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

J. A. Abecia*, I. Palacín and F. Forcada

Departamento de Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria.
Miguel Servet, 177. 50013 Zaragoza. Spain

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 (T4) 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 T4 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 (T4). 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 T4 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.

* Corresponding author: alf@unizar.es
Received: 13-12-06; Accepted: 13-03-07.
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 $2 \times 2$ 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 T4. Ewes were sheared on June 1.

To determine average fibre diameter ($\mu$m), 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 ($\mu$m; an estimate of the performance of the sample when it is spun into yarn), comfort factor (the percentage of fibres that are under 30 $\mu$m), 5% of fibres «x» $\mu$m above the mean diameter, curve ($\text{deg mm}^{-1}$; 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 125I radioimmunoassay for total T4 (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 T4 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 $N \times 1$ 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.

**T4 concentrations**

In melatonin-treated and untreated ewes, plasma T4 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 T4 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 T4 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). * = $P < 0.05$.](image-url)
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).

### Table 1. Mean (± SEM) of plasma T₄ 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.

<table>
<thead>
<tr>
<th></th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>C</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>T₄ (ng ml⁻¹)</td>
<td>3.8 ± 0.2 3.6 ± 0.2</td>
<td>4.4 ± 0.2 3.5 ± 0.1</td>
<td>3.8 ± 0.2 4.4 ± 0.2</td>
<td>4.2 ± 0.3 4.5 ± 0.3</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>7.6 ± 0.36 8.4 ± 0.38</td>
<td>1.0 ± 0.06 2.0 ± 0.38</td>
<td>3.3 ± 0.36 3.8 ± 0.26</td>
<td>4.3 ± 0.26 5.3 ± 0.1</td>
</tr>
<tr>
<td>Growth (mm)</td>
<td>0.8 ± 0.1 0.8 ± 0.0</td>
<td>25.9 ± 0.3 27.6 ± 0.5</td>
<td>22.7 ± 0.6 22.4 ± 0.5</td>
<td>22.6 ± 0.6 22.4 ± 0.9</td>
</tr>
<tr>
<td>Diameter (µm)</td>
<td>23.7 ± 0.6 22.5 ± 0.5</td>
<td>22.5 ± 0.6 21.8 ± 0.6</td>
<td>79.9 ± 1.5 73.7 ± 2.6</td>
<td>73.4 ± 1.8 74.3 ± 2.7</td>
</tr>
<tr>
<td>C. factor (%)</td>
<td>78.3 ± 1.0 75.0 ± 2.0</td>
<td>81.5 ± 1.5 72.7 ± 2.8</td>
<td>59.9 ± 1.0 66.0 ± 0.9</td>
<td>65.2 ± 1.0 60.9 ± 1.2</td>
</tr>
<tr>
<td>Curve (deg mm⁻¹)</td>
<td>83.0 ± 1.8 86.5 ± 2.0</td>
<td>82.1 ± 1.6 82.0 ± 2.5</td>
<td>81.6 ± 1.6 85.4 ± 2.1</td>
<td>84.1 ± 1.9 79.6 ± 2.4</td>
</tr>
<tr>
<td>SF⁴(µm)</td>
<td>26.4 ± 0.2 26.6 ± 0.3</td>
<td>25.5 ± 0.3 26.9 ± 0.5</td>
<td>25.9 ± 0.3 26.7 ± 0.4</td>
<td>26.7 ± 0.3 26.9 ± 0.5</td>
</tr>
<tr>
<td>5%&lt;x&gt; (µm)</td>
<td>10.7 ± 0.2 10.5 ± 0.2</td>
<td>10.3 ± 0.3 10.4 ± 0.4</td>
<td>10.4 ± 0.3 10.7 ± 0.3</td>
<td>11.1 ± 0.3 11.1 ± 0.4</td>
</tr>
<tr>
<td>Clean yield (%)</td>
<td>61.1 ± 1.3 63.8 ± 1.2</td>
<td>64.6 ± 1.3 68.4 ± 1.3</td>
<td>59.9 ± 1.0 66.0 ± 0.9</td>
<td>65.2 ± 1.0 60.9 ± 1.2</td>
</tr>
</tbody>
</table>

**Note:** (*, 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.

### 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.
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.
Since that experiment was performed using ovariec-
tomized-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 dy-
namics. Thus, the differences in the fibre diameter of
wool in the two groups can be only attributable to exo-
genous 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 T₄ levels and, in autumn, the M and C
groups differed significantly. The pattern of secretion
of T₄ 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 T₄ that
was similar to the one observed in this experiment; T₄
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 T₄ was
observed in Welsh Mountain rams (Parkinson and
Follett, 1994). In Iranian sheep, however, the highest
values of T₄ 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 T₄ profiles
were recorded in the summer (Ashutosh et al., 2001),
and Rhind et al. (2000) observed that T₄ 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 neuro-
endocrine 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 T₄ 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 corre-
related; thus, the reduction in fibre length caused by
melatonin was mediated through changes in the mecha-
nisms 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, trigly-
ecerides, fatty acids, cholesterol, mono-, and diglycerides
(Marsal et al., 2000), and there is evidence that melato-
ton 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 adminis-
stration of melatonin, concentrations of triglycerides
and phospholipids decreased, and the content of cho-
lesterol, 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 posi-
tive 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

This study was supported by grants PTR 1995-0784-OP (CICYT, Spain) and AGL-1817. We thank Dr. A. Martino (CEVA) for providing the implants.

References


PÉREZ-CLARIGET R., FORSBERG M., RODRÍGUEZ-MARTÍNEZ H., 1998. Seasonal variation in live weight, testes size, testosterone, LH secretion, melatonin and...