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Original study

The effect of the diversified signal of melatonin on milk yields in seasonally breeding sheep

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Abstract

Previous studies demonstrated that milk yields in sheep displaying strong seasonal sexual activity depend on the day length. The objective of the studies was to determine whether the introduction of melatonin in high pregnancy affects milk secretion in seasonally sheep. The studies were carried out on 60 Polish Longwool sheep. Sheep were allocated to three groups: Group I (n=20 – the control group, lambed in February), Group II (n=20 – a group of sheep lambed in June and kept under natural day-length conditions), Group III (n=20 – a group of sheep with melatonin implants injected six weeks before lambing, sheep lambed in June). Lambs were reared with mothers up to 56th day of their life. When lambs were weaned, ewes were milked mechanically twice a day up to the dry period. Once a month collective milk samples were drawn from six sheep from each group in order to determine the concentration of melatonin. Milk yields were subjected to individual checks every 10 days. The studies demonstrated that sheep lambed in February (Group I) displayed the highest milk yields in the milking period (37.8±8.1 l). The milk performance of the two other groups was lower and amounted to 30.2±9.4 litres in case of sheep lambed in June and to 29.2±7.6 litres in sheep with melatonin implants. The introduction of melatonin signal to produce a short-day condition in state of high pregnancy in ewes caused a drop of milk yields both in the period of lambs raising and during milking.

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Abbreviations: d: days, G: group, MLT: melatonin

Introduction

Sheep are animals displaying very clear features of seasonal breeding. The dependence of reproduction on the season of the year is attributable to the organism sensitivity to changes in secretion of melatonin (MLT) from the pineal gland (Reiter 1991). Melatonin is synthesized and released at night, whereas at day time the secretive activity of the pineal gland is reduced (Arendt *et al.* 1988). Until now it was believed that milk yields in mammals are affected by genetic and environmental factors. In recent years, though, a special focus has been placed on light, being the modulator of the prolactin concentration. In farm animals changes of the light day play a very important role as they determine their yields. The introduction of e.g. long photoperiod in hens enhanced egg-laying, whereas in cows it produced a higher concentration of prolactin and increased milk yields (Peters *et al.* 1978, Reksen *et al.* 1999, Dahl *et al.* 2000, Morrissey *et al.* 2008). The photoperiod is of special importance in short-day animals, in which the day length involves changes of MLT concentrations (Karsch *et al.* 1984). In the autumn and winter (short-day period) higher concentrations of MLT are produced, whereas in the spring and summer the secretion of MLT decreases. Changes in the secretion of MLT affect the secretion profile of prolactin which is responsible for entering and maintenance of lactation. In the short-day period the increased secretion of MLT contributes to a decrease of prolactin secretion, and in the spring and summer period the short signal of MLT does not inhibit the synthesis of prolactin (Lincoln *et al.* 2003). Changes in MLT secretion throughout the year modulate the secretion profile of prolactin and have an effect on sheep's milk yields. Studies carried out by Molik *et al.* (2006, 2011) on lactating sheep showed that sheep entering lactation in the period of shorter days produced a yield of milk which was lower by 50 % compared to sheep milked in the long-day period. The shorter of days caused an increase of MLT concentration and a decrease of prolactin levels, which contributed to the reduced synthesis of milk in sheep. In studies performed by Molik *et al.* (2009) an attempt was made to maintain lactation in sheep lambed in June and milked in the period of shortened days by using artificial long-day conditions (16L:8D). In these sheep the length of lactation and the amount of obtained milk was similar to yields of sheep lambed in the same period (June) and kept under natural day-length conditions. Seasonal changes in MLT secretion have a significant effect on milk-performance parameters of sheep, and because of that, by determining physiological factors which are required for milk synthesis in seasonally breeding sheep, it is possible to control the period of using them for dairy production. It is probable that such a discovery can contribute to maintaining milk yields of sheep on a profitable level in the period of shorter days, in particular now when consumers expect healthy, ecological and harmless food, to which undoubtedly sheep milk belongs.

Therefore, the objective of the studies was to verify whether the introduction of exogenous MLT during the stage of a high pregnancy affects milk secretion in sheep.

Material and methods

Animals

The study was carried out at the Experimental Station of the Department of Swine and Small Ruminant Breeding of the Agricultural University of Krakow, Poland. Polish Longwool ewes (n=60) were used for the experiment. This breed exhibits strongly seasonal reproductive activity. The animals were four to five years old and weighed 60±5 kg. Prior to the beginning of the experiment, all animals were housed in individual pens under natural photoperiodic and thermoperiodic conditions (longitude: 19°57′ E, latitude: 50° 04′ N) and had a body condition score (BCS) of 3 (on a scale from 0 to 5, where 0=emaciated and 5=obese by Russel *et al.* 1969).

Dietary treatments

Throughout the experiment, sheep were fed according to their physiological status. From the mating to the end of the fourth month of pregnancy, sheep were fed in conformity with the standards of the National Research Institute of Animal Production (Norms 1993) based on: silage (dry matter 273 g/kg, 41 g of crude protein per kg, 1.53 MJ of net energy) and hay (dry matter 860 g/kg, 118 g of crude protein per kg, 3.83 MJ of net energy) (sheep lambing in February – Group [G] I) and forage pasture (dry matter 235 g/kg, 50 g of crude protein per kg, 1.36 MJ of net energy) sheep lambing in June (G II and G III).

In order to unify feeding from the fifth month of pregnancy to the dry period, all sheep (G I, G II, G III) received 1.5 kg of pelleted concentrate (7.5 MJ of net energy and 220 g of crude protein per kg of concentrate) and supplemental hay (dry matter 860 g/kg, 118 g of crude protein per kg, 3.83 MJ of net energy) for standardization (Norms 1993). All animals had free access to water and mineral licks.

Experimental design

The ewes were randomly allocated to three groups, with 20 animals per group. Oestrus synchronization was carried out with gestagens using the Chronogest method. Polyurethane sponges impregnated with 40 mg Cronolone (Intervet, Boxmeer, The Netherlands) were inserted in sheep intravaginally for 14 d and removed at the time of 500 I.U. PMSG injections (Serogonadotropin, Biowet, Drwalew, Poland). Oestrus occurred within 48-72 h of PMSG administration, and the duration of oestrus was additionally controlled using a teaser ram. Mating after oestrus synchronization was performed on 5 September for G I and on 15 January for G II and G III. Polish Longwool rams weighing 80±5 kg were used for natural mating. Ewes of G I lambed between 20-25 February and those of G II and III lambed between 18-24 June. Sheep of the third group were inserted exogenous MLT six weeks before lambing. Subcutaneous MLT implants (18 mg of exogenous MLT; Melovine, Ceva Animal, Libourne, France) were inserted into each ewe in G III at 90-day intervals (18 mg of exogenous MLT shows a biological activity for a period of 90 d). Experimental ewes reared twins and the mean prolificacy was 190-200 %. The mean body weight of lambs was 2.6 kg and a losses was 1 %. Parturitions were not complicated and the veterinary help was not needed. Lambs stayed with their mothers until 56 d of age, afterwards they were weaned and ewes were used for milking. When the lambs were reared, the milk production of the ewes was estimated based on the lambs' weight gain from 2 to 28 d of age using a conversion factor of 4.5 litres of milk per kg of weight gain. During the milking period, ewes were milked twice a day using an Alfa-Laval milking machine. Milk yields were recorded individually at 10-day intervals. For each ewe the dry period was defined as beginning when the milk yield was less than 50 ml per day.

From day 57 of lactation until the dry period, blood was drawn from each ewe every 28 d to monitor the changes in MLT concentrations. On each sampling date, 5 ml blood samples were collected at 20 min intervals over a period of 6 h beginning at 12:00. The whole blood was sampled using a catheter, which was inserted into the jugular vein 6 h prior to the blood collection program. After centrifugation in heparinized tubes, plasma was stored at −20 °C until hormone assays were performed. Blood sampling methodology was based on previous studies (Molik *et al.* 2007, 2010, 2011, 2013).

Melatonin was assayed in unextracted plasma according to the method of Fraser *et al.* (1983), modified by Misztal *et al.* (1996). Ovine anti-melatonin serum (AB/S/01, Stockgrand Ltd., Guildford, UK), synthetic MLT (Sigma-Aldrich, St. Louis, MI, USA) as a standard and [O-methyl-3H]-melatonin (Amersham, Amersham, UK) as a tracer were used. Melatonin free plasma for calibrated curve and blanks was obtained by early afternoon bleeds from sheep and striped from endogenous MLT by activated charcoal Norit-A (Sigma-Aldrich, St. Louis, MI, USA). The range of the calibrated curve was from 15.6 to 1 000 pg/mL and the working dilution of antibodies was 1:4 000. Bound and free tracers were separated after overnight incubation at 4 °C by dextran-coated charcoal. Sensitivity of the assay was 16.8±8.0 pg/ml and the intra- and inter-assay coefficients of variations were 10.5 and 13.2 %, respectively.

Statistical analysis

The milk-performance parameters (milk yield during the first 28 d of lactation, duration of lactation, d of milking and milk production) were expressed as the means±SEM (standard error of mean). The Mann-Whitney test was used to assess the differences among groups in lactation parameters. Plasma MLT concentrations are expressed as the mean±SEM. The effects of the treatments on plasma hormone concentrations were analysed using a one-way analysis of variance (ANOVA), followed by the post-hoc least significant difference test using SAS software (SAS Institute Inc., Cary, NC, USA). The differences in lactation parameters between groups were assayed by the nonparametric ANOVA rank Kruskal-Wallis test using the same SAS software.

Results

Lactation parameters

In the first 28 d of lactation milk yields of G I and G II sheep were significantly highest ($P≤0.01$) reaching the level of 63.2±13.9 and 61.8±11.2 litres. The lowest milk yields were observed in G III sheep amounting to 44.6±11.4 litres (Table 1). The obtained results of the studies demonstrated that G I sheep had the longest lactation period of 172±18.3 d. Lactation of G II sheep was significantly shortest ($P≤0.01$) and lasted 136±10.4 d. The significantly greatest milking persistency ($P \leq 0.05$) lasting 117±17.4 d was observed in sheep of G I. Sheep of G II and sheep with MLT implants were milked for the shortest periods (80±9.8 and 95±10.4 d respectively) and identified differences were statistically significant ($P \leq 0,05$) in relation to the control G.

Table 1
Parameters characterizing lactation duration and its efficiency of Polish Longwool sheep kept under natural lighting conditions (Group I, II), sheep with exogenous melatonin implants (Group III)

Groups/parameters of lactation	Group I	Group II	Group III
Milk yield of the first 28 days of lactation	63.2^{A}±13.9	61.8^{A}±11.2	47.6^{B}±10.9
Total length of lactation, days	171^{Aa}±18.3	136^{B}±10.4	147^{b}±12.1
Days of milking, days	117^{a}±17.4	80^{b}±9.8	95^{b}±10.4
Milk production during milking, liters	37.8^{a}±8.1	30.2^{b}±9.4	29.2^{b}±57.6

Table 2
Individual control (intervals 10 days) milk yield parameters of Polish Longwool sheep kept under natural lighting conditions (Group I, II), sheep with exogenous melatonin implants (Group III)

Milking Yield- (intervals for 10 days)	Group I	Group II	Group III
1st control milk	$0.48^{a.}$±0.018	0.42^{b}±0.008	0.43^{b}±0.013
2nd	$0.45^{a.}$±0.017	0.40^{b}±0.016	0.33^{b}±0.009
3rd	$0.50^{a.A}$±0.012	0.38^{b}±0.012	0.28^{B}±0.001
4th	$0.45^{A.a}$±0.011	0.35^{b}±0.015	0.26^{B}±0.001
5th	$0.37^{a.b}$±0.012	0.30^{b}±0.001	0.23^{b}±0.015
6th	0.35^{A}±0.001	0.21^{B}±0.006	0.18^{B}±0.013
7th	0.37^{A}±0.006	0.18^{B}±0.001	0.14^{B}±0.012
8th	$0.26^{A.a}$±0.006	0.15^{b}±0.002	0.07^{B}±0.009
9th	0.20^{A}±0.009	0.02^{B}±0.001	0.06^{B}±0.001
10th	0.17±0.002	-	0.02±0.001
11th	0.10±0.001		
12th	0.03±0.001		

Based on a detailed analysis of changes in milk yields the conclusion was drawn that sheep of G I on the 10th day of milking (first control milking) produced the significantly ($P \leq 0.05$) highest amount of milk at the level of 0.48±0.018 litres of milk daily (Table 2). The least milk was obtained from sheep of G II and G III (0.42±0.008 and 0.43±0.013 litres of milk daily respectively). During the second experiment milk yield in sheep from G I amounted to 0.45±0.017 litre of milk daily and was higher ($P \leq 0.05$) than milk yields of sheep of G II and G III (0.40±0.016 and 0.33±0,009 litres of milk daily). On the 30th day of milking (third control milking) the milk yield in sheep of G I amounted to 0.50±0.012 litres of milk daily and was significantly ($P \leq 0.01$) higher than milk yields of sheep of G III (0.28±0.001 litres of milk daily) and was higher ($P \leq 0.05$) than milk yields of sheep of G II (0.38±0.012 litres of milk daily). During 10 consecutive days of milking sheep of G I still produced the highest milk yields (0.45±0.011 litres of milk daily). Significantly ($P \leq 0.01$) lower milk yields were observed in sheep of G III (0.26±0.001 litres of milk daily) and was higher ($P \leq 0.05$) than milk yields of sheep of G II (0.35±0.015 litres of milk daily). In the fifth control milking sheep of G I produced 0.37±0.012 litres of milk daily. Sheep of G II and G III, in turn, produced significantly ($P \le 0.05$) lesser milk (0.30±0.001 and 0.23±0.015 litres of milk daily respectively). As lactation progressed, milk yields dropped both in sheep entering lactation in the long-day period (G I) and in sheep milked in the period of shorter days and exposed to the effects of exogenous MLT (G II, G III). On the 70th day of milking (seventh control milking) the milk yield in sheep of G I amounted to 0.37±0.006 litres of milk daily and was significantly ($P \le 0.01$) higher than milk yields of sheep of G II and G III (0.18±0.001 and 0.14±0.012 litres of milk daily). The significantly ($P \le 0.01$) highest milk yields, though, were recorded in sheep of G I (0.26±0.006 litres of milk daily), whereas mothers in G II and III produced as little as (0.15±0.002 and 0.07±0.009 litres of milk daily). During 10 consecutive days of milking sheep of G I produced significantly ($P \le 0.01$) more milk reaching the level of 0.20±0.009 litres of milk daily, whereas sheep entering lactation in the period of shorter days, G II, produced 0.02±0.001 litres of milk daily and G III sheep yielded 0.06±0.001 litres of milk daily. During the 10th control milking the milk yield in sheep of G I amounted to 0.17±0.002 litres of milk daily and milk yields of sheep of G II and G III were 0.02±0.001 litres of milk daily.

Secretion of melatonin

An analysis of the concentration of MLT in sheep of G I milked in the long-day period revealed that the concentration of MLT was significantly ($P < 0.05$) lowest in April and May (77.8±8.9 pg/ml, 73.3±6.3 pg/ml respectively) (Figure 1). As lactation progressed and days became shorter, the concentration of MLT increased and in June (91.3±6.4 pg/ml) and July (124.7±10.1 pg/ml) it was significantly ($P \le 0.05$) higher compared to previous drawn samples. In August secretion of MLT increased reaching the level of 132.8±16.1 pg/ml. In the last month of milking, September, the concentration of MLT amounted to 133.5±16.4 pg/ml. In sheep of G II, which were milked in the period of shorter days, in August the concentration of MLT

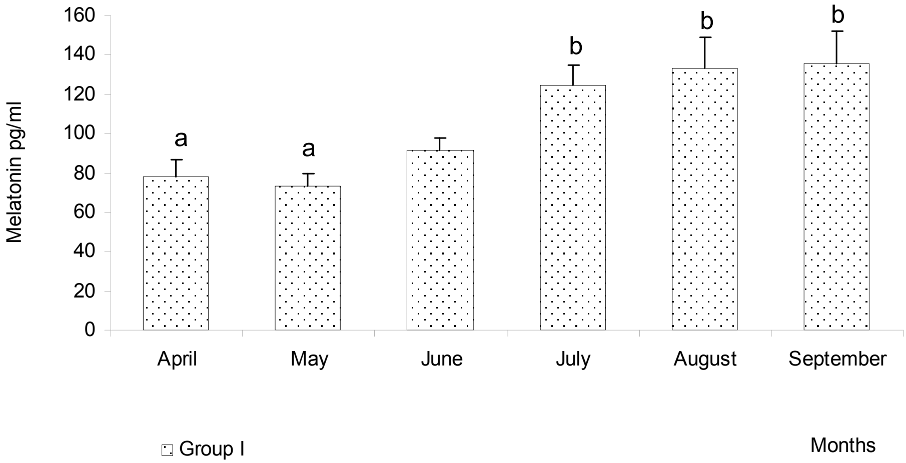

Figure 1
Mean (±SEM) plasma melatonin hormone sheep kept under natural lighting conditions (Group I)

[ABC]means in months denoted with the different letters differ significantly at $P \le 0.01$, [ab]means in months denoted with the different letters differ significantly at $P \le 0.05$. See text for statistical comparisons.

in the first month of milking equalled 22.44±8.75 pg/ml. In G III, in turn, the concentration of this hormone was 106.46±10.7 pg/ml and differences were statistically significant ($P \leq 0.001$) (Fig 2). In the second month of milking (September) the concentration of MLT in G II reached the level of 23.97±8.86 pg/ml and in G III it amounted to 97.16±10.7 pg/ml, whereas differences were statistically significant ($P \leq 0.001$). In October, on the 90th day of milking, the concentration of MLT in G II was at the level of 48.56±8.7 pg/ml, whereas in G III it amounted to 92.93±10.8 pg/ml with significant differences ($P \leq 0.001$). In the last drawn sample, in November, in G II the concentration of MLT amounted to 67.68±8.7 pg/ml and in the G with MLT implants it reached the level of 71.31±10.6 pg/ml.

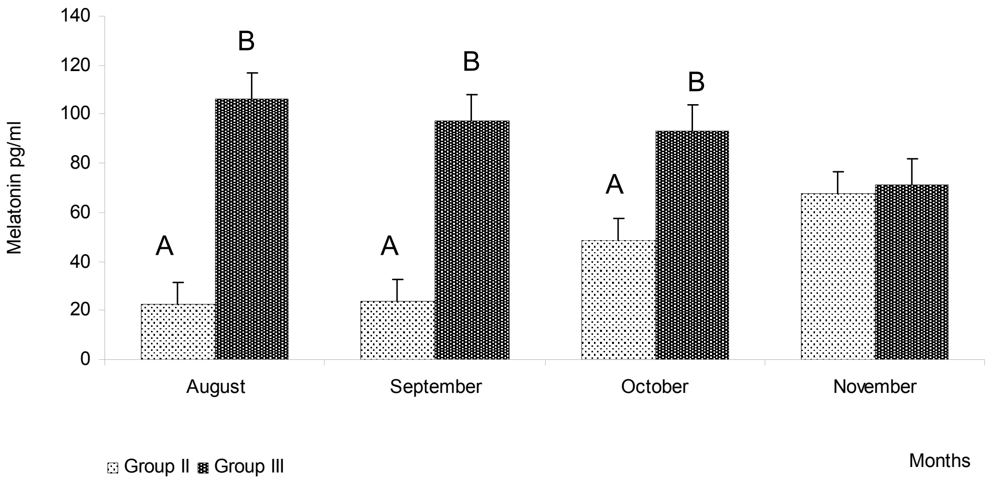

Figure 2
Mean (±SEM) plasma melatonin hormone sheep kept under natural lighting conditions (Group II) and sheep with exogenous melatonin implants (Group III)

[ABC]means in months denoted with the different letters differ significantly at $P \leq 0.01$, [ab]means in months denoted with the different letters differ significantly at $P \leq 0.05$, See text for statistical comparisons.

Discussion

In sheep, seasonal changes in secretion of MLT which are determined by the biological clock represent a signal in the annual reproductive cycle (Sweeney *et al.* 1999). The endocrine mechanism of entering and maintenance of lactation has not been fully understood. Despite that, it involves a great number of hormones, which proves that the process relies basically on the activity of hypothalamus and pituitary gland (Morgan 1996, 2000).

The present studies demonstrated that the introduction of exogenous MLT six weeks before lambing significantly decreased the sheep's milk yields in the first 28 d after rearing of lambs. At this time an increased level of secretion of MLT, which is a hormone inhibiting secretion of prolactin, did not cause a drop in milk secretion. Studies carried out by Misztal *et al.* 2008 and Górski *et al.* 2009 showed that the sucking factor, i.e. stimulation of the udder, gives rise to increased secretion of prolactin and growth hormone. In the milking period the longest lactation and the highest milk yields were observed in sheep milked in the long-day period. In these sheep MLT retained the features of the seasonal rhythm, i.e. a low level in the long-day period and a high level in the autumn and winter period. Sheep milked in the short-day period, in turn, had worse milk-performance parameters. The results of the studies conducted by Molik *et al.* 2007 demonstrated that a shift of the lactation period to the period of shorter days had a negative effect on milk-performance parameters of seasonally breeding sheep. Increased secretion of MLT in the period of shorter days contributed to lower milk secretion in sheep. The studies by Lincoln *et al.* (1992, 2000) and Misztal *et al.* (1999) carried out on barren sheep and rams demonstrated similar trends of the MLT profile. The milk-performance parameters in seasonally breeding sheep are undoubtedly affected by the profile of MLT which modulates secretion of lactothroph hormones. The conducted studies revealed that in sheep of G II the concentration of MLT started to increase from the very beginning of milking and corresponded with the endogenous rhythm. Milk yields in the first month of milking were significantly lower than those in sheep of G I. The duration of lactation and milk yields in sheep of G II were significantly lower.

The introduction of MLT implants during high pregnancy (six weeks before lambing) caused a drop of milk secretion from the very first days of milking, but the sheep had longer lactation than G II sheep. The data of milk yields between G II and III seem to be very interesting; in G III a lower milk yield was observed compared to G II. As a result of MLT implant insertion in a period of six weeks before parturition a decrease in milk field was noted. Study herein showed that introduction of exogenous MLT during the long-day period (May) did not created the state of MLT resistance during the short days and did not let maintain the milk field when day was shortened. At the beginning of the study we posted the hypothesis that introduction of the short day signal during pregnancy allow us to create the state of MLT resistance (from August till November) and would not lead to the decrease in milk yield.

The performed studies provided new information about the possibility of modulating milk secretion in sheep. The obtained results demonstrated that the introduction of a short-day signal by inserting MLT implants six weeks before lambing did not reduce the effect of the so called MLT resistance in the short-day period. Even the sucking factor in the period of lambs rearing did not overcome the inhibitory effect of MLT on the secretion of prolactin.

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