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Skin Deiodinase Profiles after Melatonin Manipulated in Chinese Inner Mongolia Cashmere Goats*

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ABSTRACT : The roles of melatonin in the control of deiodinase (MD) activity in cashmere goat skin and associated cashmere fibre growth were investigated. Eighteen half-sib Chinese Inner Mongolia cashmere wethers were allocated randomly to two groups (n = 9/group). One group was implanted subcutaneously with melatonin (2 mg/kg BW) at three 2-monthly intervals while the other group served as a control. All goats were maintained under natural photoperiodic conditions and were grazed on natural pasture. The plasma melatonin concentration showed a significant difference ($p<0.01$) between the implant group (M) and the control group (C) but plasma T_4 (or T_3) showed no significant difference ($p>0.05$). The monodeiodinase type II (MDII) activity in skin tended to increase gradually from the summer solstice to November. During July and August, the activity of MDII for the M group was higher ($p<0.05$) than that of the C group, also during this period, there was a significant positive correlation between MDII activity of skin and cashmere fibre growth rate. The monodeiodinase type III (MDIII) activity and the ratio of MDIII and MDII tended to decrease from the summer solstice to November. The ratio of MDIII and MDII for the M group was lower ($p<0.05$) than that of the C group in July and August. The cashmere fibre growth rate of the M group was significantly greater than that of the C group in July ($p<0.01$), August ($p<0.001$) and September ($p<0.05$). The cashmere fibre diameter and guard hair and body weight were not influenced ($p>0.05$) by melatonin implantation. The results demonstrate that melatonin plays an important role in the regulation of skin MD activity. Simultaneously, the cashmere fibre elongation stimulated by melatonin may result from enhanced MDII activity during a period of two months after melatonin treatment. (**Key Words :** Deiodinase, Melatonin, Goats, Cashmere Fibre, Thyroid Hormone)

INTRODUCTION

In cashmere goats, the pattern of growth and moult of the cashmere is seasonal, with growth generally beginning after the longest day (the summer solstice) and moult occurring after the shortest (the winter solstice) (Klören et al., 1993; Santiago-Moreno et al., 2004). The performance of Chinese Inner Mongolia cashmere goats was described in a previous report (Chen et al., 2001; Zhou et al., 2003; Bai et al., 2006). Research has shown marked changes of the concentration several hormones that are related to follicle activity, fibre growth, and moult (Dicks et al., 1994). However, subsequent investigations suggest that follicle activity is not directly controlled by changes in hormone concentrations (Villar et al., 1999, 2000a), and that other physiological processes are involved.

The expression of deiodinase (MD) has been investigated: the monodeiodinase type II (MDII) and the monodeiodinase type III (MDIII) were shown to be present in the skin of cashmere goats. However, the monodeiodinase type I (MDI) was not detected (Villar et al., 2000b). In Scottish cashmere goats, Rhind et al. (2004a) found seasonal variation in MD expression, with a lower level of activity of MDII during periods of long daylength (July and April) and a higher level in December. These authors postulated that differences between individual and genotype in hair follicle activity and cashmere fibre growth were partially dependent on the pattern of expression of MD in the skin.

Exogenous melatonin can initiate growth of cashmere fibre (Nixon et al., 1993). Melatonin also stimulated fibre elongation of secondary follicles *in vitro* (Ibraheem et al., 1994), and histological examination of skin also showed that fibre growth was initiated by melatonin treatment

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(Nixon et al., 1993). However, melatonin receptors were not detected in the hair follicles of goats (Dicks et al., 1996). Therefore, the mechanism by which melatonin stimulates the cashmere fibre growth is not clear, and the relationship between skin MD activity and cashmere fibre growth is also unknown.

The first aim of this study was to investigate the roles of melatonin in the control of MD activity expression associated with cashmere fibre growth. The second aim was to determine the seasonal patterns of activity of MD in high-yielding Inner Mongolian cashmere goats from June (the summer solstice) to November.

MATERIALS AND METHODS

Animals and treatment

The study was performed at the stock farm of Inner Mongolia in China (latitude 39°06'N, longitude 107°59'E). Eighteen half-sib wethers were used, that had a mean (±SD) age of 15±2.5 months and an initial mean (±SD) body weight of 33.1±2.3 kg. They were allocated randomly to two groups (n = 9/group). One group was subcutaneously implanted with the melatonin implants (2 mg/kg BW; Northeast Forest University, China) at the base of the ear at three 2-monthly intervals commencing in June 2005 (the summer solstice). The other group served as the control. During the experiment period, all goats were maintained under natural photoperiodic conditions and were grazed on natural pasture. The composition of vegetation in this area has been described (Zhou et al., 2003).

Sample collections, recording and measurement

The fibre on the mid-side of each goat was dyed using human hair dye (CCP-N6, Jingxi Chemica Company, China) at the beginning of the study. The relaxed lengths of undyed cashmere fibre and guard hair were recorded monthly. Cashmere fibre growth rate were calculated according to the method of Rhind et al. (1995). At the beginning (21 June) and end (21 December) of the experiment, fibre samples from measured areas (2 cm×2 cm) were clipped at skin level, and were separated manually into cashmere fibre and guard hair. Each of which was weighed using an electronic balance (AUW220D, Shimadzu, Japan). The cashmere fibre diameters were measured using an optical fiber diameter analyser (Auda 2000, Shenaoda Technology Company, China). The mean diameter was calculated on >200 fibres per sample. Body weight of each goat was also recorded monthly throughout the study.

Blood samples were collected at 10:00 a.m. from all experimental goats by jugular venepuncture at one-monthly intervals throughout the study. The blood (EDTA anticoagulant) was immediately centrifuged, and the plasma frozen at -20°C until assayed. Skin samples (approximately 1 cm^2) were cut monthly from the mid-side region under local anaesthesia (Procaine hydrochloride, Huabei Medicine Company, China), snap frozen in liquid nitrogen and then stored at -70°C for subsequent determination of MD activity.

Hormone assays

Plasma melatonin concentration was determined using the Melatonin ELISA kit (RE 54021, Immuno-Biological Laboratories GmbH of Hamburg, Germany). The assay procedure was done according to the instructions for use. Plasma thyroid hormone (total T_4 and T_3) were determined using the commercial solid phase ^{125}I radioimmunoassay kit (Diagnostic Products, Beijing North Institute of Biological Technology, China). Intra-assay coefficients of variation were 4.2% and 9.1% for T_4 and T_3, respectively. The respective sensitivity of the assay was 2.6 ng/ml and 0.11 ng/ml for T_4 and T_3. The assay procedure according to the protocols described (Rajendran et al., 2001; Lohakare et al., 2006).

Enzyme activity assays

The MD determinations were conducted according to the protocols previously described (Villar et al., 2000b; Hans et al., 2004). A portion of skin was first disrupted using a freezer mill (6750-230 Freezer/mill, Spex Company, USA) and the fine powder was homogenized immediately in 5 ml of sodium phosphate buffer (125 mM, pH 7.4) containing 10 mM dithiothreitol (DTT). The homogenate was centrifuged at 1,000 g for 15 min at 4°C, and the supernatants were subsequently stored at -70°C for analysis of enzyme activities.

The protein concentration of skin homogenate was determined using a Coomassie brilliant blue G-250 reagent (Sigma-Aldrich Corp., USA) and an UV-Vis Spectrophotometer (UVmini-1240, Shimadzu, Japan).

The reaction mixture (200 μl of final volume) for the measurement of MDII activity contained 1 μM $^{[125I]}rT_3$, 2 nM rT_3, 10 μl skin homogenate (approximately 1-100 μg protein), 5 mM DTT, and 1 mM PTU. The reaction mixture (200 μl of final volume) for the measurement of MDIII activity contained 5 mM DTT, 1 mM EDTA, 125 mM sodium phosphate buffer (pH 7.4), 1 nM T_3, and 10 μl skin homogenate. Both reaction mixtures were incubated for 1 h at 37°C, and the reaction was terminated by the addition of 400 μl cold ethanol (4°C) (Rhind et a1., 2004b). The MDII activity was estimated by the amounts of free ^{125}I in the supernatant; the MDIII activity was estimated using $^{[125I]}T_3$ present in the ethanol extract that was quantified by binding to a specific sheep ant-T_3 antibody (Villar et al., 2000b).

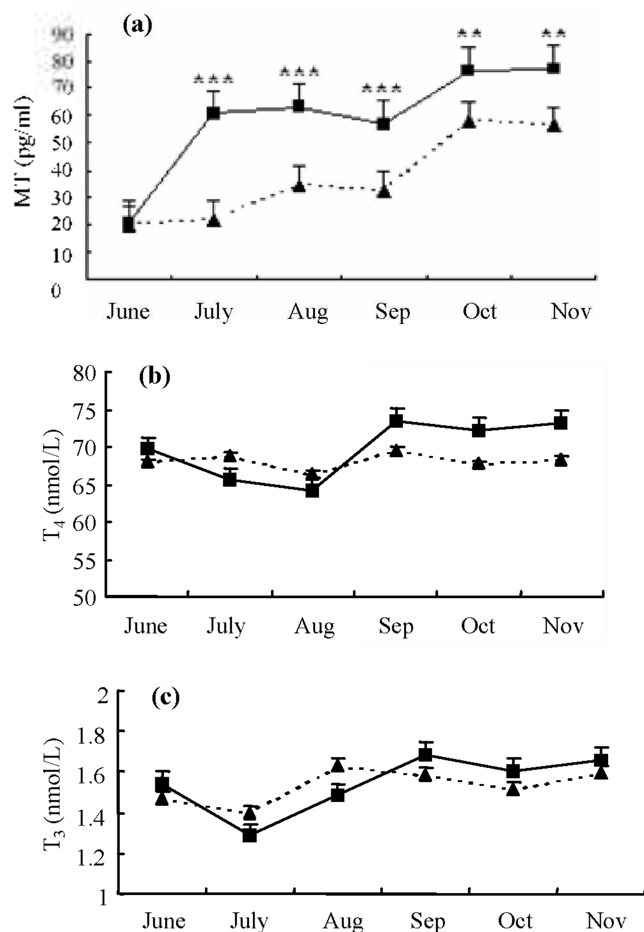

Figure 1. Both of melatonin implant groups (■) and control groups (▲) mean plasma melatonin (MT) concentration (pg/ml) (a), mean plasma thyroxine (T_4) concentration (nmol/L) (b) and triiodothyronine (T_3) (nmol/L) at monthly intervals (The samples of June was sampling before implants of melatonin) from 21 June (the summer solstice) to 21 November. Values of melatonin implant groups superscribed with *** are significant difference (p<0.001) to control groups (** p<0.01).

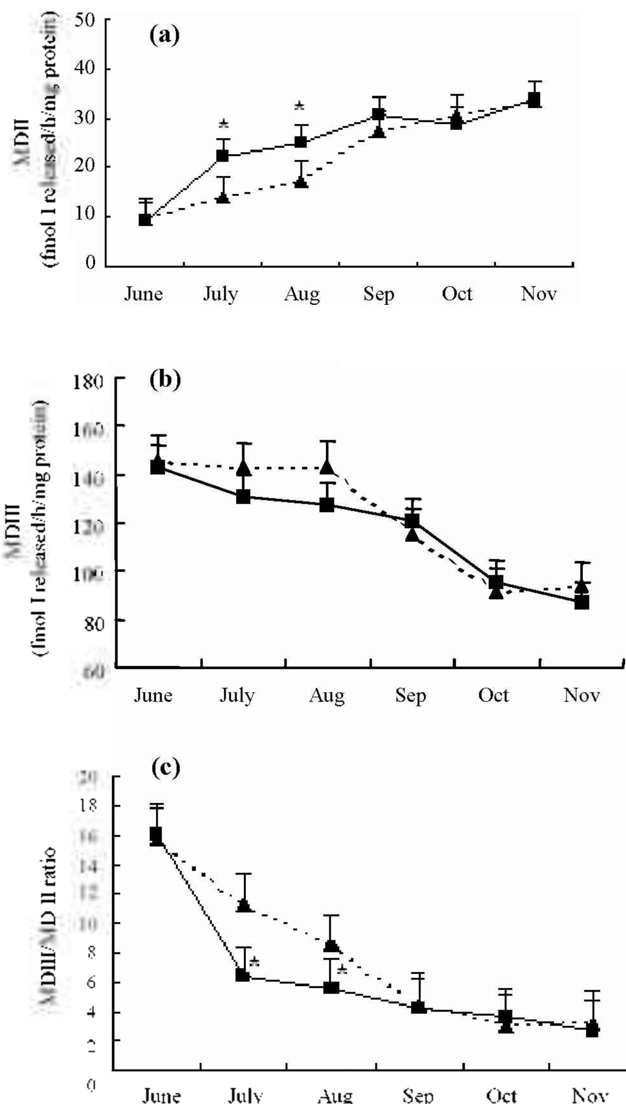

Figure 2. Both of melatonin implant groups (■) and control groups (▲) mean the monodeiodinase type II (MDII) activity (a), and the monodeiodinase type III (MDIII) activity (fmol iodine released/h/mg protein) (b), and mean MDIII/MDII ratios (c) in homogenization of the entire skin sample at monthly intervals (The samples of June was sampling before implants of melatonin) from 21 June (the summer solstice) to 21 November. Values of melatonin implant groups superscribed with * are significant difference (p<0.05) to control groups.

Statistical analysis

Data were analyzed using the t-test and ANOVA procedure of SAS (SAS, 1999). Significant means were separated using Duncan's multiple range test. Because the data exhibited a skewed distribution, the data of MDII, MDIII and MDIII/MDII were $\log_{10}$ transformed before analysis. Correlation coefficients for the MD activity and the cashmere fibre growth rate were calculated using Pearson's correlation coefficient, with animal as the experimental unit.

RESULTS

Plasma hormone concentration

Results showed no significant difference between groups before administration of melatonin in June (p>0.05) but there were significant differences (p<0.001) after administration in July, August and September. There were also significant differences (p<0.01) in October and November (Figure 1a).

The results of T_4 (Figure 1b) show that the mean plasma T_4 concentration was not consistent with seasonal changes, and there were no significant differences (p>0.05) between the M and C groups in each month. The plasma T_3 concentration exhibited a similar pattern (Figure 1c), however no significant differences (p>0.05) were detected between the two groups.

MD activity

The mean level of MDII activity was higher in the M compared with C group ($p<0.05$) in July and August, but there was no significant difference during other months. MDII activity in skin exhibited marked seasonal variation (Figure 2a), and that was a trend towards increasing MDII activity from the summer solstice to November for both M and C. There was a significant positive correlation between cashmere fibre growth rate and MDII activity of skin in July ($r = 0.79$, $p<0.05$), August ($r = 0.67$, $p<0.05$), and September ($r = 0.61$, $p = 0.08$) in the M group, and in July ($r = 0.71$, $p<0.05$) and in August ($r = 0.68$, $p<0.05$) in the C group, but there were no significant correlation in other months.

MDIII activity also exhibited marked seasonal variation (Figure 2b), gradually decreasing from the summer solstice to November for both M and C. There was no significant correlation between cashmere fibre growth rate and MDIII activity.

The result of MDIII/MDII ratios (Figure 2c) showed a seasonal variation that decreased for both the M and C groups from July to November. Of importance is the ratio of MDIII/MDII which differed significantly ($p<0.05$) between M and C in July and August. There was no significant correlation between cashmere fibre growth rate and MDIII/MDII ratio.

Profiles of body weight, cashmere fibre and guard hair

Body weight steadily increased from June to December (Table 1) but there were no significant differences ($p>0.05$) between the M and C groups. In both groups, guard hair length increased continuously each month from June to December but there were no significant differences ($p>0.05$) between the M and C groups (Table 1). The data for cashmere fibre length in the M and C groups from June to December (from the summer solstice to the winter solstice) are also presented in Table 1. There was no cashmere fibre growth before administration of melatonin on 21 June but the cashmere fibre length of the M group was significantly higher ($p<0.01$) than that of the C groups in July (C: three of the nine goats cashmere fibre outgrowth; M: the entire nine goats cashmere fibre outgrowth), August, September, October, November and December.

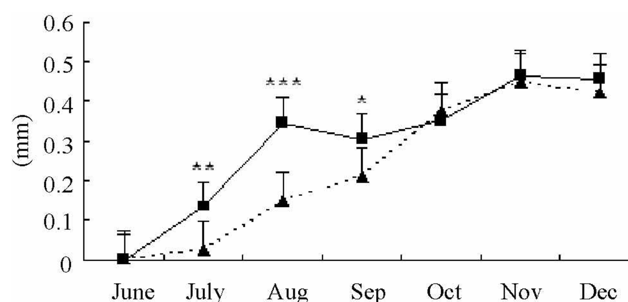

Figure 3. Both of melatonin implant groups (■) and control groups (▲) mean cashmere fibre growth rate (mm/d) at monthly intervals from July to December. Values of melatonin implant groups superscribed with *** are significant difference ($p<0.001$) to control groups (** $p<0.01$; * $p<0.05$).

The M cashmere fibre growth rate was markedly greater than that of the C group in July ($p<0.01$), August ($p<0.001$) and September ($p<0.05$). There was no significant difference between the M and C groups in October, November and December ($p>0.05$) (Figure 3). The cashmere fibre weight, guard hair weight and cashmere fibre diameters are presented in Table 2. Only the cashmere fibre weight of the M group is significant difference

Table 1. The data of body weight, guard hair length and cashmere accumulative length during experiment

Dates	Body weight (kg)		Guard hair length (mm)		Cashmere length (mm)	
	M	C	M	C	M	C
21 Jun	33.6±1.6^{c}	32.7±2.9^{d}	0.0±0.0^{g}	0.0±0.0^{g}	0.0±0.0^{f}	0.0±0.0^{f}
21 July	35.0±5.4^{c}	35.5±3.8^{d}	18.7±3.6^{f}	15.4±1.8^{f}	4.0±1.7^{f}, **	0.8±1.2^{f}
21 Aug	38.9±2.8^{b}	40.7±3.3^{c}	28.7±5.7^{e}	29.0±2.1^{e}	14.3±3.2^{e}, ***	5.2±2.1^{e}
21 Sep	43.4±1.8^{a}	42.2±3.4^{b,c}	36.2±4.8^{d}	37.4±3.8^{d}	23.4±4.6^{d}, ***	11.6±1.9^{d}
21 Oct	44.6±1.7^{a}	43.8±3.8^{b,c,a}	47.2±4.5^{c}	51.9±4.8^{c}	34.0±4.2^{c}, ***	22.9±2.7^{c}
21 Nov	45.3±2.2^{a}	45.0±3.6^{b,a}	57.3±7.4^{b}	60.3±5.7^{b}	47.9±5.2^{b}, ***	36.3±4.1^{b}
21 Dec	45.8±2.3^{a}	45.8±3.3^{a}	68.1±8.5^{a}	70.7±6.8^{a}	61.6±7.3^{a}, ***	49.0±3.6^{a}

M: the melatonin implant groups; C: the control groups; Means with different letters ($^{a, b, c, d, e, f, g}$) within columns are significant differences ($p<0.05$).
** Significant differences ($p<0.01$) to the control groups; *** Significant differences ($p<0.001$) to the control groups; all the values of body weight, guard hair length and cashmere length were mean±SD.

Table 2. The data of cashmere weight, guard hair weight and cashmere diameter from measured areas (2 cm×2 cm) at the beginning and end of the experiment

Item	Cashmere weight (mg)		Guard hair weight (mg)		Cashmere diameter (μm)
	21 Jun	21 Dec	21 Jun	21 Dec	21 Dec
M (Mean±SD)	0	162.6±13.**	101.9±11.1	195.9±30.4	15.2±0.7
C (Mean±SD)	0	132.1±20.4	108.9±10.2	206.3±35.2	14.8±0.6

M: the melatonin implant groups; C: the control groups; Means of M superscribed with ** within columns are significant difference ($p<0.01$) to C.

($p<0.01$) to the C group. However, the others are no significant differences ($p>0.05$) (Table 2).

DISCUSSION

An important role of melatonin is to mediate the regulation of seasonal rhythms by photoperiod in mammals, with melatonin production decreasing during long photoperiods (such as in summer) and increasing during short photoperiods (Vanecek, 1998). Our results show that, in Chinese Inner Mongolia cashmere goats, there were seasonal variations in plasma melatonin concentration with increasing from the June solstice to November (from long daylight to short daylight) for both groups. The data show that there were significantly enhanced plasma melatonin concentrations as a consequence of the melatonin implants. These results are similar to previous reports (Nixon et al., 1993; Santiago-Moreno et al., 2005).

Studies have already indicated that exogenous melatonin has a positive role on cashmere growth, resulting in increased cashmere production in some breeds (Teh et al., 1991; Jia, 1996); the consistent conclusion was obtained in Chinese Inner Mongolia cashmere goats (Wang et al., 2006). Our experiment supports the viewpoint that melatonin has an effect on cashmere fibre growth, especially during the three-month period after melatonin treatments. Histological examination of skin showed that structural reorganization of follicles began between days 6 and 12 after melatonin treatment, and cashmere fibres sprout by day 24; whereas untreated goats remained in the quiescent phase (Klören et al., 1993). So far, a primary cause of the initiation cashmere fibre growth and increased cashmere production after administration of melatonin is thought to be plasma prolactin concentration reduce (Dicks et al., 1995; Santiago-Moreno et al., 2004). Previous studies showed that goats skin was an active site of thyroid hormone metabolism (Villar et al., 2000b). However, the initiation and quick elongation of the cashmere fibers following exogenous melatonin administration was not associated with differences in MD activity as previously reported. In this study, the relationship between MD activity and cashmere fibre growth rate was observed from 21 June (the summer solstice) to 21 November (from proanagen to anagen) (Nixon et al., 1993).

Previous report indicated that the MDI activity was not detected in the goats skin but both MDII and MDIII activity could be detected (Villar et al., 2000b). The function of MDII is to convert T_4 to T_3, MDIII is to converts T_3 to T_2, and T_4 to reverse T_3 (rT_3) (Beckett and Arthur, 1994). MDII activity in the skin of Chinese Inner Mongolia cashmere goats exhibits seasonal variation from the summer solstice to November, which is consistent with a previous report (Rhind et al., 2004a). However, the level of MDII activity was higher in the M group compared with the C group after administration of melatonin in July and August, suggesting that the increase in MDII activity might be responsible for increased growth and elongation of cashmere fibre, especially during a period of two months after implantation. In addition, the correlation between cashmere fibre growth rate and MDII activity of skin indicates a close association. Therefore, it is likely that MDII plays an important role in initiating cashmere fibre growth, and this result supported the postulation of Rhind (2004a). The mechanism of melatonin stimulation on cashmere fibre elongation was possibly implemented, at least partly through enhanced MDII activity.

MDIII activity in skin is consistent with a previous report (Rhind et al., 2004a). However, the MDIII activity of the M group was lower than that of the C group in July and August, but the difference was not statistical significant ($p>0.05$), indicating that melatonin might inhibit MDIII activity during the initial stage of cashmere fibre growth.

The decreasing ratio of MDIII/MDII would regulate the change of T_3 and T_4 concentration within the skin, resulting in the enhanced skin T_3 concentration and reduced T_4 concentration. In the present research, the MDIII/MDII ratio presented a significant seasonal variation. This trend is consistent with previous report (Rhind et al., 2004a). However, this variation was in contrast to the seasonal change in melatonin concentration at the same stage. In particular, the ratio of MDIII/MDII differed significantly between the M and C groups after administration of melatonin. This difference may be necessary for the maintenance of T_3 bioactivity and cashmere fibre growth at that time; otherwise melatonin maybe have a important physiological function on expression of MD during the first two months after melatonin treatment. The declining MDIII/MDII ratio is consistent with increasing T_3 concentration but a previous report confirmed that melatonin treatment had no effect on plasma T_3 and T_4 concentration in goats (Dicks et al., 1995). Both T_3 and T_4 plasma concentration also showed no significant differences ($p>0.05$) in between the M and C groups within this study. However, there was a decreasing trend in plasma T_3 and T_4 concentrations after the first two months and afterwards increasing compared with M to C, but this was not statistically significant ($p>0.05$). This result suggest that plasma T_3 (or T_4) concentration maybe not be coincided with skin tissue T_3 (or T_4) concentration; the plasma T_3 (or T_4) concentration was mostly for other physiological systems.

The body weight no significant differences between the M and C groups. Previous reports also confirmed this viewpoint (Dicks et al., 1995; Jia et al., 1996). The gradual increase in body weight was possibly consistent with the increasing stage of maturity and seasons but has not been

related with melatonin treatments.

Guard hair length and weight for both groups increased during experiment, but there were no significant differences between M and C; this result was consistent with previous reports (Jia et al., 1996). At the end of the experiment (21 December), the cashmere fibre accumulative length and the cashmere production of M were significant increased than that of C, as a result of melatonin treatments. Simultaneously the cashmere fibre diameters are no significant differences ($p>0.05$), which is consistent with a previous report (Wang et al., 2006).

Implanted melatonin showed the differences in MDII activity and ratio of MDIII/MDII between M and C only in July and August, but no differences after September. The possible reason was that cashmere fibre grew fast in C group after August and physiology of goats in this period changed to increase the skin MDII activity and decrease the ratio of MDIII/MDII. Therefore, there were no differences between M and C in September, October and November. However, the correlations between circulating hormones, melatonin profile, MD activity and cashmere fibre growth are complex. Further studies are necessary to determine the mechanism of melatonin action on cashmere fibre growth and MD activity; including an investigation of the profiles of T_3 (or T_4) concentration and the site (or expression) of T_3 (or T_4) receptor in goat skin.

CONCLUSIONS

This study indicates that the skin MD activity showed a seasonal variation in Chinese Inner Mongolia cashmere goats, with the MDII activity increasing and the MDIII and MDIII/MDII ratio decreasing from June (the summer solstice) to November.

The results of this research demonstrate that melatonin played an important role in the regulation of skin MD activity. Therefore, it is hypothesized that the melatonin stimulates cashmere fibre elongation, by enhanced MDII activity during a period of two months after melatonin treatments.

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