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Melatonin levels in Holstein-Friesian dairy cow milk

Melatoningehalte in der Milch von Holstein-Friesian-Kühen

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Summary

In the present study, milk melatonin concentrations of 37 Holstein-Friesian cows were determined by ELISA bimonthly for one year to record seasonal melatonin secretion. Mean melatonin concentrations ranged from 5.4 pg/ml in February up to 11.8 pg/ml in August with statistically significantly highest values in August and June and lowest concentrations in February. However, milk melatonin concentration is not related to fat/protein ratio, milk yield, somatic cell score and the number and stage of lactation. Mean melatonin concentration was 9.7 pg/ml prior the dry period, which was statistically significantly higher compared to 6.3 pg/ml after the dry period.

Keywords: diurnal rhythm, seasonal effects, Holstein-Friesian cow

Zusammenfassung

In dieser Studie wurden die Melatoninkonzentrationen in der Milch von 37 Holstein-Friesian Kühen mit einem ELISA im Abstand von zwei Monaten über den Zeitraum von einem Jahr gemessen, um den Melatoningehalt im jahreszeitlichen Verlauf zu ermitteln. Die Melatoninkonzentrationen variierten zwischen 5,4 pg/ml im Februar und 11,8 pg/ml im August mit den statistisch signifikant höchsten Werten im August und Juni und den geringsten im Februar. Die Melatoninkonzentrationen in der Milch korrelieren nicht mit dem Fett-/Proteingehalt, der Milchmenge, der Zellzahl und der Anzahl und dem Stadium der Laktationen. Der mittlere Milchmelatoningehalt war 9,7 pg/ml vor der Trockenstehperiode und damit statistisch signifikant höher im Vergleich zu 6,3 pg/ml nach der Trockenstehperiode.

Schlüsselwörter: Tagesrhythmus , saisonale Effekte, Holstein-Friesian Kühe

Introduction

In many vertebrates the photoperiod as the predominant environmental cue is involved in the regulation of reproduction performance, body growths, improves milk yield and even alters melatonin secretion profiles (Dahl et al., 2000; Dahl et al., 2012). The neurohormone melatonin (N-Acetyl-5-Methoxytryptamine) is the major secretory product of the pineal gland, is secreted at night, is responsible for regularly sleep rhythms in humans (Brzezinski et al., 2005; Valtonen et al., 2005) and works as a free radical scavenger and antioxidant (Berra and Rizzo, 2009). The signal transduction starts with light perception at photoreceptors in the retina, which conducts signals to the suprachiasmatic nucleus via the retinohypothalamic tract and finally the pinealocytes of the pineal gland secrete melatonin (McMahon et al., 2014). It is possible to measure melatonin in blood samples, saliva and urine (Arendt, 1998) as well as in milk. Eriksson et al. (1998) report that milk melatonin concentrations confirm after 15 to 30 minutes to the blood concentrations. Light intensities below 50 lux and long wavelength lighting (above 525 nm) at night are required for high melatonin productivity (Lawson and Kennedy, 2001; Gnann, 2010) and result in 5.1 times higher serum melatonin concentrations during darkness (Stanisiewski et al., 1988). Concentrations of melatonin in serum increase rapidly (within 30 min) in response to darkness and also decrease within 30 min toward baseline concentrations when light intensity exceeds 50 lux (Buchanan et al., 1992).

Melatonin content in cow milk varies from 3.9 pg/ml up to 40 pg/ml (Eriksson et al., 1998; Valtonen et al., 2003; Valtonen et al., 2005; Kollmann et al., 2008; Milagres et al., 2014). Night-time milk or products thereof are proposed to enhance sleep quality due to the containing melatonin (Valtonen et al., 2005; Gnann 2010). Therefore, it is necessary to evaluate the melatonin content of milk. In a previous study, the melatonin concentration in raw milk of 10 Holstein-Friesian cows varied between 1.3 and 25 pg/ml. Although these variations of concentration were also observed by another sampling two weeks later, individual cows showed similar values (Schaper et al., 2015). Thus raised the question whether the melatonin production depends on individual constitution, which might be reflected in milk quality parameters. The duration of the nocturnal rise in melatonin is altered with the duration of darkness, i.e. the longer the duration of darkness, the longer the duration of increased nocturnal melatonin concentrations, which has also been proven in cows (Stanisiewski et al., 1988). This leads to the assumption of seasonal differences in melatonin production. However, there exist no data on seasonal milk melatonin concentrations of Holstein-Friesian dairy cows.

The main objective of this study was to examine, whether there are differences in milk melatonin concentrations within a period of one year. Furthermore, we evaluated if melatonin concentration correlates with fat/protein ratio, number of lactation, milk yield, and stage of lactation or the somatic cell score.

Material and methods

Animals

Thirty-seven healthy, adult dairy Holstein-Friesian cows were included in this study, which was conducted from August 2013 through June 2014. Each 9 cows were in the first, second, and third lactation while 10 cows were in the fourth lactation. They lived under equal conditions at the same farm in a cubical housing with slatted floors and were fed with mixed feed rations, hay and individual, concentrated feed dependent on the respective milk yield. The cows were milked twice a day in a double-four tandem milking parlor and the milk was daily delivered to the dairy. From the first of May until the end of September they grazed outside from 06:30 to 11:30.

Samples

Milk samples were obtained bimonthly a few days before milk quality testing to be able to interpret those data in the context of the milk melatonin level. One sample comprised altogether 200 ml mixed milk from each udder quarter. The raw milk samples were transported below 10 °C to the Institute of Food Hygiene and analyzed at the same day.

The milking started at 03:30 and lasted up to 05:30. During the whole trial (August 2013 until June 2014), the temperature and brightness in the middle of the cow shed was recorded every 30 minutes by a HOBO Pendant data logger (UA-002) to ensure that light intensity did not exceed 50 lux during milking. We could not gain milk samples for melatonin determination during the dry period and the sample of one clinical sick cow was rejected (cow 112, *Escherichia coli* mastitis in April).

Milk quality

Four to seven days after sampling the milk for melatonin determination, raw milk samples of each cow were gained and analyzed by the official milk quality testing institution (Landeskontrollverband Sachsen-Anhalt e. V. (LKV)). The testing was done according to the national regulation (Milchgüte-Verordnung). Individual data on fat/protein ratio, number of lactation, milk yield, stage of lactation and the somatic cell count were provided and collected by the LKV. The somatic cell count was converted to the somatic cell score developed by Shook (Wiggans and Shook, 1987).

Melatonin ELISA

The Melatonin direct Saliva ELISA (IBL-International GmbH, Hamburg, RE54041), was applied for quantitative determination of melatonin in milk according to Milagres et al. (2014), whereas we dilute the milk samples 1:5 as a slight modification. Although this test was originally designed for examination of saliva it was also successfully applied to detect melatonin in milk (Milagres et al., 2014; Schaper et al., 2015), serum, and whole blood (Ackermann et al., 2010). The further test was performed according to the manual of the manufacturer. The optical density was measured by a plate reader (Tecan Sunrise-Basic, Tecan Austria GmbH) at 450 nm. We developed a standard curve based on the standard dilution series (included in the ELISA kit). For each single plate, its equation was described by nonlinear fitting of the sigmoid curve using Prism 4.00 (Graph Pad Software Inc., USA) and therefore quantitative results were calculated for samples.

Data analysis

Mean milk melatonin concentrations of the months were compared by One Way Repeated Measures Analysis of Variance and followed by Bonferroni t-test, because data showed Gaussian distribution. Tests were conducted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., USA). Furthermore, Prism 4.00 (GraphPad Software Inc., USA) was used to conduct the unpaired t-test to detect differences prior and post dry period. Differences were considered to be statistically significant for p<0.05.

Pearson product-moment correlation coefficient was calculated using Excel 2007 (Microsoft Corporation) to quantify the strength of association between melatonin concentration in milk and fat/protein ratio, milk yield, number of lactation, stage of lactation, and somatic cell score.

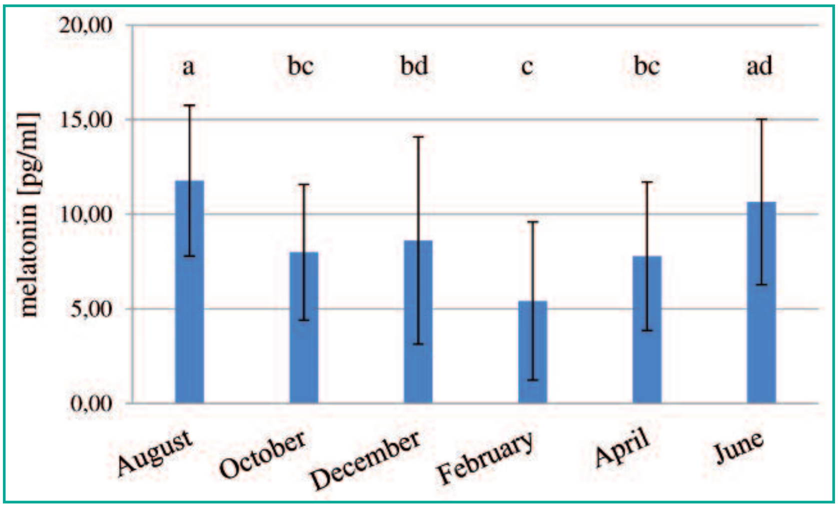

FIGURE 1: *Mean melatonin concentrations of different months in the milk of 37 Holstein-Friesian dairy cows (August 2013–June 2014). Different letters (a–d) indicate statistically significant differences (p < 0.05).*

Results

The mean melatonin concentrations of the milk from different months and the respective standard deviations are depicted in Figure 1. Mean melatonin concentrations were 11.8 pg/ml in August, 8.0 pg/ml in October, 8.6 pg/ml in December, 5.4 pg/ml in February, 7.8 pg/ml in April and 10.6 pg/ml in June. The standard deviations varied from 3.6 pg/ml in October up to 5.5 pg/ml in December. The quantity of samples was lower in December (n=26) than it has been in the other months, because one third of the test group was in the dry period. Furthermore, the letters in Figure 1 indicate statistically significant differences between the melatonin levels of the single months. Highest concentrations were obtained in August and June whereas the lowest content in raw milk was clearly shown in February. No statistically significant differences were achieved between October, December, and April. Detailed inspection on individual cows in the course of the study revealed that some values differed more or less considerable from the mean melatonin content. On the one hand, we could not detect any melatonin concentrations in some samples (October: one cow; December: five cows; February: four cows; April: two cows). On the other hand, some values were much higher than the mean (December: two cows with 16.5 pg/ml and 18.3 pg/ml; February: two cows with 13.1 pg/ml and 16.0 pg/ml; April: one cow with 15.8 pg/ml and the overall highest value in one cow in June was 25.8 pg/ml).

Table 1 shows the Pearson product-moment correlation coefficients, which quantify the strength of association between the measured milk melatonin content and the data of the milk quality testing (fat/protein ratio, milk yield, number of lactation, stage of lactation, somatic cell score). These quality parameters, as measured by the LKV, were in an expected range for Holstein-Friesian cows. The correlation coefficient of melatonin and the fat/protein ratio is –0.12, the milk yield –0.24, the number of lactation –0.21, the stage of lactation 0.24 and of the somatic cell score –0.14, respectively. Since the absolute values of r are 0.24 at most, there is no correlation between melatonin concentration and the milk quality test items.

TABLE 1: *Correlations between milk melatonin and milk quality parameters of Holstein-Friesian dairy cows.*

Parameter	Correlation coefficient, r*)
Fat/protein ratio	– 0.12
Milk yield	– 0.24
Number of lactation[a)]	– 0.21
Stage of lactation[b)]	0.24
Somatic cell score	– 0.14

*) Pearson product-moment correlation coefficient; [a)] number of previous calvings at start of the current lactation; [b)] number of days after calving

The mean melatonin concentration was 9.7 pg/ml prior the dry period, which was statistically significantly higher than after the dry period (6.3 pg/ml). Out of the 25 cows from which data prior and after the dry period could be obtained, in two of them the concentration remained the same. However, it has to be noted that for these two cows melatonin concentration was measured as 0.0 pg/ml at both time points. In milk of six cows, melatonin concentration was higher after calving compared to prior the dry period. The increase ranged from 0.3 to 12.0 pg/ml (median: 2.6 pg/ml, third quartile: 5.3 pg/ml) in the milk of the remaining 17 cows a lower melatonin concentration was observed after the dry period. The decrease ranged from 0.2 to 18.3 pg/ml (median: 3.4, third quartile: 9.0 pg/ml). The time span from the end of the dry period to the day of melatonin measurement ranged from 6 to 60 days (mean: 34.8 days; median: 38 days).

Discussion

In the present study, we investigated the melatonin production of Holstein-Friesian dairy cows in the course of one year and data were analyzed for correlation to the fat/protein ratio, milk yield, stage of lactation, number of lactation and the somatic cell score in milk. Melatonin peaks were generally recorded in the summer months (August and June), followed by a slight reduction in autumn and spring with the lowest values generally recorded in winter. In fact, the elevated melatonin concentrations in the summer months could be caused by more light hours during the day which results in a prolonged melatonin suppression. The longer this suppression during the day, the higher will be the melatonin peak at night (Stanisiewski et al., 1988; Newbold et al., 1991).

Contrary, in some other studies, no seasonal effect or rather effect of long or short photoperiods could be detected influencing the mean nocturnal melatonin concentration (Buchanan et al., 1992; Ogino et al., 2013). Buchanan et al. (1992) measured melatonin concentration in serum of sixteen Holstein females by using the direct assay, however, the animals were prepubertal calves and not cows which may account for the differences. In the study of Ogino et al. (2013), plasma of Japanese Shorthorn cattle was examined for melatonin. But they tested only four animals, so it would be unexpected to detect seasonal changes from such a low number of individuals.

In accordance to Eriksson et al. (1998) and Kollmann et al. (2008), melatonin levels in milk varied considerably between single cows. These individual differences, in spite of same living conditions in the cowshed, might be an indication that melatonin production is influenced by the individual genetic (Zarazaga et al., 1998). In a recently published study (Schaper et al., 2015), we found consistent milk melatonin concentration in individual cows as well as differences between cows which raised the question if individual melatonin secretion depends on the lactation status or single factors that are reflected in milk quality parameters. However, we could not identify any correlations between the fat/protein ratio, number of lactation, milk yield, stage of lactation or the somatic cell score and melatonin content in milk. In contrast, Eriksson et al. (1998) and Kollmann et al. (2008) explained that milk of cows in a late stage of lactation (10 month and 200 days post partum, respectively) contains more melatonin than of cows in early stage of lactation. Mean melatonin levels in our samples varied from 5.4 pg/ml in February up to 11.8 pg/ml in August. These values are low compared to 39.43 pg/ml in raw milk of ten Holstein cows milked at 02:00 (Milagres et al., 2013), 15–35 pg/ml melatonin in the milk of Holstein cows (Eriksson et al., 1998), 10-81 pg/ml plasma melatonin of prepubertal Holstein cows (Muthuramalingam et al., 2006), and about 90 pg/ml melatonin in plasma of adult pasture-bred French Friesian cows (Berthelot et al., 1990). The differences in melatonin concentration might be caused by the wavelength of the light in the cowshed. Using long-wavelength environmental lighting above 525 nm (ideal color: red; less well but possible: amber, orange or yellow) and an intensity below 50 lux ensured maximum levels of melatonin (Lawson and Kennedy, 2001; Kayumov et al., 2005; Gnann, 2010). The shed of the cows included in this study was equipped with warm-white LED spot-lights (2700 K) which may tend to reduce melatonin production (Gnann, 2010). However, the light intensity was below the limit of 50 lux during the whole milking session (03:30–05:30). Another explanation for the differences of the melatonin concentrations in our studies might be the high melatonin content of Poaceae species (Hattori et al., 1995) the cows could feed during grazing in May to September. The observation that milk samples from few cows did not contain any measurable melatonin was unexpected since this phenomenon has not been noted anywhere, yet. However, there are only few publications presenting results on melatonin concentration in milk, at all, and in some of these only mean values are presented but no individual values. So, there may have been milk samples without melatonin but they are not indicated. Cows in our study were milked in the dark between 03:30 and 05:30. Although not exceeding 50 lux, the light intensity increased with proceeding milking time. There may be differences in response to light intensity in individual cows, so that some will reduce or even stop melatonin production at lower light intensities than others and even below 50 lux. However, to prove this assumption, further research is needed.

The significant differences prior and after the dry period (9.7 pg/ml and 6.3 pg/ml melatonin in milk, respectively) must be critically examined. We only took samples in two-month intervals and, therefore, the days of lactation after calving of the single cows varied between 6 and 60 days. Within this time span melatonin concentrations could have been changed concisely. The individual differences of the milk melatonin levels between the cows, as discussed above, were also regained in the prior/post dry period values. Further studies with the same dry period intervals, in the same season and with cows in the same lactation are necessary to compare these data. Up to now, melatonin data in refer to the dry period have not been reported to our knowledge.

The direct Saliva ELISA (IBL-International GmbH, Hamburg, RE 54041) was previously applied by Milagres et al. (2014) and in our former study (Schaper et al., 2015) resulting in plausible values for melatonin concentrations in milk. However, deviant results of the cited studies may be due to usage of different methods (radioimmunoassay, ELSIA), test media (serum, plasma, milk) or breeds and different stages of development (prepubertal dairy heifers, Japanese shorthorn cattle, Ayrshire cows, French Friesian cows, Holstein-Friesian cows (Berthelot et al., 1990; Buchanan et al., 1992; Eriksson et al., 1998; Muthuramalingam et al., 2006; Ogino et al., 2013)).

In summary, the photoperiod seemed to influence melatonin concentrations in milk of Holstein Friesian dairy cows. However, because we could track the course of only one year, further studies over an extended period are necessary to describe seasonal effects on the milk melatonin concentration more precisely. Moreover, the milk melatonin content seems not to be associated with the fat/protein ratio, number of lactation, milk yield and stage of lactation or the somatic cell score since no correlations could be detected.

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Conflict of interest

We declare that there are no conflicts of interest.

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