Melatonin concentration in cow’s milk and sources of its variation

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Abstract

The effects of weather/season (winter and summer), milk production (high and low), and milking time (night and day) on melatonin levels were evaluated. The melatonin in the milk from 30 individual cows, the bulk tank milk (BTM) from 16 farms, and ultra-high temperature (UHT) processed milk of 12 brands over two seasons were also assessed. The melatonin average concentration was 6.98 pg/mL in the cows’ milk, 4.71 pg/mL in the BTM, and 5.62 pg/mL in the UHT milk. In the night milk, the melatonin concentration averaged 14.87 pg/mL, whereas it was 6.98 pg/mL in the total daily milk. In winter, the milk melatonin concentration was, on average, 74.7% higher than in the summer (13.89 vs. 7.95 pg/mL). The night milk from the low production group in winter also had a higher concentration of melatonin (41.94 pg/mL). The variables related to illuminance, such as the time of milking and season of the year, had considerable effects on the melatonin concentration in milk. Collecting and marketing melatonin-rich milk could benefit populations and agribusiness.

Introduction

Melatonin is a neurohormone produced from the essential amino acid tryptophan by the pineal gland, and has a role in regulating circadian rhythm and sleep (Tosini and Fukuhara 2003). In addition, there are studies that suggest that it acts as mood modifier, antioxidant, and anticancer agent. It is also thought to be involved in osteoporosis prevention (Di Bella et al. 2013; Amstrup et al. 2015; De Crescenzo et al. 2017).

Melatonin biosynthesis is under the control of light and, in cattle, five to 10 lux seems to be the threshold above which synthesis is inhibited (Muthuramalingam et al. 2006). The number of hours of darkness and the photoperiod also dramatically affect synthesis (Dahl 2010). In addition, melatonin biosynthesis is sensitive to light wavelength because synthesis is suppressed by short light wavelengths (Cajochen et al. 2005; Asher et al. 2015).

Melatonin is amphiphilic, which means that it can freely diffuse through biological membranes (Simonneaux and Ribelayga 2003; Yu et al., 2016) into the circulatory system and from the bloodstream into the milk. Therefore, there is a relationship between blood and milk concentrations (Vanecek 1998; Castro et al. 2011), which means that milk is an important source of melatonin for the consumer. Kollmann et al. (2008) found about 40% of the blood melatonin concentration in the milk of cows producing approximately 32 kg/milk/day.

Melatonin is produced under dark conditions and only has a half-life of about 30 min (Singh et al. 2011). Therefore, milking time can affect the concentration of melatonin in milk (Milagres et al. 2014; Asher et al. 2015). In some countries, milk from night milking is rich in melatonin and has been commercialized as a sleep aid for people, such as Lullaby Milk in Ireland, Sleepiz and iNdream3 in New Zealand, Ingman Dairy’s Night Time Milk in Finland, and Dreamerz in the United States (Boland 2010).

This study investigated whether the daily cow milk yield, milking time, and seasonal weather variations could affect the melatonin concentration in milk from individual cows. Seasonal melatonin levels in the bulk tank milk from farms and branded ultra-high temperature (UHT) processed milk were also compared.

Material and methods

Milk samples were collected from 30 individual Holstein cows, 16 bulk tanks, and 12 brands of UHT processed milk during two collection periods, June (WINTER) and January (SUMMER). The milk samples from individual cows and bulk tanks were collected in Castro, in the southern Brazil, with a Cfb temperate climate (IAPAR, 2018), where there is the higher Brazilian milk production, the average productivity in 2017 was 8250 kg/cow/year (IBGE 2017), Holstein breed, mostly is confined with three daily milkings and balanced complete diets.

The samples of individual cows were collected in a commercial farm from 15 cows in the high-production group (HIGH) and 15 in the low-production group (LOW). In each collection period, two representative samples from each cow milking were collected. The first sample was collected when the cow was milked between 05:00 and 6:00 am (NIGHT) and the other was taken from the milk produced from the three daily milkings (TOTAL). One sub-sample was used for the milk physicochemical analysis and another for melatonin determination.
All the cows had an average productivity greater than 11660 kg/305 lactation days and the cows in the low production group were more than 276 days in milk. All cows were kept in the same free-stall barn, which had a 6 m ceiling height and pens containing sand beds. The diets were provided ad libitum, balanced according to nutritional requirements. The high and low milk production group diets contained 48.90 and 60.45% forage, 17.46 and 16.92% crude protein, 21.64 and 25.19% neutral detergent fibre, 38.65 and 35.70% non-fiber carbohydrates, 5.08 and 4.11% ether extract, and 96 and 97% of their tryptophan requirements as metabolizable protein, respectively.

The illuminance in the free-stall barn was measured by a portable digital luxmeter (Minipa do Brasil Ltda, São Paulo, Brazil) equipped with a sensor for visible light. It had a 3% precision level and recorded automatic readings (datalogger) at 4-minute intervals. The equipment took readings at a height of 1.5 m from the floor and was placed beside the free-stall pen for 48 h.

The samples used for the cow's milk physicochemical analyses were packed in 70 mL plastic containers that contained the preservative Bronopol® (2-bromo 2-nitropropane 1.3-diol). The fat, protein, lactose, and urea contents were determined by infrared absorption using the automated Bentley 2000® system (Bentley Instruments Inc., Chaska, Minnesota, USA), and the somatic cell count (SCC) was undertaken by an electronic counter that was part of the flow densitometry equipment in the Somacount 500® system (Bentley Instruments Inc. Chaska, Minnesota, USA). The non-fat content was determined as the difference between the total solids and fat contents.

The bulk tank milk samples were collected from 16 dairy farms in the same region (Castro, Paraná, Brazil) and the collection dates were the same for all the cows. Before collection, the milk was homogenized in the tanks. The UHT milk samples were collected in packages that are available for sale in supermarkets. Samples from 12 different brands that had been processed in different farms were collected from individual cows were collected.

The bulk tanks and UHT milk samples were frozen at −20°C immediately after collection without the addition of preservatives. Due to the freezing, the methodologies used to determine the composition of nutrients of these samples were different from those used for milk from individual cows. The samples were analyzed in the laboratories of the Universidade Norte do Paraná (UNOPAR) for protein content (Kjeldahl), fat (Gerber), and total solids (oven drying), according to the AOAC (1995) methodologies. The lactose concentration was determined by the lactose-methylamine method using a spectrophotometer at 540 nm (Nickerson et al. 1975).

The milk samples from individual cows, the bulk tanks, and the UHT milk that were used to determine the melatonin concentration were packed in 100 mL plastic containers, without the addition of preservatives, and frozen at −20°C immediately after collection. For analysis, the samples were thawed and centrifuged at 3300 × g for 15 min at 4°C to separate out the fat. The melatonin concentration was evaluated in the skimmed milk as per Kollmann et al. (2008) using the enzyme-linked immunosorbent assay (ELISA) technique (RES4041; IBL International, Hamburg, Germany).

A completely randomized design was used. The data were analyzed using descriptive statistics, the Kolmogorov–Smirnov test for normality, and by analysis of variance. The means were compared by Tukey's test at the 5% significance level. The Statistica 13.1 (Statssoft 2016) statistical software package was used to undertake the analyses. A 2 × 2 × 2 factorial arrangement was adopted to analyze the melatonin concentration in an individual cow’s milk. There were two seasons (winter and summer), two levels of productivity (high and low), and two sample collection times (night and total). The melatonin concentrations in UHT milk, the bulk tank milk, and the total daily milk from individual cows were analyzed in a 2 × 3 factorial arrangement. The factors were the two seasons and three sources of milk. Results are presented as means with their associated standard deviation.

Results and discussion

The maximum concentration of melatonin in the individual cow's milk was 41.94 pg/mL in the milk from the low production group during the winter season and night milking, whereas the minimum concentration was 2.69 pg/mL in the total daily milk from high production cows group during the summer season.

Milk time and season of the year affected the melatonin concentration in individual cow’s milk (Figure 1). In the night milk, the melatonin concentration (14.87 ± 7.69 pg/mL) was, on average, 113% higher than the total daily milk concentration (6.98 ± 3.05 pg/mL). During the winter, the melatonin concentration in total daily milk was, on average, 74.7% higher than in the summer (13.89 ± 8.38 vs. 7.95 ± 3.45 pg/mL).

The cow productivity level only showed a trend towards higher melatonin levels in milk from the low production group (11.75 ± 8.34 vs. 10.10 ± 5.40 pg/mL in the high production group), which suggests that the daily amount of milk produced may have a diluting effect on the melatonin concentration. A dilution effect of high milk yield on melatonin concentration was expected, but the high milk production variation in the low production group (Table 1) may explain why this result was not significant.

The only significant interaction was observed between season, milking time, and milk yield. Milk collected during the winter after night milking and from the low productivity cows (n = 15) contained more melatonin (mean: 21.93 ± 11.06 pg/mL) than the other combinations.

The melatonin concentration in the night milk was lower in this study than the levels reported by Milagres et al. (2014) who studied 10 cows. They reported a mean production of 25 L milk/day in the winter season (June) and found that the melatonin concentrations were 39.43 and 4.03 pg/mL in the milk sampled after milking at 2 am and 3 pm, respectively. Asher et al. (2015), who studied cows that produced 34.34 ± 1.34 L milk/day, reported an average melatonin concentration of 30.70 ± 1.79 pg/mL in the milk when cows were kept for 13 h under 5.08 ± 0.04 lux of light with a wavelength of 548 ± 5.12 nm. Valtonen et al. (2001) recorded melatonin levels of 56.4 pg/mL milk when the cows were kept for 17 h in the dark.

In this study, the time between sunrise and the sunset was 10 h 30 min in the winter and 13 h 30 min in the summer. The light levels during the day were, on average, 593.97 ± 306.73 lux. At night, the cows had initially about two hours of lights on, followed by five hours of lights off, and then five
hours of lights on when the night milkings were made (Figure 2). The mean illuminance during the night was 0.27 ± 0.11 lux with the lights off and 5.95 ± 0.36 lux when the lights were on.

In melatonin biosynthesis, the enzyme N-acetyltransferase regulates the conversion of serotonin to melatonin and light causes rapid inhibition of the activity of this enzyme (Klein and Weller 1972). The threshold for illuminance, above which melatonin synthesis is inhibited, is not well defined. Lawson and Kennedy (2001) and Muthuramalingam et al. (2006) observed a decrease in the melatonin level when the illuminance was 50 lux, compared to 0 lux. Muthuramalingam et al. (2006) observed that an illuminance of 5–10 lux did not affect the plasma melatonin levels. However, in a later study, Bal et al. (2008) also did not observe a decrease in melatonin when the illuminance was 40–60 lux, compared to 0–5 lux. These results suggest that 5–10 lux is an appropriate limit that will prevent the inhibition of melatonin synthesis in bovines.

A short light wavelength decreases melatonin synthesis (Cajochen et al. 2005; Asher et al. 2015). The wavelength determines the perceived visual colour, which ranges from approximately 390 nm (purple colour) to 780 nm (red colour) in humans (Sjaastad et al. 2010). Jacobs et al. (1998) observed an amplitude range of 451–555 nm in cows. The sodium vapour lamps used in the free-stall lightning have a wavelength of 589.3 nm (yellow light) and, therefore, it can be inferred that the wavelength of the lamp used minimized the inhibition of the melatonin synthesis in cows.

The melatonin content did not significantly differ in the bulk tank milk (4.08 pg/mL), UHT milk (4.16 pg/mL), and in the total daily milk from individual cows (5.24 pg/mL) collected in the summer period (Figure 3).

During the winter, the melatonin concentration in the bulk tank milk (5.35 pg/mL) was lower than in the individual cow’s milk (8.72 pg/mL), which may be due to the night lighting being better managed for melatonin synthesis at the farm. The fact that UHT milk melatonin content was not significantly different from the bulk tank and individual cow’s milk suggests that the UHT process does not significantly change the melatonin content in milk. Normally, during UHT processing, the milk is subjected to 140°C for 2 s, but this can vary from 138 to 145°C for 1–10 s (Deeth and Lewis 2017). Milagres (2012) evaluated the effect of 75–95°C on the kinetics of melatonin degradation and found the rate of degradation (k) ranged from 0.013–0.173 /min, which indicated that melatonin was highly stable.

The overall averages of milk composition are in accordance with current Brazilian legislation (Brasil 2011). However, the high production group produced milk with fat and protein contents that were below the levels required by current legislation (Table 1).

The urea levels between 12.90 and 15.60 mg/dL milk (Table 1) suggest that protein nutrition was adequate (Doska et al. 2012), and the SCC values indicated that the mammary glands were healthy (Hagnestam-Nielsen et al. 2009; Flere et al. 2016).

Table 1. Means of nutrient and urea compositions, and the somatic cells count (SCC) in the total daily milk from cows in the low (Low) and high (High) production groups during winter and summer.

| Parameter           | Winter     | Summer    | CVa       | Winter     | Summer    | CVa       |
|---------------------|------------|-----------|-----------|------------|-----------|-----------|
| Number in group      | High       | Low       | High      | Low        | High      | Low       | CVa       |
| Milk, kg/cow/day     | 49.53      | 31.09     | 57.67     | 14.95      | 45.66     | –         | –         |
| Fat, %               | 2.95       | 3.96      | 2.74      | 3.96       | 25.02     | –         | –         |
| Protein, %           | 2.97       | 3.43      | 2.73      | 3.41       | 12.59     | –         | –         |
| Lactose, %           | 4.50       | 4.66      | 4.62      | 4.45       | 5.97      | –         | –         |
| Total solids, %      | 11.36      | 13.07     | 11.05     | 12.88      | 10.19     | –         | –         |
| Urea, mg/dL          | 15.60      | 12.90     | 13.12     | 13.02      | 18.35     | –         | –         |
| SCC, x 1000/mLb      | 59.12      | 44.62     | 72.80     | 158.88     | –         | –         | –         |

CV = coefficient of variation; Geometric means.
Table 2 shows the composition of the milk nutrients in the bulk tank and UHT processed milk. These values comply with current Brazilian legislation (Brasil 2011) and are similar to those reported by Ribas et al. (2014), who investigated 1,950,034 bulk tank samples in the state of Paraná and found content percentages of 3.74 ± 0.69 fat, 3.22 ± 0.27 protein, 4.40 ± 0.23 lactose, and 12.29 ± 0.85 total solids.

Several studies have demonstrated the effectiveness of exogenous melatonin in clinical human treatments, including inducing sleep and improving its quality. The results were dose-dependent and varied from 0.05–5 mg (Ferracioli-Oda et al. 2013; Andersen et al. 2015; Andersen 2016). The range represents at least 10 times the night secretion of melatonin, which is 10 µg/night (Huether 1993). Although these doses are much
higher than those possible through consuming milk with a high melatonin concentration, some studies have demonstrated that consuming melatonin in milk also has beneficial effects (Dela Peña et al. 2015). Valtonen et al. (2005) in Finland evaluated sleep quality and the diurnal activity levels in people consuming an average of 0.61 L milk/day with a melatonin concentration of 15.3 ± 3.1 pg/mL. They concluded that the milk improved sleep quality and diurnal activity levels. More recently, Bae et al. (2016) studied the effects of consuming a glass of night milk containing 1000 pg of melatonin and 58.24 mg of tryptophan and compared it to normal milk containing 100 pg of melatonin and 47.5 mg of tryptophan. They observed that sleep satisfaction increased and daytime sleepiness decreased.

### Conclusion

The variables related to illuminance have greater effects on the melatonin concentration in cow’s milk than other factors. Higher melatonin concentrations were obtained after night milking during winter when the night period is longer. The UHT processed milk available for sale in supermarkets had a melatonin concentration that was similar to the milk from individual cows and from bulk tanks on farms. The melatonin concentrations obtained from the night milk were similar to those reported in the literature as being beneficial to human health. Collecting the milk from the night milking into a separate tank allows for the processing and commercialization of high-melatonin milk.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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### Table 2. Means of nutrient composition of milk collected from the bulk tanks and UHT processed milk during winter and summer.

| Parameter     | Winter Tanks | UHT Tanks | Summer Tanks | UHT Tanks | CVa |
|---------------|--------------|-----------|--------------|-----------|-----|
| N             | 16           | 12        | 16           | 12        | –   |
| Fat, %        | 3.63         | 3.21      | 3.30         | 2.99      | 12.44 |
| Protein, %    | 3.11         | 2.90      | 3.10         | 2.82      | 8.49 |
| Lactose, %    | 4.97         | 4.79      | 4.53         | 4.83      | 7.45 |
| Total solids, %| 12.52        | 12.18     | 11.91        | 12.50     | 7.51 |

*aCV = coefficient of variation.*
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