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The effects of exogenous melatonin on wool quality and thyroid function in Rasa Aragonesa ewes

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Abstract

The effect of melatonin on wool quality and thyroid function was studied. Ten ewes received a melatonin implant (M) on March 2004, and 10 ewes which were not implanted served as control (C). At monthly intervals over 12 months, fibre length and growth were calculated, and plasma thyroxine (T_4) concentrations were measured. Wool samples (~N = 2000 fibres) were collected and fibre diameter, standard deviation and coefficient of variation of fibre diameter, spin fineness, comfort factor, 5% of fibres «x» μm above the mean diameter, curve, and clean yield were measured. In summer, M ewes had significantly lower plasma T_4 concentrations than C ewes, with significant group ($P < 0.05$) and season ($P < 0.01$) effects. Melatonin ewes produced shorter wool than C ewes ($P < 0.001$) and, in autumn, the differences were statistically significant. Fibre growth did not differ significantly between groups. Throughout the year, M ewes produced wool with a fibre diameter (mean = 26.5 ± 0.2 μm) that was significantly ($P < 0.01$) shorter than C wool (means = 27.2 ± 0.5 μm). Overall, the melatonin treatment had a significant ($P < 0.01$) effect on mean comfort factor, and the differences between groups were significant ($P < 0.05$) in summer and autumn. The overall annual mean curve value of wool produced by M ewes (80.9 ± 1.7) was significantly ($P < 0.01$) lower than wool produced by C ewes (82.8 ± 1.3) and, in winter, the difference between groups was significant ($P < 0.05$). In conclusion, exogenous melatonin in spring positively affected medium- and long-term measures of wool quality. The physiological processes mediating these mechanisms remain to be elucidated.

Additional key words: fibre diameter, thyroxin, wool growth.

Resumen

Efecto de la melatonina exógena sobre la calidad de la lana y la función tiroidea en ovejas Rasa Aragonesa

Para estudiar el efecto de la melatonina sobre la calidad de la lana, 10 ovejas de raza Rasa Aragonesa recibieron un implante de melatonina (M) en marzo de 2004; otras 10 ovejas, sin implante, se consideraron control (C). A intervalos mensuales, se determinaron la longitud y el crecimiento de la fibra de lana y se determinaron los niveles plasmáticos de tiroxina (T_4). Además, se midieron diámetro, desviación estándar y coeficiente de variación de la fibra; *spin fineness*, *comfort factor*, 5% de fibras de «x» μm por encima del diámetro medio, curva y rendimiento al lavado. Durante el verano, las ovejas M presentaron niveles plasmáticos de T_4 significativamente menores que el lote C, con un efecto del grupo ($P < 0{,}05$) y la estación ($P < 0{,}01$). Las ovejas tratadas produjeron lana más corta que las control ($P < 0{,}001$) y en otoño, las diferencias fueron significativas. El crecimiento de la fibra no difirió significativamente. A lo largo del año, las ovejas M presentaron un diámetro de fibra (media = 26,5 ± 0,2 μm) significativamente más corta ($P < 0{,}01$) que la producida por las control (media = 27,2 ± 0,5 μm). De manera global, el tratamiento con melatonina tuvo un efecto significativo ($P < 0{,}01$) sobre el *comfort factor*, con diferencias en verano y otoño ($P < 0{,}05$). El valor medio de la curva de la lana de las ovejas M (80,9 ± 1,7) fue significativamente ($P < 0{,}01$) más bajo que el del lote C (82,8 ± 1,3) y, en invierno, la diferencia entre grupos fue significativa ($P < 0{,}05$). En conclusión, el implante de melatonina en primavera afecta de manera positiva, a medio y largo plazo, a la calidad de la lana.

Palabras clave adicionales: crecimiento de la lana, diámetro de fibra, tiroxina.

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Introduction

Sheep and other ruminants present an important control by photoperiod of some of their physiological processes, such as reproductive cycles (Yeates, 1949), spring moult (Zeuner, 1963), pelage growth (Ryder, 1964), changes in appetite and weight (Lincoln and Ebling, 1985), and horn growth (Lincoln, 1990). In most breeds of sheep, wool growth varies seasonally and is correlated with changes in photoperiod, temperature, and nutrition. The regulation of wool growth through those factors can result from systemic changes or to localized responses at the level of the wool follicle (Winder *et al.,* 1995). Sumner *et al.* (1994) suggested that most of the variation in wool growth rate is due to differences among sheep breeds.

The neuroendocrine system associated with reproduction receives photoperiodic information from the circadian secretion of melatonin by the pineal gland (Bittman *et al.*, 1983) and melatonin is used to advance the breeding season of sheep (Haresign *et al.*, 1990). The use of melatonin to influence seasonal reproductive cycles might pose a problem in fibre producer species, such as goat, because a sparse summer coat during winter can be a side effect of melatonin treatments (Deveson *et al.*, 1992). Exogenous melatonin is effective in shortening the coat maturation period in growing chinchillas and causing changes in the hair follicle cycle (Lanszki *et al.*, 2002). Moreover, when administered to German Angora rabbits around the summer solstice, melatonin treatments are effective in suppressing the normal summer decrease in wool production, which results in a significant improvement in wool production traits (Lanszki *et al.*, 2001).

In Mediterranean countries, spring shearing coincides with the time when melatonin implants are used to induce reproduction, and the exogenous melatonin might influence the pattern of wool production and the quality of the wool produced. A preliminary study of the effects of melatonin implants during the spring equinox suggested that wool growth rate was not affected (Abecia *et al.*, 2001). Advancing to the winter solstice the time at which Rasa Aragonesa ewes are given melatonin implants (Forcada *et al.,* 2002) raises questions about the effects of this hormone on wool production. Although the convention of improving sheep reproduction by treating ewes with melatonin in spring does not appear to have a negative effect on wool production in breeds that have different types of wool, melatonin treatments during the winter solstice might have a negative effect on wool growth in spring, without having an effect on fibre diameter (Abecia *et al.*, 2005).

The Spanish sheep breed Rasa Aragonesa has medium wool, which produces a medium-quality fleece that is white and has square strands. In Spain, it is the second most common breed (more than 2.5 million). Previous studies have focused on the effect of exogenous melatonin on the short- and medium-term effects on wool fibre characteristics (Abecia *et al.,* 2001, 2005). In this study, the objective was to determine whether implanting exogenous melatonin in sheep in spring affects the seasonal pattern of wool fibre growth and quality throughout the year. No effect of melatonin treatment on plasma concentrations of thyroxine (T4), neither evidence of an association between plasma thyroid hormone concentrations and changes in the hair follicle cycle in general, or in the spring moult in particular of Cashemere goats has been observed (Dicks *et al.,* 1995). However, since T4 influences the mechanisms that control wool growth (Donald *et al.,* 1994; Hynd, 1994; Williams, 1995) T4 concentrations were measured throughout the year.

Material and Methods

The study was conducted at the experimental farm of the University of Zaragoza, Spain (latitude 41° 41' N), which meets the requirements of the European Union for Scientific Procedure Establishments.The Animal Experimentation Ethics Committee of the University of Zaragoza approved all of the protocols.

Animals

On 8 March 2004, 20 Rasa Aragonesa ewes were stratified according to live weight (LW), body condition (BC) score (Russel *et al.*, 1969) and fiber length, and assigned by the use of a randomization protocol to the control or melatonin groups. Thus, they were divided into the melatonin (M) treatment group (LW 56.4 ± ± 1.0 kg, BC 2.8 ± 0.1, fibre length 2.6 ± 0.1 mm, n = 10), which received at the base of the left ear a single subcutaneous implant containing 18 mg melatonin (Melovine®, CEVA Salud Animal, S.A., Barcelona, Spain), or were assigned to the control (C) group (LW 56.1 ± 1.4 kg, BC 2.8 ± 0.1, fibre length 2.7 ± 0.1 mm, n = 10), which did not receive an implant. All of the animals were fed to meet their liveweight maintenance requirements and the experiment lasted 12 months (March to February).

Measurements

Fibre length was measured at 1-month intervals using the method of Rhind and McMillen (1996). At the start of the experiment, areas of wool on the midside of each sheep were dyed using black commercial hair dye. To measure the rate of wool growth, at monthly intervals, the relaxed length of the undyed portion of the fibres in those areas was measured to the nearest millimetre using callipers. At that time, on the right mid-side, a 2 × 2 cm patch of fleece was clipped to skin level and fibre characteristics were measured using the Optical Fibre Diameter Analysis (OFDA) method (Natural Fibre Centre, Olds, AB, Canada). In addition, blood samples were collected and assayed for T_4. Ewes were sheared on June 1.

To determine average fibre diameter (µm), standard deviation (variation in fibre diameter within a sample), and coefficient of variation (variation in fibre diameter throughout a fleece), about 2000 fibres were measured from each sample of fleece. Other fibre measurements taken on each sample included spin fineness (µm; an estimate of the performance of the sample when it is spun into yarn), comfort factor (the percentage of fibres that are under 30 µm), 5% of fibres «x» µm above the mean diameter, curve (deg mm^{-1}; the degree of the bend of the fibre snippet), which is correlated with crimp frequency, and clean yield (%), which is based on bone-dry, extractive-free wool fibre.

Thyroid hormone measurements were performed in a single assay using the Coat-A-Count® solid-phase ^{125}I radioimmunoassay for total T_4 (Diagnostic Products, Los Angeles, CA, USA), which has been validated in sheep (Moenter *et al.*, 1991). The intra-assay coefficient of variation was 8.7 % and the detection limit threshold was 2.5 ng ml^{-1}.

Statistical analysis

The measurements of plasma T_4 concentrations and wool fibre characteristics were categorized by season: spring (April, May, and June), summer (July, August, and September), autumn (October, November, and December), and winter (January and February). To determine whether the response to melatonin treatment varied by season, the comparison was performed using a factorial ANOVA and the following fixed effect model: $Y = Xb + e$; where Y is the N × 1 vector of records, b denotes the fixed effects in the model (four seasons and two treatments) with the associated matrix X, and e denotes the vector for residual effects.

Results

Liveweight and BC

At the end of the experiment, the LW and BC scores (both 2.8 ± 0.1) of melatonin-treated (LW of 56.3 ± 1.3 kg) and untreated ewes (LW of 56.2 ± 1.5 kg) were statistically indistinguishable.

T_4 concentrations

In melatonin-treated and untreated ewes, plasma T_4 concentrations varied markedly throughout the year and were lowest in summer (Fig. 1). Significant group ($P < 0.05$) and season ($P < 0.01$) effects were detected and, in autumn, melatonin-treated ewes had significantly lower plasma T_4 concentrations than did untreated ewes (Table 1).

Fibre length, growth, and diameter

Consistently, melatonin-treated ewes produced wool that had a shorter fibre length (Fig. 2A) than did control ewes (group effect $P < 0.001$) and, in autumn, the difference was statistically significant. Although fibre growth

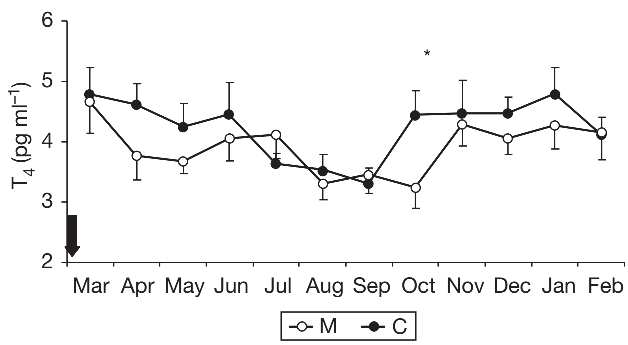


Figure 1. Mean (± SEM) plasma T_4 concentrations (pg ml^{-1}) in melatonin-treated (M, n = 10) and untreated (C, n = 10) Rasa Aragonesa ewes in northern Spain (41ºN). Treatment ewes were given a single dose of melatonin on 8 March (arrow). * = $P < 0.05$.

Table 1. Mean (± SEM) of plasma T_4 concentrations and wool fibre measurements from melatonin-treated (M, n = 10) and untreated (C, n = 10) Rasa Aragonesa ewes in northern Spain (41ºN). Treatment ewes were given a single dose of melatonin on 8 March and all of the ewes were sheared on 1 June

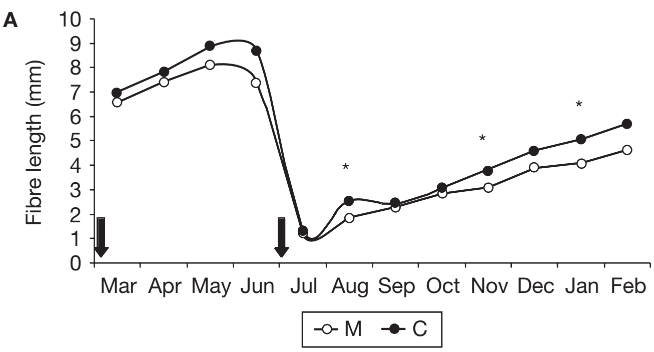
	Spring		Summer		Autumn		Winter		G[1]	S[2]	G × S
	M	C	M	C	M	C	M	C			
T_4 (ng ml^{-1})	3.8 ± 0.2	4.4 ± 0.2[g]	3.6 ± 0.2[c]	3.5 ± 0.1[h]	3.8 ± 0.2[ac]	4.4 ± 0.2[bg]	4.2 ± 0.3[d]	4.5 ± 0.3[g]	*	**	ns
Length (mm)	7.6 ± 0.3[ac]	8.4 ± 0.3[bg]	1.8 ± 0.0[d]	2.0 ± 0.3[h]	3.3 ± 0.3[ae]	3.8 ± 0.2[bi]	4.3 ± 0.2[fj]	5.3 ± 0.1	***	***	ns
Growth (mm)	0.9 ± 0.0	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	ns	***	ns
Diameter (µm)	26.5 ± 0.2	27.1 ± 0.4	25.9 ± 0.3[a]	27.6 ± 0.5[b]	26.3 ± 0.3	27.2 ± 0.5	27.5 ± 0.3	27.0 ± 0.5	**	ns	*
CV[3] (µm)	23.7 ± 0.6	22.5 ± 0.5	22.5 ± 0.6	21.8 ± 0.6	22.7 ± 0.6	22.4 ± 0.5	22.6 ± 0.6	22.4 ± 0.9	ns	ns	ns
C. factor (%)	78.3 ± 1.0	75.0 ± 2.0	81.5 ± 1.5[a]	72.7 ± 2.8[b]	79.9 ± 1.5[a]	73.7 ± 2.6[b]	73.4 ± 1.8	74.3 ± 2.7	**	ns	ns
Curve (deg mm^{-1})	83.0 ± 1.8	86.5 ± 2.0	82.1 ± 1.6	82.0 ± 2.5	81.6 ± 1.6	85.4 ± 2.1	84.1 ± 1.9[a]	79.6 ± 2.4[b]	**	ns	ns
SF[4] (µm)	26.4 ± 0.2	26.6 ± 0.3	25.5 ± 0.3	26.9 ± 0.5	25.9 ± 0.3	26.7 ± 0.4	26.7 ± 0.3	26.9 ± 0.5	ns	ns	*
5%[5] (µm)	10.7 ± 0.2	10.5 ± 0.2	10.3 ± 0.3	10.4 ± 0.4	10.4 ± 0.3	10.7 ± 0.3	11.1 ± 0.3	11.1 ± 0.4	ns	ns	ns
Clean yield (%)	61.1 ± 1.3[c]	63.8 ± 1.2[g]	64.6 ± 1.3[ad]	68.4 ± 1.3[bhi]	59.9 ± 1.0[ae]	66.0 ± 0.9[bi]	56.2 ± 1.0[e]	60.9 ± 1.2[j]	***	***	ns

([a,b]), ([c,d]), ([e,f]), ([g,h]), ([i,j]) mean $p < 0.05$. [1] Group. [2] Season. [3] Coefficient of variation. [4] Spin fineness. [5] 5% of «x» µm above the mean diameter. * P< 0.05. ** P < 0.01. *** P < 0.001.

did not differ significantly between melatonin-treated and untreated ewes (Fig. 2B), the effect of season was highly significant (P < 0.001), and the seasons were ranked as follows: summer < autumn < winter < spring. Exogenous melatonin had a significant effect on fibre diameter (Fig. 2C) (overall means for M and C ewes were 26.5 ± 0.2 and 27.2 ± 0.5, respectively, P < 0.01), and melatonin-treated ewes had lower values than did untreated ewes throughout the year. In C and M ewes, the seasonal patterns in fibre diameter were inverted. Control ewes had their highest and M ewes their lowest mean fibre diameters in summer (Table 1).

Other fibre measurements

Neither melatonin treatment nor season (Table 1) has a significant effect on the mean coefficient of variation of the fibre (Fig. 3A), spin fineness (Fig. 4A), or 5% of «x» µm above the mean diameter (Fig 4B). Melatonin treatment did have a significant (P < 0.01) effect on mean comfort factor (Fig. 3B) and, in summer and autumn, the M and C groups were significantly (P < 0.05) different (Table 1). Over the entire year, the mean curve values of M (80.9 ± 1.7) and C (82.8 ± 1.3) ewes (Fig. 3C) were significantly (P < 0.01) different



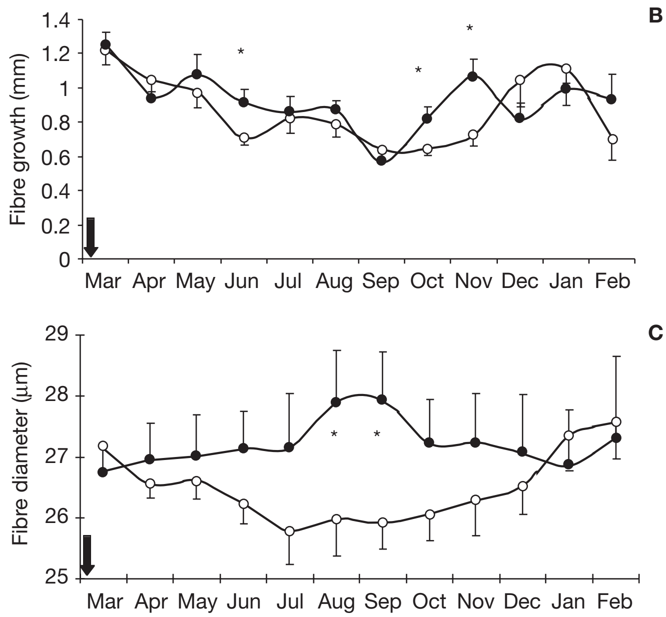


Figure 2. Mean (± SEM) fibre length (mm) (A), monthly growth (mm) (B), and diameter (µm) (C) in melatonin-treated (M, n = 10) and untreated (C, n = 10) Rasa Aragonesa ewes in northern Spain (41ºN). Treatment ewes were given a single dose of melatonin on 8 March (left arrow) and all of the ewes were sheared on 1 June (right arrow). * = P < 0.05.

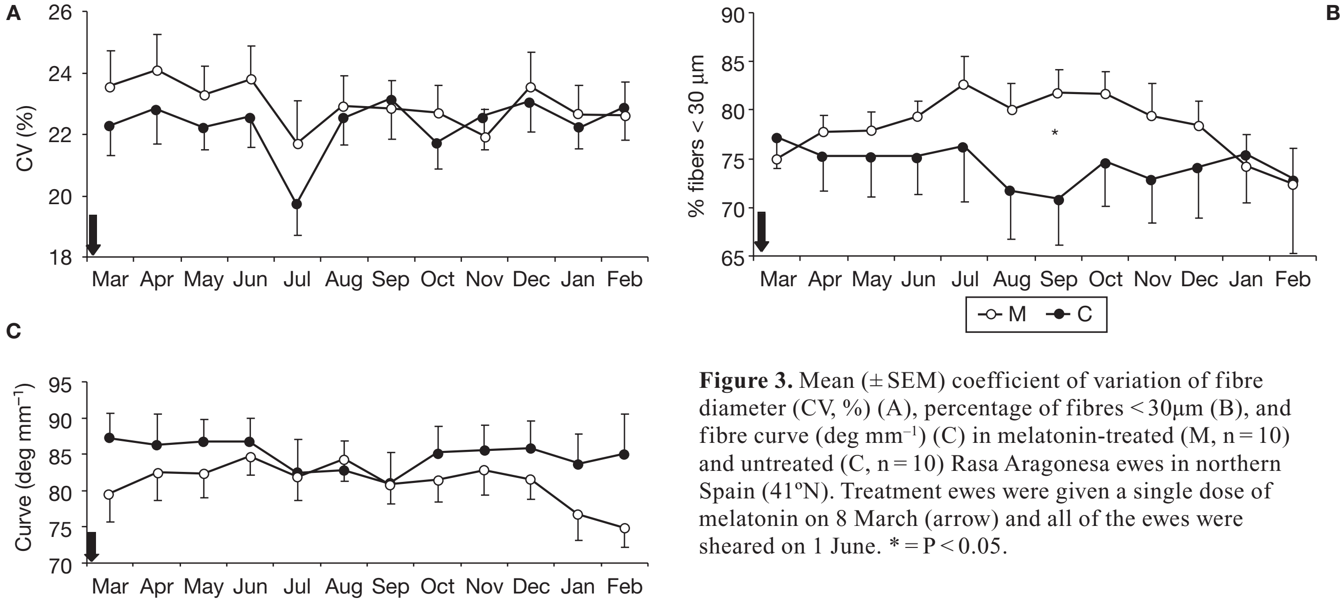


Figure 3. Mean (± SEM) coefficient of variation of fibre diameter (CV, %) (A), percentage of fibres < 30µm (B), and fibre curve (deg mm^{-1}) (C) in melatonin-treated (M, n = 10) and untreated (C, n = 10) Rasa Aragonesa ewes in northern Spain (41ºN). Treatment ewes were given a single dose of melatonin on 8 March (arrow) and all of the ewes were sheared on 1 June. * = P < 0.05.

and, in winter, the groups differed significantly (P < 0.05). Finally, mean bone-dry yield (Fig. 4C) was significantly affected by treatment and season (P < 0.001) as melatonin-treated ewes had lower values in each of the four seasons.

Discussion

The most remarkable result of this study was the significant effect of exogenous melatonin on fibre diameter, which resulted in a lower diameter in treated ewes throughout the year. Consequently, as reflected in some of the wool quality parameters, specifically, the percentage of fibres less than 30 µm and spin fineness, wool quality improved. To our knowledge, this is the first study to demonstrate such an effect of exogenous melatonin on wool quality in sheep, although it has been described in other species, e.g., goats (Deveson *et al.*, 1992), chinchillas (Lanszki *et al.*, 2002), or rabbits (Lanszki *et al.*, 2001). Apparently, these results contradict observations of the same group using the same breed (Abecia *et al.*, 2005), where exogenous melatonin did not show any effect on fibre diameter.

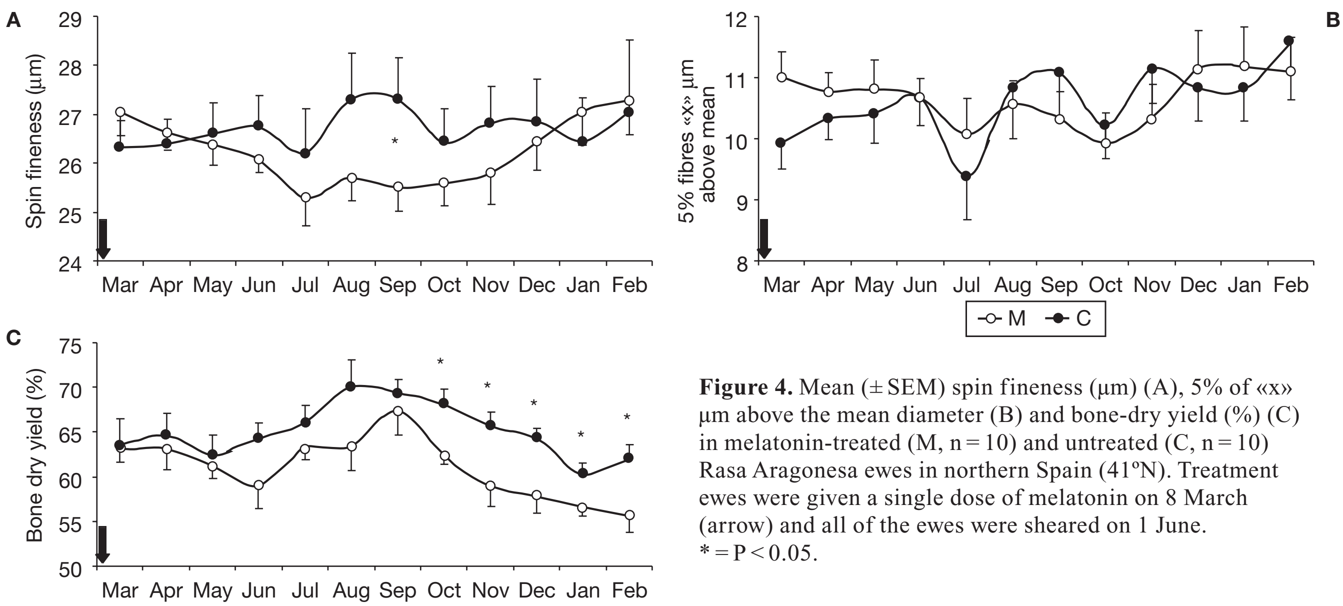


Figure 4. Mean (± SEM) spin fineness (µm) (A), 5% of «x» µm above the mean diameter (B) and bone-dry yield (%) (C) in melatonin-treated (M, n = 10) and untreated (C, n = 10) Rasa Aragonesa ewes in northern Spain (41ºN). Treatment ewes were given a single dose of melatonin on 8 March (arrow) and all of the ewes were sheared on 1 June. * = P < 0.05.

Since that experiment was performed using ovariectomized-oestradiol implanted ewes, the absence of any other ovarian effect on the mechanisms involved in the control of wool follicle development could mask the actual effect reported in the present experiment.

A positive genetic correlation between fibre diameter and liveweight has been demonstrated (for a review, see Adams and Cronje, 2003). Moreover, Birrell (1992) found a relationship between the production of clean wool, feed intake, pasture quality, and liveweight dynamics. Thus, the differences in the fibre diameter of wool in the two groups can be only attributable to exogenous melatonin because both groups were fed the same diet, and their LW and BC values were essentially the same throughout the experiment.

Exogenous melatonin had a significant effect on mean plasma T_4 levels and, in autumn, the M and C groups differed significantly. The pattern of secretion of T_4 in this study was similar to that observed in another study of Rasa Aragonesa ewes (Abecia *et al.*, 2005), although in that experiment sampling ended in August. In Corriedale sheep, Pérez-Clariget *et al.* (1998) observed a seasonal pattern in the secretion of T_4 that was similar to the one observed in this experiment; T_4 concentrations were highest at the end of winter and in spring, and lowest from the end of summer to mid-autumn. A similar pattern in the secretion of T_4 was observed in Welsh Mountain rams (Parkinson and Follett, 1994). In Iranian sheep, however, the highest values of T_4 were recorded from early summer through autumn and the lowest values occurred at the end of spring and in early summer (Zamiri and Khodaei, 2005). In tropical sheep breeds, the highest T_4 profiles were recorded in the summer (Ashutosh *et al.*, 2001), and Rhind *et al.* (2000) observed that T_4 concentrations were higher in the spring and summer than in the autumn. Thrun *et al.* (1997) hypothesized that, to promote seasonal reproductive suppression in the ewe, thyroid hormones are necessary only during a short interval late in the breeding season and the reproductive neuroendocrine axis is not equally responsive to thyroid hormones at all times of the year. If that is true, it suggests there is a critical period of responsiveness during which thyroid hormones must be present for anoestrus to develop, and it might explain the different seasonal patterns in the secretion of T_4 reported in the literature.

In this study, the marked seasonal pattern of wool growth was unaffected by exogenous melatonin, which is consistent with our previous observations in the same breed at the same latitude (Abecia *et al.*, 2001, 2005). Winder *et al.* (1995) found that the regulation of wool growth by environmental factors appears to be extrafollicular and there is no carryover of the *in vivo* growth rate when follicles are isolated *in vitro* (when systemic signals are absent) and treated with melatonin and prolactin. They concluded that the selective breeding of domesticated sheep has suppressed the response of follicles to regulation by melatonin. That interpretation is supported by the findings of Dicks *et al.* (1996), who found that melatonin receptors are not present on the hair follicles or associated structures, and those of McCloghry *et al.* (1992), who found that pinealectomy had no effect on wool growth and wool follicle density in Merino lambs. Yet, Hales and Fawcett (1993) found a strong, positive correlation between wool production and pineal blood flow, which they concluded might be indicative of an enhancement of wool production by the pineal gland, e.g., by melatonin, because the pineal gland can be involved in cyclic changes in the growth of hair and wool.

In this study, exogenous melatonin had a negative effect on fibre length, but no apparent effect on wool growth; however, melatonin significantly reduced fibre curve. Fibre curve and fibre length are positively correlated; thus, the reduction in fibre length caused by melatonin was mediated through changes in the mechanisms controlling fibre curve, rather than to a direct effect on the mechanisms regulating fibre growth.

Exogenous melatonin significantly reduced the clean yield (the ratio of clean to dirty wool), which is surprising. When lanolin and wax content increases, dust content and penetration increase, and clean yield is reduced. The natural fat of sheepskin consists of a variety of components, including lanolin, waxes, triglycerides, fatty acids, cholesterol, mono-, and diglycerides (Marsal *et al.*, 2000), and there is evidence that melatonin can affect the fat components of skin, at least in Wistar rats (Gribanov *et al.*, 1999). In that species, total skin lipid content increased over 24 h after administration of melatonin, concentrations of triglycerides and phospholipids decreased, and the content of cholesterol, cholesterol esters, and free fatty acids increased by the end of the second day. It was concluded that blood and subcutaneous fat, as well as changes in the metabolic interrelationships among skin lipids, are involved in the response of skin to increases in the concentration of melatonin.

In conclusion, exogenous melatonin implanted in spring in Rasa Aragonesa ewes had a significant positive effect on wool quality in the medium- and long-term. The physiological processes that underlie the role of melatonin in improving some of the factors that define wool quality remain to be elucidated.

Acknowledgments

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