Effects of melatonin on Dairy Herd Improvement (DHI) of Holstein cow with mastitis

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Research

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Abstract

Background: Mastitis is a common disease in cows breeding. The milk quality will be significantly reduced with increased milk somatic cells which often occur in cows with mastitis. In the current study, we reported that melatonin significantly reduced the milk somatic cell count in Holstein cow with mastitis. In addition, the nutritional value of the milk also significantly improved by melatonin treatment.

Results: The results showed that melatonin significantly suppressed the milk somatic cell score under any of the tested conditions. A long term of melatonin application (one year) to the cows with mastitis suppressed the somatic cell score by roughly 63% which was more efficiency than that of some antibiotic treatments. Melatonin treatment also improved the milk nutritional value by reducing its fat but increasing its lactose and protein contents.

Conclusions: Melatonin application significantly improved DHI. The beneficial effects of melatonin on DHI are likely attributed to the antioxidant and anti-inflammatory activities of melatonin.

Background

DHI (Dairy Herd Improvement) is a standard measurement used to evaluate the milk productive performance of the dairy cows [1]. DHI measurement includes the parameters of milk somatic cell number (SCC), milk yield, protein, fat, lactose, dry matter, urea nitrogen of the milk [2]. DHI establishment is an important approach to improve dairy herd performance and to breed high-yielding cow herds [3]. Among the parameters measured in the DHI, the SCC is a most important indicator to reflect the milk quality and the status of breast health of the cows [4]. Many factors influence the milk SCC. These include environmental stresses, number of fetuses, air humidity, feeding management, nutrition level, ect. [5, 6]. Among the environmental stresses, the seasonal alterations have a significant impact on the SCC [7]. For example, high temperature (hot stress) in summer often causes reduced milk production and elevated SCC [8]. Cows in different ages exhibit variable DHI indexes[9]. This partially relates to the fact that cows are sexually matured at the first mating time, but they still do not reach fully physical maturity, thus, with the continuous body development, the somatic cell score exhibits regular changes with the gestational times[10]. In addition, cows in the different lactation periods including early, peak, middle and late lactation periods have different milk production and physiological characteristics which also result in variable DHI values. For example, the SCC in the early stage of lactation is higher than that in the peak and middle stages of lactation[11].

SCC, lactose, fat, protein and urea nitrogen indexed in the DHI determine milk quality[12]. The higher the SCC, the lower the milk quality is[13]. When the milk SCC exceeds 200,000 /mL, the cow will be diagnosed as the mastitis. The extremely high SCC always indicates the serious breast infection of the cows[14]. Lactose is a unique ingredient in milk and is present in many infant formula and dairy products[15]. Milk fat is a complex mixture of different types of fat which is the main source of energy of the milk[16]. The milk protein is mainly casein, with small amounts of albumin and globulin[17] which is an important protein source of human diet. Urea nitrogen reflects the efficiency of nitrogen and protein metabolism in rumen. The urea nitrogen content provides accurate information as to how much milk should be consumed based on the daily protein diet requirement[18]. Changes in any of these factors mentioned above will affect the quality of raw milk and the quality of its processed products[19]. The milk with balanced composition ratio of these factors is considered to have suitable nutritional value [20].

To produce high quality milk with low SCC is a priority agenda for dairy famers as well as researchers. In the previous study, our group have found that melatonin application reduces milk SCC[21] and this study is the continuation of the previous work to investigation whether melatonin administration can also increase the milk nutritional value. This aspect has not been reported till now.

Melatonin (MT) is originally classified as an endocrine hormone mainly produced by the pineal gland in vertebrates. Currently, it is found that many cells, organs and tissues have the capacity to synthesize melatonin. These include

gastrointestinal tract[22], retina[23] bone marrow [24], skin[25], oocytes[26], etc. The main sites of melatonin synthesis are the mitochondria[27] and thus, actually all cells appear to have the ability to synthesis melatonin. Melatonin production exhibits an apparent circadian rhythm with the peak at dark and baseline level during the day[28]. Therefore, melatonin has been used to mimic the photoperiod signal to improve milk yield[29]. Few studies have reported the effects of melatonin supplement on milk quality in cows evaluated by DHI which is considered as the best index to reflect the milk quality in the field. In the current study, thus, we have adopted DHI to systemically evaluate melatonin's effects on the milk quality. The results are reported following.

Methods And Material

Chemicals

Melatonin is purchased from sigma company (Louis, MO, USA), and anhydrous ethanol is purchased from sinopharm group chemical reagent co., LTD (Shanghai, China). Melatonin was dissolved in ethanol and diluted with normal saline while in a darkroom. The solvent was prepared with 4.64 mg/mL melatonin.

Animals

Holstein cows with normal reproductive cycle, disease free and similar body condition were selected for the study. The cows were fed with TR diet (NRC2001) for 3 times at 06:00, 12:00 and 18:00 respectively, and after each feeding, the cows were allowed to enter the outside yard to drink and free activities.

Study Design

Based on the monthly reports of the DHI measurement, cows with the milk SCC 0.3-1 million /mL which are classified as mastitis were selected for the studies. In the current study, the effects of seasonal changes were also considered as to the melatonin treatment on milk quality. Melatonin was injected subcutaneously at 10am with the dose of 9.3 mg/cow/day, for 4 consecutive days. Then, 15 days later, the fresh milk was collected for DHI analyses. The general information on the study has been listed in the Table 1.

Table 1
General information of the cows used in the study

category	seasons				age structure						lactation period		
group	spring	summer	autumn	winter	2	3	4	5	6	7	peak	middle	late
Cattle NO.(MT)	90	74	83	81	23	80	66	56	27	19	15	38	59
Cattle NO.	728	702	662	686	507	681	601	502	159	62	21	54	98
Note: initial lactation period: 1–15 days after calving; Peak lactation period:16–100 days; Metaphase of lactation: 101–200 days; End of lactation: 201 days - no milk.													

DHI Measure

The 40 mL of fresh milk were collected and mixed with 2–3 drops of saturated potassium dichromate solution stored in 2–7°Cfor further analysis. DHI determination was performed by National Milk Product Standard Sanction Laboratory located in Beijing animal husbandry station. by use of the DHI measure instrument (MilkscanFT+&FC, denmark).

Statistical analysis

Somatic cell number (SCC) was converted into somatic cell score (SCS) using the formula: $SCS = \log_2(SCC/100) + 3$. All data were presented as means $\pm$ SEM. Multivariate analysis of variance was used for the comparison of multiple mean among groups and followed by the Paired t-test for the comparison of single mean between the groups. (SPSS software) $P < 0.05$ was considered as significant difference.

Results

1. Influence of seasonal changes on milk DHI index.

The results showed that the highest SCS occurred in Summer which was significantly higher ($p < 0.05$) than that in Winter (Fig. 1.a). The milk yield in Spring was significantly higher ($p < 0.05$) than that in summer and autumn (Fig. 1.b). The Ratio of protein in summer was the lowest ($p < 0.05$) among spring, autumn, and winter (Fig. 1.c). Lactose content in autumn was significantly higher ($p < 0.05$) than that in spring (Fig. 1.d). The dry matter content in summer was significantly lower than that in spring and autumn (Fig. 1.f). No significant differences were observed in fat and urea nitrogen content in different seasons (Fig. 1. e and g).

2. Effects of melatonin on milk DHI index with different seasons

The profound effect of melatonin on milk SCS was observed in all seasons, that is, melatonin significantly reduced the SCS in different seasons, with the maximum effect in Summer (Fig. 2. a). Melatonin application significantly ($p < 0.05$) increase the protein content only in Autumn (Fig. 2.c), and lactose content in Spring, summer and autumn (Fig. 2.d) ($p < 0.05$). Interesting, melatonin treatment significantly reduced milk fat content only in Spring ($p < 0.05$) (Fig. 2.e). Melatonin treatment had no significant effect on milk production, dry matter and urea nitrogen in different seasons (Fig. 2. b, f and g).

3. Influence of cow's age on milk DHI index

The results show that age is a reliable factor to impact the SCS. SCS was gradually increased with age. The oldest cows (7 years old) had the greatest SCS value and the youngest cows (2 years old) exhibited the lowest among other age groups (Fig. 3.a). The highest milk production was observed in the 3 year-old cows. The 7-year-old cows had the lowest milk yield among other age groups (Fig. 3.b). The milk protein content was relatively high in cows of 4 years old compared to the other age groups (Fig. 3.c). Milk lactose content had the trend decreased with age (Fig. 3. d). In contrast, the milk fat content increased with the age, but it rapidly declined in cows of 7 years old (Fig. 3. e). No significant differences were identified in dry matter and urea nitrogen contents among the different age groups (Fig. 3. f, g).

4. Effect of melatonin on milk DHI index in cows of different ages

Melatonin application significantly reduced SCS in all age groups, except in age of 6 year old group in which this decrease did not reach significant difference (Fig. 4.a). The results also showed that melatonin treatment significantly increased milk lactose content in cows at 3 and 4 years old groups compared to rest of the age groups (Fig. 4.d). The milk fat was significantly reduced by melatonin treatment only in cows at 3-year-old group compared to other groups (Fig. 3.e). No significant differences were detected on the daily milk production, protein, dry matter content and urea nitrogen content among the different age groups after melatonin treatment (Fig. 3.b,c,f and g).

5. Influence of lactation stages on milk DHI index.

The results showed that the lactation stages had a profound influence on the milk SCS. SCS was significantly increased as the lactation stage advanced with the highest SCS in the end of the lactation stage (Fig. 5.a). The milk yield in the middle lactation stage was significantly higher than that in the other stages (Fig. 5.b). The fat content in the early lactation stage was significantly higher than that in the end lactation stages (Fig. 5. e). No significant differences were detected as to milk protein, lactose, urea nitrogen content and dry matter in different lactation stages (Fig. 5. c, d, f and g).

6. Effects of melatonin on DHI index in cows with different lactation stages

Melatonin treatment reduced the milk SCS in the cows at the early lactation stage, but this decrease did not reach to significant difference. However, melatonin treatment highly significantly suppressed the SCS in middle and end lactation stages compared to the controls (Fig. 6.a). Melatonin treatment significantly elevated the milk lactose content at the early and end lactation stages compared to the controls (Fig. 6.d). No significant differences were detected on the milk yield, protein, fat, dry matter and urea nitrogen in the different lactation stages after melatonin treatment (Fig. 6. b, c, e, f and g).

7. Effect of long term of melatonin application (over one year) on milk DHI index in cows.

The effects of long term of melatonin application (12 months) on the DHI index were investigated. The cows were treated with melatonin for an entire year and their DHI indexes were analyzed. The data showed that this long term of melatonin treatment reduced milk SCS by 63.54% ($p < 0.01$), fat content by 11.31% ($p < 0.05$), urea nitrogen content by 11.08% ($p < 0.05$) and increased milk protein content by 3.79% ($p < 0.01$) and lactose content by 4.74% ($p < 0.01$), respectively, compared to the untreated controls (Fig. 7). No significant differences were detected on the daily milk production and milk dry matter after melatonin application.

Discussion

How to evaluate the quality of fresh milk is a challenge for dairy industry since it is still debatable for justification of levels of nutrition and residues of antibiotics in milk[30]. Nevertheless, DHI index is adopted for the entire dairy industry to evaluate the milk quality [31]. DHI index reflects dairy production performance and fresh milk quality in the individual cow. In the current study, we have systemically investigated the influences of seasonal changes, age and lactation stages on the DHI in the cows with potential mastitis. Most importantly, in the paralleled studies, the effects of melatonin on the DHI have also been studied under all of these environmental and physiological conditions. As we reported in the results that all of these alterations differentially impacted the DHI. For example, the reduced milk protein, fat and dry matter were found in the Summer (Fig. 1). These results were justified by the increased water intake of the cows in Summer to dilute these parameters [32]. The most significant alteration caused by seasonal changes, age and lactation stage was the SCS. The Summer, the advanced age and the end stage of lactation all significantly enhanced the milk SCS. These results were consistent with the previous reports. Green MJ *et al.* reported the highest SCS being found in Summer among other seasons [8]. Actually, Summer has the longest photoperiod among other seasons and animals exposed to the long photoperiod will drop their melatonin production [33]. This may be an additional reason to have a higher milk SCS in Summer. The increased SCS was also observed followed the increased lactation time due to the poor milking practices and breast management [34].

Milk SCS is an important index of breast health and milk quality. The higher the SCS and the lower the milk quality is [35]. Once the SCS was excessive of 3×10^5 cells/mL, it indicated the clinical mastitis of the cows and also increased the elimination rate of cows suffered for the mastitis[36]. Use of antibiotics is the conventional treatment for the mastitis and it effectively reduces the SCS[37]. However, frequently use of antibiotics leads to drug resistance of the animals and the residues of antibiotics in the milk are the safety concerning for human health [38]. Identification of remedies which can replace antibiotics to target the mastitis is an urgent task for researchers. We realized that the major etiologies of mastitis are associated with oxidative stress and pro-inflammatory reaction after infection [39]. Thus, in the current study, melatonin is selected for this purpose. Melatonin is a potent free radical scavenger and antioxidant [40]. It detoxifies a spectrum of reactive oxygen species (ROS) and reactive nitrogen species (RNS). It is also an anti-inflammatory molecule and its anti-inflammatory activities has been well documented [41]. The most notable changes observed in this study was the suppressive effect of melatonin application on the milk SCS. No matter in what conditions including in the seasonal alterations, increased age and advanced lactation stage melatonin uniformly and significantly reduced the milk SCS compared to the untreated controls. The long term of melatonin application (one year) to the cows yielded remarkable result that melatonin suppressed the milk SCS by 63.54% (Fig. 7). This SCS suppressive effect of melatonin was

consistent with the previous report which showed that subcutaneous injection of melatonin lowered the SCS and cortisol level in cows with subclinical mastitis [21]. It is known that majority of the milk somatic cells are derived from the neutrophils [42]. Many studies have reported that melatonin has the capacity to reduce the neutrophil infiltration into the infected tissue and then subsides the inflammatory reaction [43]. This may be also applied to the mastitis and reduce the milk SCS with melatonin treatment. In addition, oxidative stress is an important promoter of mastitis and melatonin has been reported to lower the oxidative stress in the mastitis of cows [44]. Thus, the milk SCS lowering effect is mainly attributed to the antioxidative and anti-inflammatory activities of melatonin [45]. In the study, we also observed that melatonin application modified some nutritional compositions of milk. It reduced the milk fat but increased the milk lactose and protein contents. Milk fat is mainly composed of triglycerides with nutritional value [46] and modification of its content is also the main target in dairy breeding [47]. Reports as to the effects of melatonin on milk fat are not consistent. Some reported that melatonin had no effect on milk composition [48] and others showed that melatonin indeed reduced the amount of fat in mid-lactating milk [49]. In this study, we observed that melatonin decreased milk fat content in different seasons, ages and lactation periods. Molecular mechanism study has identified that melatonin inhibits the mTOR signaling pathway through MT1 receptors in bovine mammary epithelial cells and via this pathway melatonin suppresses the synthesis of milk fat [50]. This observation provides a new basis for applying melatonin to regulate milk fat metabolism.

Lipid, sugar and protein can be inter transformation each other in body [51]. The decreased milk fat can be used to synthesize milk lactose and protein [52] to improve nutritional value of milk as observed in the study. In conclusion, the influence of seasonal changes, age and lactation stages on the DHI of dairy cows has been systemically studied. All of these factors impact the values of DHI. Melatonin application significantly improves the DHI under any condition mentioned above. The most profound effect of melatonin is to suppress the SCS. The long term of melatonin application (one year) to cows reduced the milk SCS by 63.54%. Such a great effect of melatonin on milk SCS has not reported previously. In addition, melatonin application improved the milk quality by reducing fat and increasing the protein and lactose. The beneficial effects of melatonin are likely attributed to its antioxidative and anti-inflammatory activity. It seems that melatonin may be a suitable candidate to replace the antibiotics to treat the mastitis in dairy cows. For this purpose, the further investigation is warranted.

Conclusions

In conclusion, our results indicate that a long term of melatonin application to the cows with mastitis improved DHI, in particular reduced SCS in milk. The effect of melatonin to low the SCS is comparable the antibiotics and in some cases, it is even better than that of antibiotics. Considering the safety of melatonin with none or low side effects to animal or human health, the development of melatonin products and their application in dairy farming can greatly improve industrial benefits.

Abbreviations

DHI: Dairy Herd Improvement; SCC: Somatic cell number; SCS: somatic cell score; ROS: Reactive oxygen species; RNS: reactive nitrogen species

Declarations

Authors' contributions

HW, SYY, LZ, GSL designed the study;

HY, WKM, PYJ performed the experiments;

TkW, JW, KR, YQL, HM contributed the materials;

HW wrote the manuscript;

HW, SY Y analyzed the data.

All the authors approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

All experimental protocols concerning the handling of animals were performed in accordance with the requirements of the Institutional Animal Care and Use Committee at the China Agricultural University (permission number:AW20151203-01).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The influences of seasonal changes on the DHI index in cows with potential mastitis. A:somatic cell score change : Spring (4.51±0.04), Summer(5.12±0.46), Autumn(4.31±0.20) Winter(3.76±0.28). B:change of milk yield: Spring (34.88±0.14,kg/d), Summer(33.60±0.19,kg/d), Autumn (33.61±0.33,kg/d), Winter (34.16±0.22,kg/d). C: protein content changes: Spring (3.45%±0.02), Summer (3.31%±0.03), Autumn(3.53%±0.02), Winter (3.47%±0.05). D: lactose content changes: Spring (4.99%±0.02), Summer (5.05%±0.02), Autumn(5.14%±0.01), Winter (5.08%±0.07). E: change of fat content: Spring(4.65%±0.17), Summer(4.23%±0.15), Autumn (4.60%±0.01) Winter (4.43%±0.32). F: change of dry matter content :Spring(13.79%±0.13), Summer (13.29%±0.17), Autumn (13.97%±0.02), Winter (13.68%±0.23). G: change of urea nitrogen content: Spring(14.61±1.98,mg/dL), Summer(14.25±2.26,mg/dL), Autumn(12.49±1.11,mg/dL), Winter (16.64±2.79,mg/dL). All data were represented by mean±standard error,(n=728 in Spring,n=702, in Summer,n=662 in Autumn,and n=686 in Winter),*p<0.05.

Figure 2

effects of melatonin treatment on DHI in cows with mastitis during different seasons. A. somatic cell score change: Spring (5.96 ± 0.08 VS 4.89 ± 0.15), Summer (6.06 ± 0.12 VS 4.09 ± 0.20), Autumn (6.03 ± 0.11 VS 4.55 ± 0.16), Winter (6.12 ± 0.11 VS 4.34 ± 0.17). B. change of milk yield: Spring (32.08 ± 1.04 VS 32.11 ± 1.15 , kg/d), Summer (34.09 ± 1.06 VS 32.69 ± 1.00 , kg/d), Autumn (32.88 ± 1.02 VS 32.07 ± 1.05 , kg/d), Winter (31.67 ± 1.11 VS 31.82 ± 1.17 , kg/d). C. protein content changes: Spring ($3.46\% \pm 0.04$ VS $3.49\% \pm 0.04$), Summer ($3.45\% \pm 0.06$ VS $3.45\% \pm 0.04$), Autumn ($3.51\% \pm 0.05$ VS $3.64\% \pm 0.05$), Winter ($3.39\% \pm 0.05$ VS $3.43\% \pm 0.05$). D. lactose content changes: Spring ($4.89\% \pm 0.02$ VS $4.94\% \pm 0.02$), Summer ($4.96\% \pm 0.03$ VS $5.06\% \pm 0.02$), Autumn ($4.99\% \pm 0.03$ VS $5.07\% \pm 0.04$), Winter ($4.70\% \pm 0.07$ VS $4.84\% \pm 0.04$). E. fat content changes: Spring ($5.18\% \pm 0.12$ VS $4.78\% \pm 0.14$), Summer ($4.48\% \pm 0.16$ VS $4.20\% \pm 0.12$), Autumn ($4.80\% \pm 0.11$ VS $4.68\% \pm 0.13$), Winter ($4.82\% \pm 0.10$ VS $4.75\% \pm 0.11$). F. change of dry matter content: Spring ($14.23\% \pm 0.14$ VS $13.92\% \pm 0.15$), Summer ($13.61\% \pm 0.17$ VS $13.42\% \pm 0.13$), Autumn ($14.00\% \pm 0.13$ VS $14.10\% \pm 0.14$), Winter ($13.68\% \pm 0.10$ VS $13.65\% \pm 0.12$). G. change of urea nitrogen content: Spring (13.08 ± 0.39 VS 14.35 ± 0.45 , mg/dL), Summer (16.59 ± 0.64 VS 14.19 ± 0.50 , mg/dL), Autumn (12.14 ± 0.43 VS 11.72 ± 0.36 , mg/dL), Winter (17.39 ± 0.47 VS 15.94 ± 0.51 , mg/dL). All data were represented by mean ± standard error, (n=90 in Spring, n=74 in summer, n=83 in autumn, n=81 in winter) * p < 0.05.

Figure 3

DHI changes of Holstein cows at different ages: A: somatic cell score : 2 years old (4.04 ± 0.24), 3 years old (4.33 ± 0.21), 4 years old (4.94 ± 0.15), 5 years old (4.77 ± 0.20), 6 years old (4.69 ± 0.19), 7 years old (5.27 ± 0.17). B: milk yield: 2 years old (32.94 ± 0.58 , kg/d), 3 years old (35.67 ± 0.66 , kg/d), 4 years old (31.86 ± 0.34 , kg/d), 5 years old (34.74 ± 0.56 , kg/d), 6 years old (34.26 ± 0.75 , kg/d), 7 years old (30.15 ± 1.57 , kg/d). C: protein content changes: 2 years old ($3.30\% \pm 0.03$), 3 years old ($3.35\% \pm 0.02$), 4 years old ($3.55\% \pm 0.50$), 5 years old ($3.46\% \pm 0.07$), 6 years old ($3.41\% \pm 0.05$), 7 years old ($3.45\% \pm 0.75$). D: lactose content changes: 2 years old ($5.17\% \pm 0.02$), 3 years old ($5.08\% \pm 0.02$), 4 years old ($4.95\% \pm 0.05$), 5 years old ($4.84\% \pm 0.06$), 6 years old ($4.96\% \pm 0.03$), 7 years old ($4.94\% \pm 0.04$). E: changes in fat content: 2 years old ($4.46\% \pm 0.14$), 3 years old ($4.48\% \pm 0.10$), 4 years old ($4.73\% \pm 0.12$), 5 years old ($4.75\% \pm 0.08$), 6 years old ($4.92\% \pm 0.14$), 7 years old ($4.51\% \pm 0.24$). F: dry matter content changes: 2 years old ($13.64\% \pm 0.14$), 3 years old ($13.61\% \pm 0.10$), 4 years old ($14.01\% \pm 0.15$), 5 years old ($13.87\% \pm 0.10$), 6 years old ($13.98\% \pm 0.13$), 7 years old ($13.61\% \pm 0.21$). G: changes in urea nitrogen content: 2 years old (14.35 ± 0.86 , mg/dL), 3 years old (14.01 ± 0.15 , mg/dL), 4 years old (14.61 ± 1.01 , mg/dL), 5 years old (14.74 ± 1.04 , mg/dL), 6 years old (15.19 ± 1.09 , mg/dL), 7 years old (14.86 ± 1.37 , mg/dL). All the data were represented by mean $\pm$ standard error, (2 years old n=507; 3 years old, n=681; 4 years old, n=601; 5 years old n=502; 6 years old n=159; 7 years old n=62) *p<0.05.

Figure 4

effects of melatonin on DHI at different ages: A: somatic cell score : 2 years old (5.30 ± 0.27 VS 3.87 ± 0.33), 3 years old (5.70 ± 0.15 VS 4.31 ± 0.18), 4 years old (5.89 ± 0.17 VS 4.68 ± 0.18), 5 years old (5.50 ± 0.18 VS 4.04 ± 0.19), 6 years old (5.63 ± 0.26 VS 5.19 ± 0.27), 7 years old (5.91 ± 0.20 VS 5.03 ± 0.29). B: milk yield: 2 years old (32.06 ± 0.95 VS 32.12 ± 1.63 , kg/d), 3 years old (31.72 ± 1.04 VS 32.11 ± 0.96 , kg/d), 4 years old (30.85 ± 1.19 VS 30.03 ± 1.22 , kg/d), 5 years old (35.03 ± 1.54 VS 33.56 ± 1.52 , kg/d), 6 years old (33.71 ± 2.45 VS 35.19 ± 2.67 , kg/d), 7 years old (33.54 ± 1.73 VS 30.34 ± 2.08 , kg/d). C: protein content changes: 2 years old ($3.37\% \pm 0.07$ VS $3.41\% \pm 0.09$), 3 years old ($3.43\% \pm 0.04$ VS $3.44\% \pm 0.04$), 4 years old ($3.57\% \pm 0.06$ VS $3.66\% \pm 0.06$), 5 years old ($3.44\% \pm 0.06$ VS $3.55\% \pm 0.07$), 6 years old ($3.38\% \pm 0.07$ VS $3.38\% \pm 0.07$), 7 years old ($3.20\% \pm 0.06$ VS $3.32\% \pm 0.07$). D: lactose content changes: 2 years old ($5.20\% \pm 0.04$ VS $5.19\% \pm 0.03$), 3 years old ($4.95\% \pm 0.03$ VS $5.03\% \pm 0.02$), 4 years old ($4.80\% \pm 0.04$ VS $4.94\% \pm 0.03$), 5 years old ($4.80\% \pm 0.04$ VS $4.86\% \pm 0.05$), 6 years old ($4.85\% \pm 0.05$ VS $4.91\% \pm 0.06$), 7 years old ($4.90\% \pm 0.06$ VS $4.93\% \pm 0.06$). E: fat content changes: 2 years old ($4.65\% \pm 0.24$ VS $4.54\% \pm 0.26$), 3 years old ($4.73\% \pm 0.12$ VS $4.36\% \pm 0.11$), 4 years old ($4.97\% \pm 0.16$ VS $4.69\% \pm 0.13$), 5 years old ($4.83\% \pm 0.14$ VS $4.62\% \pm 0.16$), 6 years old ($5.14\% \pm 0.18$ VS $5.27\% \pm 0.21$), 7 years old ($5.16\% \pm 0.37$ VS $4.66\% \pm 0.37$). F: dry matter content change: 2 years old ($13.91\% \pm 0.22$ VS $13.87\% \pm 0.27$), 3 years old ($13.82\% \pm 0.12$ VS $13.57\% \pm 0.13$), 4 years old ($14.19\% \pm 0.17$ VS $14.18\% \pm 0.15$), 5 years old ($13.82\% \pm 0.17$ VS $13.87\% \pm 0.18$), 6 years old ($14.14\% \pm 0.20$ VS $14.13\% \pm 0.24$), 7 years old ($13.80\% \pm 0.36$ VS $13.48\% \pm 0.32$). G: changes in urea nitrogen content: 2 years old (12.78 ± 0.90 VS 13.90 ± 0.80 , mg/dL), 3 years old (14.09 ± 0.53 VS 13.58 ± 0.46 , mg/dL), 4 years old (14.30 ± 0.61 VS 13.32 ± 0.49 , mg/dL), 5 years old (14.95 ± 0.52 VS 14.09 ± 0.59 , mg/dL), 6 years old (15.38 ± 0.90 VS 15.79 ± 0.94 , mg/dL), 7 years old (16.47 ± 1.64 VS 15.11 ± 1.34 , mg/dL). All the data were represented by mean $\pm$ standard error, (2 years old n=23; 3, n = 80; 4, n = 66; 5 years old n = 56; 6 years old n = 27; 7 years old n=19). *p<0.05.

Figure 5

Effects of lactation stages on DHI A. Somatic cell score in different lactation stages: at the peak lactation stage (2.26 ± 0.31), at the middle lactation stage (3.08 ± 0.25) and at the end lactation stage (3.13 ± 0.19). B: Milk yield: at the peak of lactation (33.19 ± 2.09 , kg/d), at the middle of lactation (38.93 ± 1.19 , kg/d) and at the end of lactation (32.14 ± 0.93 , kg/d). C: Protein content: at the peak of lactation ($3.42\% \pm 0.05$), at the middle of lactation ($3.40\% \pm 0.03$) and at the end of lactation ($3.43\% \pm 0.03$). D: lactose content : during peak lactation ($5.18\% \pm 0.04$), during middle lactation ($5.18\% \pm 0.03$) and at end lactation ($5.17\% \pm 0.02$). E: Fat content: at the peak of lactation ($4.36\% \pm 0.15$), at the middle of lactation ($4.13\% \pm 0.11$) and at the end of lactation ($4.00\% \pm 0.08$). F: Dry matter content: at the peak of lactation ($13.65\% \pm 0.14$), at the middle of lactation ($13.40\% \pm 0.12$) and at the end of lactation ($13.30\% \pm 0.08$). G: Urea nitrogen content: at the peak of lactation (12.09 ± 0.51 , mg/dL), at the middle of lactation (12.54 ± 0.50 , mg/dL) and at the end of lactation (11.82 ± 0.33 , mg/dL). All data were expressed by mean $\pm$ standard error, (n=21 in peak lactation. n =54 in middle lactation; End lactation n=98). *p<0.05.

Figure 6

effect of melatonin on DHI in different lactation stages A: Somatic cell score: peak stage of lactation (2.86 ± 0.32 VS 2.04 ± 0.24), middle stage of lactation (3.92 ± 0.25 VS 2.00 ± 0.17), and e end of lactation (4.34 ± 0.18 VS 1.99 ± 0.15). B: Milk yield: at the peak of lactation (32.93 ± 2.34 VS 38.80 ± 2.06 , kg/d), at the middle of lactation (39.98 ± 1.35 VS 39.08 ± 1.79 , kg/d) and at the end of lactation (31.58 ± 1.22 VS 31.25 ± 1.29 , kg/d). C: Protein content: at the peak of lactation ($3.47\% \pm 0.06$ VS $3.35\% \pm 0.06$) at the middle of lactation ($3.45\% \pm 0.04$ VS $3.45\% \pm 0.05$) at the end of lactation ($3.49\% \pm 0.04$ VS $3.40\% \pm 0.04$). D: lactose content s: at the peak of lactation ($5.15 \pm 0.05\%$ VS $5.25\% \pm 0.04$) at the middle of lactation ($5.14\% \pm 0.03$ VS $5.19\% \pm 0.02$) at the end of lactation ($5.12\% \pm 0.03$ VS $5.20\% \pm 0.02$). E: Fat content: at the peak of lactation ($4.45\% \pm 0.19$ VS $3.87\% \pm 0.26$), at the middle of lactation ($4.20\% \pm 0.12$ VS $4.22\% \pm 0.11$) and at the end of lactation ($4.07\% \pm 0.08$ VS $4.18\% \pm 0.12$). F: Dry matter content: at the peak of lactation ($13.76\% \pm 0.16$ VS $13.17\% \pm 0.29$) at the middle of lactation ($13.49\% \pm 0.14$ VS $13.56\% \pm 0.14$) at the end of lactation ($13.38\% \pm 0.09$ VS $13.47\% \pm 0.13$). G: Urea nitrogen content: at the peak of lactation (11.93 ± 0.65 VS 11.12 ± 0.64 , mg/dL) at the middle of lactation (12.67 ± 0.65 VS 12.11 ± 0.54 , mg/dL) at the end of lactation (11.45 ± 0.38 VS 12.04 ± 0.38 , mg/dL). All data were expressed by mean $\pm$ standard error, (N = 15 at the peak, n = 38 at the middle and n = 59 at the end of lactation) *p < 0.05.

Figure 7

influence of long term of melatonin administration on DHI Arrows indicated the directions of the changes and all these changes are significant ($p < 0.05$). Somatic cell score changes are highly significant ($p < 0.01$), $n = 328$.