriate to the design of the included studies. As part of the assessment of the risk of bias, the main potential sources of bias will be taken into account, including sample selection, characteristics of the experimental design, the existence and suitability of the comparator group, control of confounding factors, measurement methods and the reporting of results. The risk of bias will be assessed independently by two assessors, and any discrepancies will be resolved by consensus.

8. Notable Results

The main result of interest will be the concentration of melatonin in cow's milk. Secondary outcomes will include melatonin concentration in relation to nutritional and technological factors, such as supplementation with protected or unprotected L-tryptophan (only if the supplemented doses of unprotected L-tryptophan had an effect on the parameters analysed), the timing of milking, storage conditions and milk processing methods. The methodological and experimental characteristics that may contribute to variations in results between studies will also be analysed.

9. Summary of the Data

The data extracted from the eligible studies will be summarised narratively and organised according to the main categories of factors investigated: nutritional factors, technological factors and milk processing factors. The results will be presented in a comparative format using tables and graphs, highlighting the trend in melatonin concentration, the experimental conditions and the main methodological characteristics of the studies.

10. Subgroup Analyses and Sensitivity Analyses

Depending on the number and characteristics of the studies included in the final analysis, subgroup analyses will be carried out by classifying the studies according to the type of factor under investigation, such as nutritional factors, technological factors and processing factors. Where relevant, studies will be analysed separately on the basis of their methodological characteristics and quality. No subgroup or sensitivity analyses will be carried out, as a narrative synthesis approach will be used in the review.

11. Data Management and Analysis

The data identified and extracted will be organised into a standardised database, which will enable the centralisation of study characteristics and relevant results. The data will be checked for completeness and consistency prior to analysis. The analysis will involve com-

paring and narratively synthesising the results, highlighting the similarities and differences between the included studies and the factors that may explain the heterogeneity of the results.

12. Changes to the Protocol

Any changes to the original protocol made during the course of the review will be documented and justified. Any relevant changes to the eligibility criteria, search strategy, methods for assessing the risk of bias or approach to data synthesis will be reported transparently in the final version of the review.

13. Ethics and Conflicts of Interest

This study is a systematic review of published scientific literature and does not involve the direct recruitment or handling of animals, nor the collection of biological samples for the purposes of this research. Consequently, no procedures requiring ethical approval are necessary for the conduct of the systematic review. Any conflicts of interest on the part of authors and sources of funding will be disclosed transparently, in accordance with the requirements of the journal to which the manuscript is submitted.