Institute of Wildlife Ecology

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Coat change in red deer hinds under the impact of exogenous melatonin, n-3 and n-6 PUFA supplementation

Diploma Thesis

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Submitted by

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Abbreviations:

ALA	Alpha linolenic acid
CW	Calendar week
DHA	docosahexaenoic acid
EB	Energy balance
EFS	Essential fatty acid(s)
FA(s)	Fatty acid(s)
LA	Linoleic acid
NST	Non-shivering thermogenesis
PUFA	Polyunsaturated fatty acid(s)
SCFA	Short chain fatty acid(s)
SCPB	Stratum corneum permeability barrier
Serca	Sarcoplasmic/endoplasmic reticulum calcium ATPase
T_b	Inner core body temperature
VFI	Voluntary food intake

1. Introduction

Mammals, especially in the northern latitudes, are confronted with a variety of challenges regarding their energy balance (EB) due to environmental changes throughout the year. To survive and maintain the population, they're dependent on adapting as best as they can, especially to the harsher conditions in winter. The animals need to adjust not only to way lower ambient temperatures, but also the changing food supply, which is not only scarcer in winter, but of lower quality, containing less usable energy. Summertime, on the other hand, must be used efficiently to accumulate fat reserves and reproduce. If the animals do not go into hibernation or daily torpor, they need to protect themselves at harsh winter conditions using other energy saving mechanisms, such as good thermal insulation by means of winter fur to maintain a high inner core body temperature (T_b) compatible with life, with a lower energy supply due to changing food supply. Voluntary daily food intake (VFI) is adapted, metabolic rate and digestion are optimized, T_b is lowered, and thus also the rumen temperature in ruminants is lowered and fat reserves built (Arnold et al. 2004, Arnold 2020, Turbill et al. 2011). Metabolic adaptations are also found when food supply gets restricted (Heydon et al. 1993, Loudon 1994). To be able to deal with the seasonal and environmental challenges, it is necessary to set these optimization processes in motion, right in time. This means adapting body condition and behavior before the external surroundings are already suboptimal. Animals must therefore be able to anticipate seasonal and environmental events early enough (Arnold et al. 2004, Arnold et al. 2018, Arnold 2020, Lincoln et al. 2003).

Seasonal cycles like molting are regulated melatonin-mediated by photoperiod (Arnold et al. 2018, Lincoln et al. 2003). Adaptions like coat change can not only be triggered by a colder environment, because the energy consumption during the coat change and an increased energy demand at already prevailing low temperatures, imbalances would be provoked (Lincoln et al. 2003, Pévet 2003, Xie et al. 2017).

Melatonin, secreted only during hours of darkness, is a direct mediator of photoperiod and thus part of the impetus to initiate the necessary seasonal adaptations (Arnold 2020, Heydon et al. 1993, Lincoln et al. 2003, Wehr 1997). In a study from 2003, measuring only the concentration of melatonin produced by the organism, adult Iberian red deer showed a high sensitivity to seasonal and daily changes in the concentration of this hormone. Factors influencing melatonin concentration are the respective body weight, season, photoperiod, and susceptibility to stress (García et al. 2003). Other studies have demonstrated the possibility of manipulating seasonal changes in red deer by exogenous melatonin administration. Physiological processes like coat change or the onset of the breeding season could thereby be anticipated and are likely to be directly related to the melatonin concentration in the body (Fisher et al. 1988, Heydon et al. 1995, Milne et al. 1990).

However, in addition to melatonin as the central pacemaker hormone in seasonal adaptation, the effects of polyunsaturated fatty acids (PUFA), n-3 and n-6 fatty acids (FA) in particular, on the body in the seasonal rhythm have been subject of numerous studies lately. n-3 and n-6 FA are indispensable in seasonal adaptation through their involvement in the maintenance of e.g membrane fluidity, onset of hibernation or it's quality (Arnold, Giroud et al. 2015, Giroud et al. 2013). A direct connection of n-3 and n-6 FAs on melatonin balance, and thus the annual rhythm of molt, could already be proven: In a former study a n-3 deficiency in diet seemed to significantly lower the melatonin level in the body of Syrian hamsters (Lavialle et al. 2008). On the other hand, exogenous melatonin administration (along with a standard diet) enhances the integration of n-6 in phospholipids (Pita et al. 2002). On top of that, both in human and veterinary medicine, the positive effects of n-3 and n-6 FAs on heart, skin and hair health are known qualities that are also significant for the coat change and coat quality (McCusker and Grant-Kels 2010, Scardino et al. 1999, Yang et al. 2019).

In addition to the direct effects of PUFA and melatonin on mammalian seasonality and body condition, there are other physiological processes that are important to consider in the context of energy management and metabolism. The significant energy expenditure required for a molt is described primarily for birds, but each individual must expend energy to produce new material such as fur (Buttemer et al. 2020). Furthermore, reproduction and milk production is generally associated with an increased energy expenditure or even a negative EB for the organism (Vries and Veerkamp 2000). Previous studies have already shown that lactating hinds have an increased need for food, which could be a disadvantage in generating necessary fat reserves depending on the food supply (Heydon et al. 1993, Heydon et al. 1995).

Because of the known effect of n-3 and n-6 on hair quality, skin health along with the effects on seasonal adaptation and melatonin secretion, we wanted to test, how n-6 or n-3 FA dietary supplementation influences molting in red deer (*Cervus elaphus*). We expect the n-6 group taking longer to shed their fur, due to the PUFAs metabolism slowing effects (Arnold et al. 2004, Arnold et al. 2011, Arnold, Giroud et al. 2015, Pita et al. 2002). The positive effects of n-3 PUFA on skin health, hair quality and the metabolism-stimulating properties, we expect a slightly faster coat change for these animals in summer and winter molting (Arnold et al. 2004, Arnold 2020, Lavialle et al. 2008, Scardino et al. 1999). In addition, we expect differences in onset of the coat change in the different diet groups under exogenous melatonin administration, since an inadequate n-3 level, or an imbalance manages to negatively influence the melatonin level in the body. Therefore, in the n-3 group, the coat change could be anticipated even further than in the n-6 group (Lavialle et al. 2008). As reproduction, lactation and a higher age have negative effects on EB and metabolism, those variables could negatively influence shedding, and we expect those to provoke slower coat change (Sguizzato et al. 2020, Shimokata and Kuzuya 1993, Vries and Veerkamp 2000).

2. Literature overview

The red deer (*Cervus elaphus*) is a species of deer found primarily in Central Asia, Europe and North Africa. Hinds reach a shoulder height of up to 120 cm and don't have antlers. As an intermediate grazing type, the red deer adapts its diet to the prevailing conditions. While in the summer fiber-poor and protein-rich food is favored, winter diet is based on rather protein-poor feed, since the metabolism is being reduced. The gestation period for red deer is about eight and a half months, calves are usually born between May and June (Niethammer 1986).

2.1.Basic information about red deer coat and molting

Without the influence of exogenously added drugs or feed additives, the seasonal coat change and molt of red deer was analyzed in Scotland in the 1970s. The results showed shedding beginning in February, with some excess hair that could be carefully pulled out. By April, the first hairs of the summer coat were visible, and the actual coat change began in May. The end of the transition from winter to summer coat occurred between June and July, with all individuals having finished in August. The winter coat transition began in September and most males were finished by October, while it took longer for hinds. All animals finished molting by December (Ryder 1977).

In general, red deer show a double coat in winter, consisting of coarse upper hairs, called guard hairs, and finer, softer under hairs. They show a morphology of typical keratin fibers. Underhairs don't have a pith, except at the tip of some hairs, and are only lightly pigmented with a tip of darker color. Coarse upper hairs are heavily medullated, except at the tip and root, and pigment density varies among the different fibers. In addition to these two hair types, there are three other, less common, fiber types: short, straight, dark hairs; guard hairs with a finer diameter and diameter constriction; long, straight, heavily pigmented guard hairs (Woods et al. 2011).

The undercoat of New Zealand red deer showed several similarities to cashmere fibers and other so-called luxury specialty fibers, such as merino wool. The undercoat of the red deer studied was similar in measured diameter, ultrastructure, and crimp characteristics (Woods et al. 2011). Since exogenously supplied melatonin has already been studied in connection with cashmere quality and yield, results of this work could also be of interest for wool industry (Yang et al. 2019)

2.2. Seasonal challenges and changes and their influencing factors, melatonin, n-3 and n-6 FA in mammals and especially red deer

The daily and annual routine in the lives of individuals is regulated by certain rhythms, which can be subdivided respect to their endogenous and exogenous influencing factors. Many biological processes are purely endogenous, i.e., controlled by the body itself, and exist even without environmental stimuli and then vary only slightly in their timing scheme. Endogenously controlled biological rhythms are also referred to as the "internal clock." These internal rhythms are vital for animals to anticipate the cycle of the year and it's environmental variations (Arnold et al. 2018, Gwinner 1986, Lincoln et al. 2003, Wehr 1997).

Analogous to endogenously controlled rhythms, there are also rhythms influenced and controlled by exogenous components, like by photoperiod. The exogenous stimulus is important to determine whether a certain acclimatization must be carried out or not. Seasonal cycles in behavior and physiology, like molting, are regulated by melatonin-mediated photoperiod-time measurement (Arnold et al. 2018, Lincoln et al. 2003).

All vertebrates are capable of alternately registering brightness and darkness, and thus day length, with the help of the pineal gland ((Lincoln et al. 2003, Tordjman et al. 2017, Wehr 1997). The pinealocytes within, which are probably a modified form of photoreceptors, receive information about light conditions via the retina and then synthesize melatonin during darkness (Salomon et al. 2015, Tordjman et al. 2017). Thus, genetically pre-programmed adaptions, such as weight gain or loss, change of VFI and molting are triggered (Arnold et al. 2018, Lincoln et al. 2003). Therefore, a change in melatonin secretion can have far-reaching consequences for the individual. In mammals, just like in humans, a reduced melatonin level can cause severe problems like sleep apnea or even sleep-related breathing disorders (Xie et al. 2017).

To investigate the effects of melatonin on various physiological processes, especially molting, specifically in red deer, several studies have already been conducted, which shall be elucidated more precisely subsequently (Fisher et al. 1988, Heydon et al. 1995, Milne et al.).

In 1988, 3 groups of red deer of different sex and age were treated with subcutaneous melatonin implants (dose of 18mg every 30 days). Yearling deer tended to advance their growing season and onset of calving but showed no difference in live weight. Yearling hinds treated with melatonin shed their coats earlier, but mean coat length was similar in treated and untreated animals. Lactating hinds also showed earlier shedding and earlier onset of calving. Their calves showed a normal growth rate (Fisher et al. 1988).

When treated orally with exogenous melatonin, beginning in mid-summer, red deer in Britain in the 1980s also showed several changes. They had less undercoat than untreated individuals, and the color of their coats was paler and patchy. Both the onset of the breeding season and the seasonal cycle of VFI were advanced. These effects were associated with lower T_3 concentrations and a rapid decrease in plasma prolactin. The increase in live weight at the end of October was higher in control animals than in melatonin-treated animals (Milne et al. 1990).

In another study in the United Kingdom, melatonin was able to induce an earlier (on average 35 days earlier) transition from summer to winter coat in lactating red deer with low feed intake, although lactating red deer with low grazing availability showed a delay in winter coat change when not treated with melatonin. Nevertheless, melatonin treatment did not significantly affect live weight nor food intake. The fact that melatonin treatment advances the onset of the breeding season could be confirmed, and plasma prolactin levels were lowered first in melatonin-treated hinds (Heydon et al. 1995).

In another study, measuring only the concentration of melatonin produced by the body, adult Iberian red deer showed a high sensitivity to seasonal and daily changes in the concentration of the hormone. Factors influencing melatonin concentration were the respective body weight, season, differences in sunrise and sunset, and susceptibility to stress (García et al. 2003).

In contrast to the previously analyzed differences in the seasonal rhythm of red deer under melatonin manipulation, there are those physical and behavioral changes and adaptations that were observed independently of exogenous melatonin administration, dependent on normal photoperiod. For example, red deer endure an extreme lowering of peripheral body temperature to, at minimum, 15 degrees Celsius subcutaneously, VFI can be lowered by half on average in winter, controlled by photoperiod (Arnold 2020, Loudon 1994). The digestive tract can be significantly reduced in size, as are some viscera, e.g. the liver, but digestion is simultaneously optimized by longer digestion times, a higher short chain fatty acid (SCFA) absorption rate and increased expression of transport proteins. The reduced VFI is also related to a reduced physical activity during foraging, which is intended to further conserve energy (Arnold 2020).

In 2011, the influence of seasonal changes on red deer without the influence of exogenously administered melatonin was investigated to distinguish between seasonal, endogenously mediated, and environmental influences. The animals were able to lower their heart rate, T_b and rumen temperature and lost body weight during winter. The decreasing or fluctuating heart rate was significantly related to the lowered rumen core temperature. However, heart rate was not only seasonally reduced in winter, independent of pellet feed, body mass, physical activity, and air temperature, but also further reduced in response to food restriction. During the first 7.9 days of food restriction, heart rate decreased and eventually stabilized. Rumen core temperature and body mass decreased significantly slower than heart rate during feed restriction (16.7 and 19.2 days, respectively) before stabilizing, while physical activity was reduced for only 3.5 days before remaining at this point. It could be shown that in the animals of this study, heart rate decreased proportionally to the decreasing rumen temperature (average 3.5 beats / min per -0.1 degrees rumen temperature). However, protein-rich diets slightly increased heart rate in both summer and winter. Despite unrestricted access to feed (pellets), the animals lost body mass during winter (Turbill et al. 2011). Similar observations were made in Svalbard reindeer, which under arctic conditions, were able to adapt to these incredibly harsh conditions and lower both their heart rate and body temperature even further and longer than previously observed in ungulates. During the period of best food availability, these animals showed peaks in heart rate and inner body temperature or rumen temperature. It is assumed that in such times the reindeer mainly try to build up fat reserves (Arnold et al. 2018).

But how is the body able to adapt in such ways to harsh winter conditions?

To withstand such a decrease in T_b and rumen temperature at all, and thus save energy, the body must adapt at cellular level. Mammals are capable of changing the FA composition of cell membranes as a reaction to environmental conditions or changes (Ruf and Arnold 2015). To provide the necessary membrane fluidity at low temperatures, FAs with a low melting point are incorporated into the phospholipids of the membrane. This effect is also referred to as homeoviscous adaptation (Ernst et al. 2016, Hazel 1995, Ruf and Arnold 2008). The term of non-shivering thermogenesis (NST) also plays an important role in these contexts. This possibility of producing heat without muscle tremors is possible either by means of an uncoupling protein in the brown adipose tissue, which shall not be further discussed in this work, or by SERCA activity (sarcoplasmic/endoplasmic reticulum calcium ATPase = SERCA). (Nowack et al. 2017)

At this point, in addition to the importance of photoperiod and melatonin, the role of PUFA in seasonality comes up. In several studies, effects of n-3 and n-6 FA on torpor, hibernation, but also the overwintering of non-hibernating mammals could already be worked out (Arnold et al. 2011, Arnold 2020, Arnold, Giroud et al. 2015, Nowack et al. 2017, Ocloo et al. 2007).

Omega FAs are characterized by more than one C=C double bond and are further subdivided into n-3 and n-6, which differ in the position of the first C double bond. n-3 FA have a C double bond at the 3rd position of methyl-end of the chain, whilst n-6 FAs have it in 6th position. Their importance is particularly evident in the fact that mammals cannot synthesize these FAs themselves and are therefore dependent on their supply through diet (Arnold et al. 2011, Arnold, Giroud et al. 2015). Neither are mammals capable of transforming n-6 into n-3, because they're missing the specific enzyme (Simopoulos 2009). The initial fats of the two FAs classes are alpha-linolenic acid (ALA) for n-3 and LA for n-6 PUFA, also called essential fatty acids (EFS). Lipid mediators, prostaglandins, lipoxins, leukotrienes, and various enzymes, among others, are formed from ALA and LA and their derivatives. Especially in human medicine, positive effects of omega FAs on e.g. inflammation, skin health and autoimmune diseases have been detected several times (Balić et al. 2020, Kaczmarski et al. 2013). n-3 and n-6 FA and their derivatives are, in most contexts, very different or even opposite in their effects to the mammalian organism. (Arnold, Giroud et al. 2015, Balić et al. 2020, McCusker and Grant-Kels 2010)

LA is found as a usable resource for humans and mammals in a number of seeds, germs and oils (for example, sunflower, corn, grape seed). In humans LA is involved in the formation of the stratum corneum permeability barrier (SCPB), as a major component of the extracellular lipid matrix that forms it. LA is essential for the homeostasis and therefore health of the skin (McCusker and Grant-Kels 2010). Additional to that, n-6 FA have a pro-inflammatory effect via their prostaglandin formation (Arnold, Giroud et al. 2015).

In hibernating mammals, the ability to reach lower T_b were observed in association with increased n-6 supplementation. Metabolic rate could be further reduced, longer torpor times were observed and the animals energy expenditure could be reduced, during time of hibernation (Giroud et al. 2015, Giroud et al. 2018). Furthermore, n-6 FA appear to positively affect SERCA activity, whereby increased calcium uptake into the sarcoplasmic reticulum of the cardiomyocite and thus myocardial function can be ensured (Arnold 2020, Arnold, Giroud et al. 2015, Ruf and Arnold 2008). In addition to all these properties, however, there are also direct points of overlap between melatonin and n-6 in the body. In rats, an increase in n-6 concentration in phospholipids of liver and plasma has previously been demonstrated after oral melatonin administration. It is likely that this effect is due to the antioxidant effect of melatonin (Pita et al. 2002). In this study, sunflower oil was used as a LA feed supplement for red deer at the Institute of Wildlife Ecology, Vienna.

ALA, n-3 PUFA, is found in green leafy vegetables, canola and flaxseed, and their oils, among others, and is thus accessible to humans and mammals (McCusker and Grant-Kels 2010). The most important representatives of the n-3 metabolism are eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), docosapentaenoic acid (DPA, 22:5 n-3) (McCusker and Grant-Kels 2010, Simopoulos 2009, Wehrmüller 2008). Linseed oil was subsequently used as a feed supplement for red deer at the Institute of Wildlife Ecology, Vienna.

In contrast to LA, ALA is associated with inhibited SERCA activity and higher rumen core temperature. In addition, n-3 PUFA were shown to have predominantly negative effects on hibernation. DHA levels increase due to n-3 supplementation and are known to be able to prevent or delay hibernation (Giroud et al. 2018). Even though n-3 FA have a negative effect on SERCA activity, they are essential in daily and annual rhythms. In Syrian hamsters, after a low-n-3 diet, not only a markedly decreased melatonin level at night induced by a change in membrane phospholipid composition could be detected, but the animals also suffered from hyperactivity (Lavialle et al. 2008). Apart from that, n-3 not only modulates neural and cardiac function positively, but also has anti-inflammatory effects and can be used for improved wound healing, skin regeneration and in preventive skin health. Some skin diseases in pets such as seborrhea, for example, are due to a nutritive deficiency of n-3 acids (McCusker and Grant-Kels 2010, Scardino et al. 1999, Simopoulos 2002, Simopoulos 2009).

In alpine marmots, a considerable membrane remodeling during hibernation was observed, which was largely independent of the immediate intake of n-6 or n-3 with food. It was concluded that n-6 PUFA are likely to stabilize cardiac activity during hibernation and n-3 PUFA improve oxidative capacity. Animals with higher n-6 concentrations had higher rewarming rates. They also showed an improved oxidative capacity at the end of winter, due to significantly higher n-3 concentration towards the end of hibernation, compared to a significantly higher n-6 concentration at the beginning. In summary, it could probably not be beneficial for animals in seasonal rotation to feed only one feed supplement, either n-3 or n-6, but to achieve an optimal balance of the two FAs in the body (McCusker and Grant-Kels 2010, Ruf and Arnold 2008). Additionally, phospholipids of liver and plasma in rats showed an increased n-6 level after oral administration of melatonin, what is likely to happen due to the antioxidant effect of the hormone (Pita et al. 2002).

Both LA and ALA are common components of ruminant rations. They are hydrogenated in the digestive tract of ruminants and are available to consumers through milk and meat (Maia et al. 2007).

In addition to the direct effects of PUFA and melatonin on mammalian seasonality and body condition, there are other physiological processes that are important to consider in the context of energy management and metabolism. Molting, lactation, particularly every cell division in the body is dependent on energy availability and reserves (Buttemer et al. 2020, Kalucka et al. 2015). Previous studies have already shown that lactating hinds have an increased need for feed, which could be a disadvantage in generating necessary fat reserves depending on the feed supply (Heydon et al. 1993, Heydon et al. 1995). In addition, body weight and body composition are a significant indicator of energy consumption. Resting activity, thermogenesis and activity are directly related to EB i.e. intake and consumption, which is why reduced consumption has a positive effect on weight gain (Fonseca et al. 2018).

Another body condition and metabolism influencing factor is age. Body composition changes in favor of fat content and metabolic rate declines with age (Shimokata and Kuzuya 1993). By growing older, apart from possible nutritional issues due to tooth problems i.e., increased problems of the pituitary gland may occur. In horses, for example, this is a frequently diagnosed problem in older animals, associated with shedding problems such as incomplete molting or many too long hairs (Brosnahan and Paradis 2003).

3. Material and methods

This study was conducted from 10th of September 2018 until 14th of October in 2019, in an enclosure adjacent to the Research Institute for Wildlife Ecology, Vienna (48.21°N, 16.37°E). The procedures were carried out in accordance with the Austrian legislation as assessed and approved by the institutional ethics commission of the University of Veterinary Medicine Vienna (experiment number: BMWFW-68.205/0191-WF/V/3b/2017).

3.1.Study animals

Data of 16 adult red deer hinds (*Cervus elaphus)*, born between 2008 and 2015, was collected and evaluated. The study hinds were part of a larger herd of red deer (herd of maximum 45 red deer in total, 26 females, one adult stag, one juvenile stag and 17 calves), living in the ~45ha research enclosure under semi-natural condition of the Research Institute of Wildlife Ecology. The area includes besides pastureland (~3 ha) also oak beech forest. Only 16 red deer received the n-3 or n-6 food supplementation and melatonin implants, therefor only those were included in the study.

The animals were marked individually with colored collars for identification from a distance. All experimental animals were continuously subjected to a training schedule ensuring regular use of an automated feeding station (see below) and approach upon call. All red deer hinds received self-made constant release subcutaneous melatonin implants, located craniolateral of the Manubrium sterni, between 27-05-2019 and 02-07-2019. The implants, with a dosage of 1 g of melatonin ((Alfa Aesar, AAJ6245206), consisted of a silicone envelope (Dow Corning Silicone elastomer membrane, 7-4107) and an airtight silicone sealant (Dow Corning, Silastic Medical Adhesive Silicone, Type A) and measured 4 x 4,5 cm. Melatonin implants were implanted under anesthesia. Anesthesia was administered intramuscularly (IM) via hand injection or blow pipe (BLOW 1.25 Model Zoo; Dan-Inject, Denmark) of medetomidine (0.1 mg/kg; Medetomidine-HCL 2 %, magistral formulation, Richter Pharma AG, Vienna, Austria) combined with tiletamine-zolazepam (3 mg/kg; Zoletil100, Virbac, Österreich GmbH, Austria). Anesthesia was terminated 60 minutes after induction by administering the medetomidine antagonist (5 mg Atipamezol for each 1 mg of medetomidine (Atipamezol = Narcostop©, Richter Pharma AG, Wels, Austria)). During the entire anaesthesia, animals were administered nostrilly 100% oxygen (2l/minute) and vital parameters (e.g. f_H, mean arterial blood pressure, respiration rate, exhaled CO_2, arterial oxygen saturation, rectal temperature) and sufficient depth of surgical anaesthesia were monitored. Each animal was kept under observation until fully recovered and observed occasionally over the subsequent 24 hours for any signs of narcotization issues (for details see Einwaller et al., 2020).

3.2.Study design

In 2018, data collection of animals without exogenous melatonin was made during molt from summer to winter coat. During 2019, data collection was made from animals in molt from winter to summer and from summer to winter coat with melatonin implants. Sunflower oil and linseed oil was used as a n-6 and n-3 feed supplement, respectively.

The 16 hinds were subdivided in terms of body size and social rank, allocated to two different food groups. These groups received, in addition to natural forage, red deer pellets (Trophy Lucerne Apple, Garant) immersed either with 10% linseed oil, containing high concentrations of ALA, or with 10% sunflower seed oil, rich in LA (Tab.1). The study animals were trained to enter an automated feeding station, where animals received their assigned food by registration of the ear transponder. In this station, the animals were automatically weighed, and the net pellet intake, date and time (MEZ) were recorded.

Feeding groups were swapped once a year in June, to ensure all hinds received ALA and LA supplementation, respectively, during the annual cycle of seasonally changing body mass. In addition, pellets were delivered *ad libitum (ad lib.)* or restricted during alternating periods of four weeks. During periods of restricted feeding, the animals received per day only 20% of the average daily pellet consumption during the preceding period of *ad lib*. Feeding (Tab. 2).

Table 1: Classification of feeding groups with supplemented oil type due to the period of supplementation

Individual	Oil supplement 06.2018 – 06.2019	Oil supplement starting 06.2019
Anna	Sunflower	Linseed
Emma	Sunflower	Linseed
Fina	Sunflower	Linseed
Kinari	Sunflower	Linseed

Pixie	Sunflower	Linseed
Sabrina	Sunflower	Linseed
Suki	Sunflower	Linseed
Tori	Sunflower	Linseed
Amaia	Linseed	Sunflower
Arsinoe	Linseed	Sunflower
Bärbel	Linseed	Sunflower
Lilli	Linseed	Sunflower
Mimi	Linseed	Sunflower
Ronia	Linseed	Sunflower
Yuffie	Linseed	Sunflower
Yuna	Linseed	Sunflower

Table 2: Feeding schedule of *ad libitum* or restricted feeding and time period of melatonin administration

Year	2018				2019									
Month	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.
4	a	r	a	r	a	r	a	r	a	a	r	a	r	a
4	r	a	r	a	r	a	r	a	r	a	a	r	a	r
4	a	r	a	r	a	r	a	r	a	a	r	a	r	a
4	r	a	r	a	r	a	r	a	r	a	a	r	a	r

a=ad libitum feeding; r=restricted feeding; dark shaded fields = melatonin implants being administered to all individuals at the beginning of June

The body weight measured in the feeding station were stored in a computer together with individual ID. As pictures were taken weekly, the weekly average of body mass of each red deer was used for statistical analyses.

In calendar week (CW) 42 2018 the red deer of the control group had a mean live body weight of 146 +/- 6 kg; min=117 kg; max=205 kg) and 144 +/- 5 kg, min= 118; max= 183) in melatonin treated group 2019. Hinds had an average body mass of 150 +/- 6 kg, min = 117 kg, max = 210 kg) in in the control group, versus 146 +/- 5 kg, min= 118 kg, max =186 kg) in the melatonin group, respectivly CW 37 in 2019.

As reproductive activity might also influence coat change with and without melatonin administration, we analyzed this in both the control and melatonin treatment group (Tab.3).

Table 3: Reproduction status of all hinds in 2018 and 2019 in combination with the mothers' birthyear

Individual	Birthyear of mother	Reproduction 2018 (control)	Reproduction 2019 (melatonin)
Amaia	2015	Yes	No
Anna	2015	Yes	Yes
Arsinoe	2010	Yes	Yes
Bärbel	2009	Yes	Yes
Emma	2010	Yes	Yes
Fina	2011	Yes	Yes
Kinari	2008	Yes	No
Lilli	2008	Yes	No
Mimi	2010	Yes	No
Pixie	2009	Yes	Yes
Ronia	2008	Yes	Yes
Sabrina	2008	Yes	Yes
Suki	2009	Yes	Yes
Tori	2009	Yes	Yes
Yuffie	2015	Yes	No
Yuna	2009	Yes	Yes

The course of coat change was determined from pictures taken weekly from each individual. Those pictures were taken during the respective coat change period. Individuals were identified on the pictures by their individually colored collars.

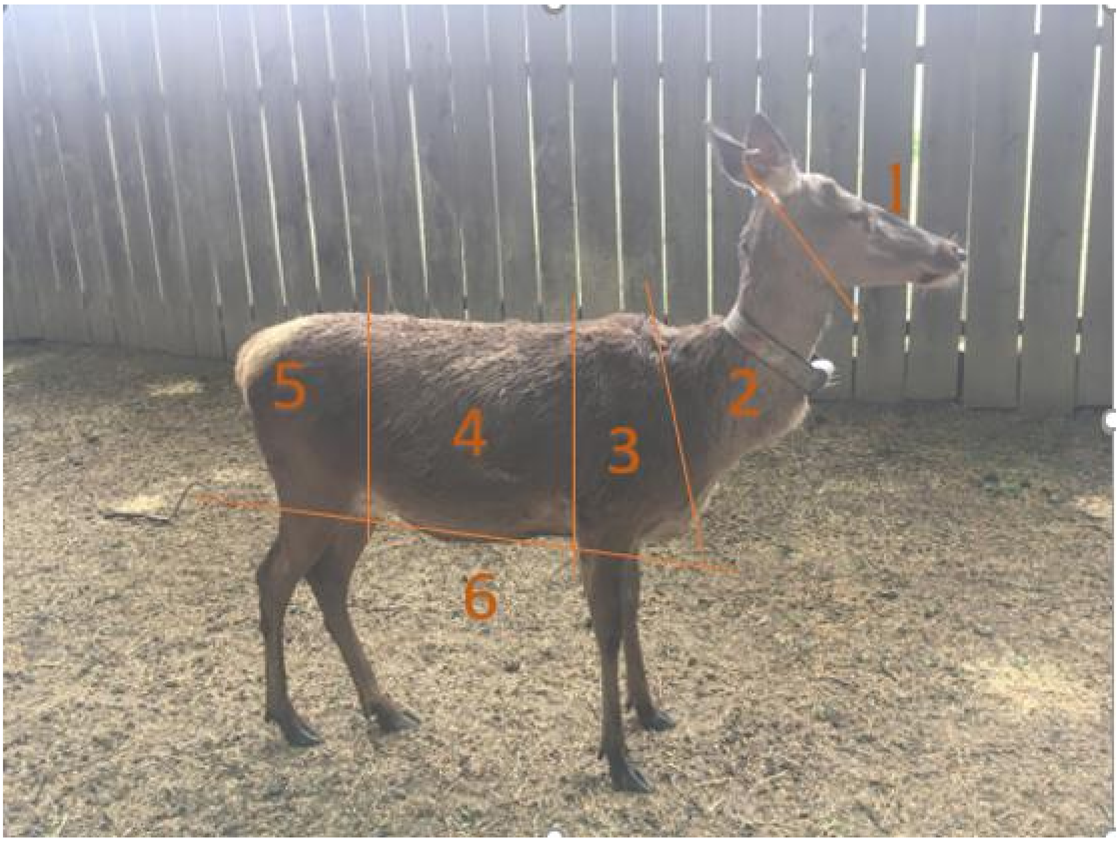
To standardize evaluation of the pictures, every picture was analyzed in random order, in the same room, under the same source of light, and with the same computer screen. To check the reliability of the evaluation all pictures of two red deer hinds were analyzed a second time two weeks after the first evaluation, under similar conditions utilizing the same equipment.

The state of coat shedding was assessed at six different body compartments on an ordinal scale from 0-6. The definition of body compartments did not conform to anatomical correct technical terms in favor of easier evaluation (Pic. 1).

Definition of body compartments:

1. Head – From the tip of the nose to the vertical line between the base of the ear and ventral edge of the mandible
2. Neck – from the vertical line between the base of the ear and ventral edge of the mandible to the line between the cranial edge of the withers and cranial line of the breast muscles
3. Thorax – from the line between withers and the cranial breast muscles to the caudal edge of the shoulder blade and elbow, excluding extremities
4. Abdomen and back – from the caudal edge of the shoulder blade to the vertical line of the knee fold
5. Croup and flank – from the vertical line of the knee fold to the tip of the tale, excluding extremities
6. Extremities – from the horizontal line of the elbow / knee to the claw

In the following text, the term "control group" represents molting in fall 2018 (summer to winter coat), while the "melatonin group" describes molting in fall 2019 (summer to winter coat). The coat change in spring 2019 will be referred to as "summer 2019", as the animals cannot be classified according to melatonin / control criterion, due to the implantation date of the melatonin preparations.



Pic. 1: Example of the division of the body compartments, Ronia, CW 18, 2019

Definition of the ordinal evaluation scale:

0 = still complete coat (summer or winter)

1 = lose hair in one area of the compartment, less than 1/3 of the compartment

2 = one single area of changed fur visible

3 = lose hair in different areas of the compartment, more than 1/3 of the compartment

4 = more than one area of changed fur visible, less than 1/3 of the whole compartment

5 = extensively changed fur, more than 1/3 of the compartment

6 = all fur in the compartment changed, only excluding some remaining loose hair

The week of change from value 0 to 1 was considered as “begin of the coat change”. The end of the coat change was defined as the respective CW in which the individual reached or exceeded the scale value 5 for the first time. The difference in weeks between begin and end of coat change is considered “duration of coat change”.

Altogether, three different cycles of coat changes were evaluated, the change from summer to winter fur in 2018 and 2019, and the change from winter to summer fur in 2019.

Pictures were excluded from analysis if the collar color of the individual could not be identified unambiguously, or due to picture quality.

Only images were included in the analysis in which at least 4 body compartments could be evaluated due to the image quality or angle of capture.

3.3.Statistical analysis

Statistical analyses were performed with R statistics ((R Core Team 2020, R version 3.6.2 (20 19-12-12)).

The package “ordinal” (Christensen 2019) was used for the analyses of the time course of molt, measured on an ordinal scale (see above), and potentially influencing factors. Scoring of different regions of the body during a given week of the year, nested within individual ID, was thereby modeled as random effect to account for repeated measurements.

Before starting analysis, reliability of the evaluation was checked by analyzing the evaluation and re-evaluation of all pictures of two red deer hinds, using Kendall’s rank correlation tau. Results were significant with tau = 0.994, $p<0.001$, and $z = 24.27$ and evaluation of the pictures therefore reliable. All data are represented with +/- s.e.m.

4. Results

The end and duration of shedding in the melatonin vs. control group and the temporal differences of molting in the different body compartments, independent of melatonin, were compared. Additionally, we analyzed the influence of food oil supplementation and tested with a linear mixed model the possible influence of all available variables (reproduction, weight, daily pellet intake, age, feed oil supplementation) on coat change.

4.1.Comparison of end of coat change and duration in control and melatonin group

In the evaluation of the coat change, significant differences were found between the individuals, their body regions, and between the control group, as well as the melatonin treated animals Start, end and duration of the coat change of the individuals in the respective coat change periods got compared. Here, the differences are shown when either coat change status = 5 (Tab. 4) or coat change status = 6 (Tab. 5) is used as a criterion for the end of shedding. The values show that there is significantly more evaluable data for the coat change status = 5 than for the status = 6 (individuals reaching coat change status 5: control group: 14; summer 2019: 16; melatonin group: 16; individuals reaching coat change status 6: control group: 8; summer 2019: 10; melatonin group: 10). In addition, the fluctuations in the start of the coat change in summer 2019 and melatonin group, in contrast to a uniform start (CW 37) in the control group, give first indications that the animals were already in the middle of the coat change at the start of the recordings in 2018. All data represent the average value of the coat change status of all evaluated body compartments in each individual.

Table 4: Comparison of the start, end and duration in calender weeks/weeks of the coat change from summer to winter in both 2018 and 2019 and winter to summer in 2019 , coat change status = 5 was defined as end of coat change

Hinds Name	S CG	E CG	D CG	S S 2019	E S 2019	D S 2019	S MG	E MG	D MG
Amaia	-	39	-	12	21	9	34	34	0
Anna	-	-	-	14	20	6	32	33	1
Arsinoe	37	39	2	13	24	11	32	32	0

Bärbel	37	38	1	13	25	12	29	33	4
Emma	-	39	-	13	21	8	30	32	2
Fina	37	40	3	12	19	7	31	33	2
Kinari	37	40	3	13	20	7	33	35	2
Lilly	-	37	-	12	21	9	30	35	5
Mimi	37	39	2	14	25	11	28	32	4
Pixie	37	41	4	14	22	8	30	32	2
Ronia	38	42	4	14	24	10	31	32	1
Sabrina	37	42	5	14	21	7	32	36	4
Suki	37	40	3	14	24	10	31	33	2
Tori	37	40	3	13	22	9	31	32	1
Yuffie	39	-	-	16	22	6	31	36	5
Yuna	40	42	2	14	21	7	31	34	3

S: start coat change; E: end coat change; D: Duration of coat change; CG: control group; MG: melatonin treatment group S2019: summer 2019

Table 5: Comparison of the start, end and duration in calender weeks/weeks of the coat change from summer to winter in both 2018 and 2019 and winter to summer in 2019, coat change status = 6 was defined as end of coat change

Hinds Name	S CG	E CG	D CG	S S 2019	E S 2019	D S 2019	S MG	E MG	D MG
Amaia	-	-	-	12	22	10	34	36	2
Anna	-	-	-	14	23	9	32	35	3
Arsinoe	37	42	5	13	25	12	32	-	-
Bärbel	37	42	5	13	26	13	29	-	-
Emma	-	41	-	13	-	-	30	38	8

Fina	37	43	6	12	-	-	31	-	-
Kinari	37	42	5	13	23	10	33	36	3
Lilly	-	43	-	12	-	-	30	41	11
Mimi	37	43	6	12	-	-	28	-	-
Pixie	37	-	-	14	24	10	30	34	4
Ronia	38	-	-	14	26	12	31	34	3
Sabrina	37	-	-	14	24	10	32	41	9
Suki	37	42	5	14	-	-	31	35	4
Tori	37	-	-	13	-	-	31	39	8
Yuffie	39	-		16	25	9	31	-	-
Yuna		-		14	25	11	31	-	-

S: start coat change; E: end coat change; D: Duration of coat change; CG: control group; MG: melatonin treatment group S2019: summer 2019

To compare the progress of winter coat shedding of melatonin treated hinds with the control group, the date of end of coat change was analyzed. Here, the end of the shedding period was chosen as the basis for evaluation, since at the beginning of data collection of the control group (CW37) most deer were already in the middle of shedding (average coat change status 2,95 +/- 0,47 weeks). Therefore, in this year neither the start week nor the duration of the change of coat could be clearly identified. Coat change was completed significantly earlier (averagely 6.72 weeks) under the impact of melatonin implants, considering coat change status 5 = end of coat change (Fig. 1; Wilcox Test, p<0,001).

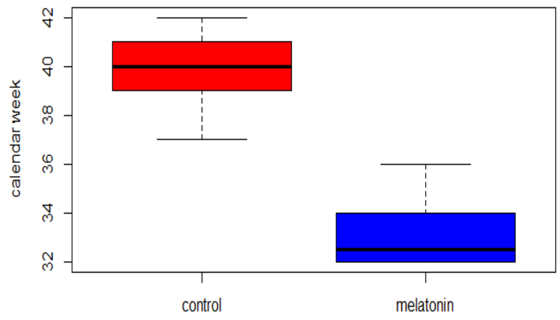


Fig. 1: Completion of winter coat change in control (red) and melatonin group (blue), coat change status = 5 was defined as end of coat change.

When coat change status = 6 was defined as end of coat change, premature shedding by 6.72 weeks on average under melatonin treatment was shown, with an average shedding end in CW 39,86 in 2018 and in CW 33,14 in 2019 (Fig.2: Wilcox Test, p< 0,063).

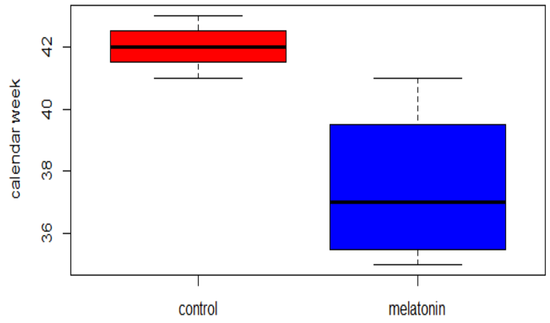


Fig. 2: Completion of winter coat change in control (red) and melatonin group (blue), coat change status = 6 was defined as end of coat change.

To compare not only the end time of coat change, but also its progress between the control and melatonin group, the coat change status in control and melatonin group in CW 37 were compared. The mean score in week 37 of melatonin treated hinds was on average 2.8 points higher than in the control group (Fig.3; Wilcox Test, p=0.003).

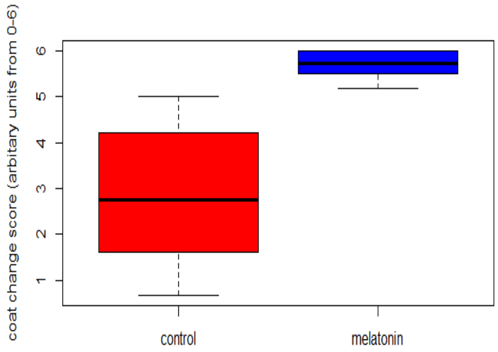


Fig. 3: Coat change scores in CW 37 (summer to winter coat), in control (red) and melatonin (blue) treatment group.

In figure 4, the progress of the coat change in the control and melatonin group is shown (Fig. 4), analyzing all coat scores available in all body compartments, and the influencing factors are examined (Tab. 6).

Changes of coat scores followed a sigmoidal pattern from begin to completion (Fig. 4). Coat changes occurred significantly earlier in 2019 due to melatonin treatment, after simultaneously considering later change in heavier and reproducing individuals, and not significant effects of daily pellet intake and age (Tab. 6).

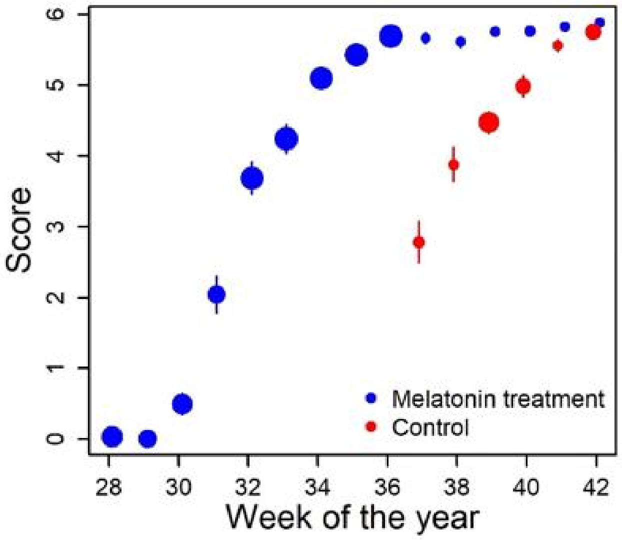


Fig. 4: Course of the change from the summer into the winter coat during 2018, and after melatonin treatment in 2019. Plotted are means calculated over all body compartments and individuals ± standard error of the mean (s.e.m.). Symbol size increases proportionally with sample size.

Table 6: Result of cumulative link mixed modeling of coat change scores, change from summer to winter coat in 2019 ((Model: Influence of the factors “added feed oil” (supplementation), “body mass”, “pellet-intake”, “reproduction” and “age” on coat change score (Score-week), whereby the interaction with CW and the Individual animal is taken in account)

Interaction effect	**Coefficient**	**Standard error**	**z-value**	**p-value**
Week:year “2019”	-0.166	0.085	-1.937	0.053
Week:body mass	0.003	0.002	1.814	0.070
Week:daily pellet intake	0.006	0.020	0.304	n.s.
Week:age	-0.039	0.016	-2.410	0.016
Week:reproduction „yes“	-0.973	0.115	-8.445	<0.001

4.2. Timing of the coat change in the different body compartments

Previous studies have already shown that deer do not change their coat in all body areas at the same time (Ryder, 1977). We have investigated these circumstances for our individuals, too, and represent the values of the respective animal from only one body compartment, as defined above, per CW. The results show there’s a significance difference in the time of ending coat change, between flank and croup with the time of ending coat change with the head (p-value= 0,003), as well as flank and croup and neck (p-value = 0,005). Head and neck are being shed earlier than flank and croup (Fig. 5).

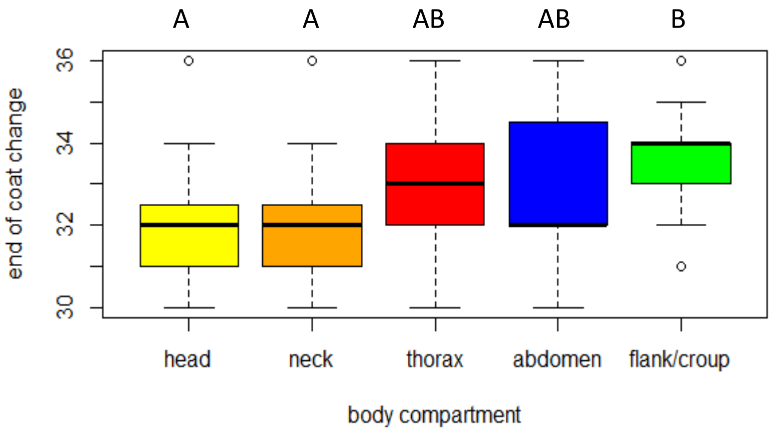


Fig. 5: End of coat change of the different body compartments in relation to the CW. Coat change status =5 was defined as end of coat change; Friedmann post-hoc Test). Different letters above the boxplots resemble a significant difference between groups.

The following tables illustrate the coat change data for the different body regions of each individual. Here, a temporal difference in the coat change between head, neck and the flank / croup can be seen (Tab. 7-12).

Table 7: Comparison of start and end of coat change in compartment 1 = head, change from summer to winter coat in control and melatonin group and winter to summer coat in 2019. End of coat change was defined as coat change status =5.

Hinds Name	Start control group (CW)	End control group (CW)	Start summer 2019 (CW)	End summer 2019 (CW)	Start melatonin group (CW)	End melatonin group (CW)
Amaia	-	39	14	18	34	34
Anna	-	-	15	15	32	32
Arsinoe	-	39	15	16	32	32
Bärbel	-	39	14	15	32	32
Emma	-	39	13	17	31	31
Fina	-	39	17	18	31	32
Kinari	-	38	15	16	33	33
Lilli	-	38	15	19	31	31
Mimi	38	38	16	17	30	32
Pixie	-	38	15	17	30	30
Ronia	-	38	16	20	32	32
Sabrina	38	40	15	15	36	36
Suki	38	38	19	19	31	31
Tori	-	37	-	15	31	31
Yuffie	-	39	19	19	32	33
Yuna	-	40	15	17	31	32

CW: calendar week

Table 8: Comparison of start and end of coat change in compartment 2 = neck, change from summer to winter coat in control and melatonin group and winter to summer coat in 2019 End of coat change was defined as coat change status =5.

Hinds Name	Start control group (CW)	End control group (CW)	Start summer 2019 (CW)	End summer 2019 (CW)	Start melatonin group (CW)	End melatonin group (CW)
Amaia	-	39	13	17	34	34
Anna	-	-	14	19	32	32
Arsinoe	-	37	15	17	32	-
Bärbel	-	37	14	25	32	32
Emma	-	39	15	18	31	31
Fina	-	39	17	20	-	31
Kinari	37	39	15	19	33	33
Lilli	-	37	12	19	31	32
Mimi	37	38	14	21	30	30
Pixie	37	41	16	18	30	31
Ronia	-	38	19	25	32	32
Sabrina	37	42	15	18	-	36
Suki	38	38	18	22	31	31
Tori	-	37	15	21	31	31
Yuffie	-	39	21	22	33	34
Yuna	-	40	17	20	31	32

CW: calendar week

Table 9: Comparison of start and end of coat change in compartment 3 = thorax, change from summer to winter coat in control and melatonin group and winter to summer coat in 2019 End of coat change was defined as coat change status =5.

Hinds Name	Start control group (CW)	End control group (CW)	Start summer 2019 (CW)	End summer 2019 (CW)	Start melatonin group (CW)	End melatonin group (CW)
Amaia	39	-	14	18	34	34
Anna	-	-	14	19	32	33
Arsinoe	37	39	13	20	-	32
Bärbel	37	38	14	20	-	32
Emma	-	39	16	20	31	31
Fina	37	42	14	18	31	33
Kinari	38	39	16	19	34	34
Lilli	37	38	13	21	31	32
Mimi	38	42	15	25	-	30
Pixie	38	41	15	20	31	32
Ronia	38	42	15	20	31	32
Sabrina	37	38	16	21	33	36
Suki	37	42	15	23	32	33
Tori	37	40	15	21	31	32
Yuffie	39	-	18	21	34	36
Yuna	40	42	15	19	31	34

CW: calendar week

Table 10: Comparison of start and end of coat change in compartment 4 = abdomen, change from summer to winter coat in control and melatonin group and winter to summer coat in 2019 End of coat change was defined as coat change status =5.

Hinds Name	Start control group (CW)	End control group (CW)	Start summer 2019 (CW)	End summer 2019 (CW)	Start melatonin group (CW)	End melatonin group (CW)
Amaia	39	-	12	20	34	34

Anna	-	-	14	22	32	32
Arsinoe	-	37	13	24	-	32
Bärbel	37	38	13	23	29	32
Emma	-	39	16	24	31	32
Fina	37	42	14	19	-	32
Kinari	37	40	15	20	34	34
Lilli	-	37	13	22	31	36
Mimi	37	39	15	25	-	30
Pixie	37	41	14	22	31	31
Ronia	39	42	16	24	31	32
Sabrina	37	41	16	21	33	35
Suki	37	40	15	25	31	32
Tori	37	40	15	22	31	36
Yuffie	-	-	18	23	33	36
Yuna	40	42	15	20	32	34

CW: calendar week

Table 11: Comparison of start and end of coat change in compartment 5 = flank / crupper, change from summer to winter coat in control and melatonin group and winter to summer coat in 2019 End of coat change was defined as coat change status =5.

Hinds Name	Start control group (CW)	End control group (CW)	Start summer 2019 (CW)	End summer 2019 (CW)	Start melatonin group (CW)	End melatonin group (CW)
Amaia	-	39	13	21	34	34
Anna	-	-	14	20	32	32
Arsinoe	37	39	13	26	32	36
Bärbel	-	37	13	24	32	33
Emma	-	39	15	22	30	33

Fina	37	42	12	19	32	34
Kinari	37	42	13	20	34	35
Lilli	-	37	12	21	30	33
Mimi	-	39	22	25	28	34
Pixie	38	40	14	23	31	31
Ronia	39	40	14	22	32	32
Sabrina	37	40	16	22	33	34
Suki	-	37	14	21	32	34
Tori	37	40	13	22	31	35
Yuffie	39	-	16	23	31	36
Yuna	40	42	14	21	33	34

CW: calendar week

Table 12: Comparison of start and end of coat change in compartment 6 = extremities, change from summer to winter coat in control and melatonin group and winter to summer coat in 2019 End of coat change was defined as coat change status =5.

Hinds Name	Start control group (CW)	End control group (CW)	Start summer 2019 (CW)	End summer 2019 (CW)	Start melatonin group (CW)	End melatonin group (CW)
Amaia	-	39	14	18	34	34
Anna	-	-	14	15	32	32
Arsinoe	-	39	14	17	-	32
Bärbel	37	38	14	19	-	32
Emma	-	40	17	18	31	31
Fina	-	39	15	17	-	31
Kinari	38	40	13	18	35	35
Lilli	38	42	13	18	31	31
Mimi	38	38	15	20	30	32

Pixie	40	41	14	17	31	31
Ronia	39	42	17	22	32	32
Sabrina	38	40	14	15	32	32
Suki	37	38	14	20	32	32
Tori	-	37	13	18	31	31
Yuffie	-	39	19	21	34	36
Yuna	-	42	14	19	33	34

CW: calendar week

4.3.The influence of oil supplementation on coat change

Additionally, we looked at the effects of dietary omega FAs on duration and end of shedding (Fig.6 A-D).

In summer 2019, animals supplemented with linseed oil, changed their coat in 7,75 +/- 0,45 weeks, (min = 6 weeks, max = 10 weeks) whilst those supplemented with sunflower oil changed their fur in 9,34 +/- 0,73 weeks (min= 6 weeks, max = 12 weeks). No significant correlation between either supplementing ALA or LA and coat change duration could be shown in the model (Fig. 6A: Wilcox test, p-value = 0,92). Additionally, the end of the coat change during summer 2019 in both oil groups was analyzed. When animals got supplemented with linseed oil, the first hind reached scale point 5 in CW 19, whereas in the group supplemented with sunflower oil the first hind reached scale point 5 in CW 21. On average, animals ended coat change in CW 21,125 +/- 0,59 (min = CW 19, max = CW 24). The linseed supplemented group ended coat change in CW 22,875 +/-0,64 (min = CW 21, max= CW 25). Although the linseed supplemented deer changed their coat prior to the sunflower supplemented deer in summer 2019, but this difference was not significant (Fig.6B: Wilcox Test, p = 0,96). The same parameters, end and duration of the coat change, were analyzed correspondingly for the melatonin group. To change from summer to winter coat, the linseed-melatonin group apparently needed averagely 2 +/- 0,33 weeks (min = 0, max = 4) and the sunflower-melatonin group needed 2,63 +/- 0,73 weeks (min = 1, max = 5). Correlation between food supplement and duration of coat change was not significant (Fig 6C: wilcox test, p value = 0,802). The sunflower-melatonin group averagely ended their shedding in CW 33,38 +/- 0,56 (min = CW 32, max = CW 36), whilst the lins eed-melatonin hinds ended coat change averagely in CW 33,25 +/- 0,53 (min = CW32, max = CW36). No significant correlation between end of coat change and food supplement oil could be found (Fig.6D: Wilcox Test, p value=0,658).

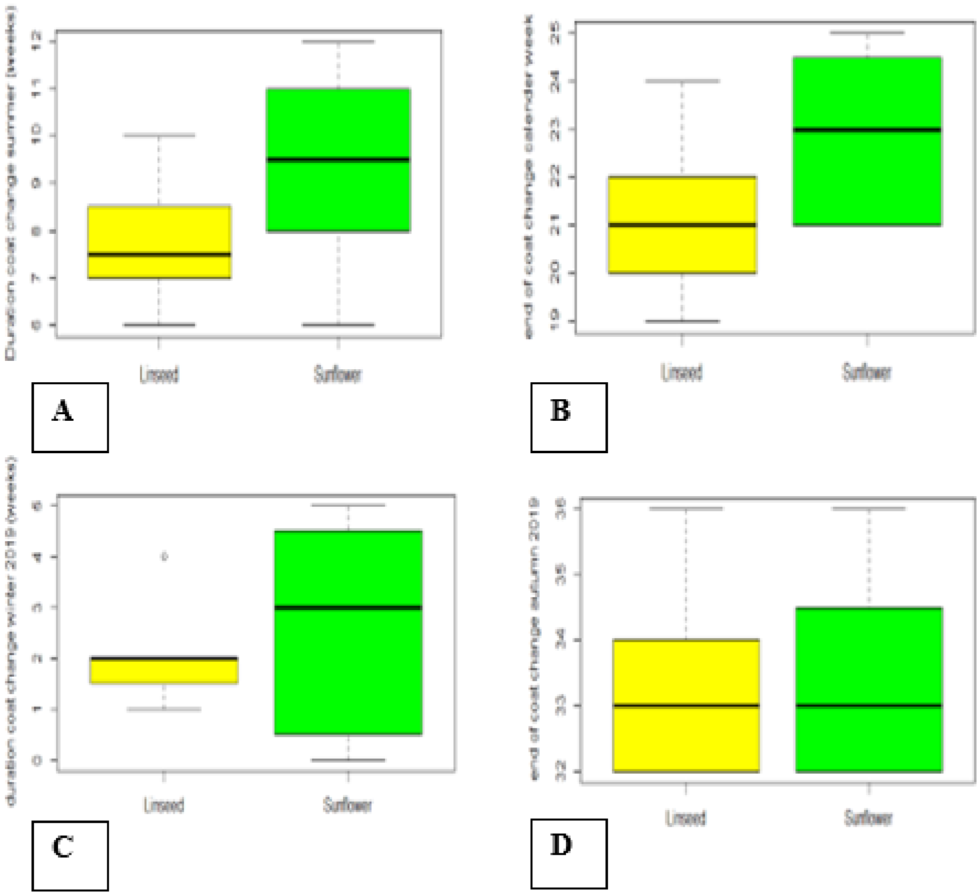


Fig. 6: Comparison of coat change duration in summer 2019 of both food oil supplement groups (coat change score: 5, boxplots, yellow = linseed oil, green = sunflower oil). (**A**) End of the coat change summer 2019 of both food oil supplement groups (coat change score:5; boxplots; yellow = linseed oil, green = sunflower oil) (**B**), Comparison of the coat change duration of both food oil supplement groups in the melatonin group (coat change score: 5, boxplots, yellow = linseed oil, green = sunflower oil, melatonin treatment started) (**C**), End of the coat change of both food supplement groups in the melatonin group (coat change score 5; boxplots; yellow = linseed oil, green = sunflower oil, melatonin treatment started) (**D**)

To check if coat changes were influenced by supplementation of either linseed or sunflower oil, both coat changes occurring 2019 were analyzed considering the effect of oil supplementation (Fig. 7). The change from winter to summer coat in spring occurred before melatonin treatment, whereas the subsequent change from summer to winter coat occurred after melatonin treatment. Coat change scores followed a sigmoidal pattern from begin to completion (Fig.7).

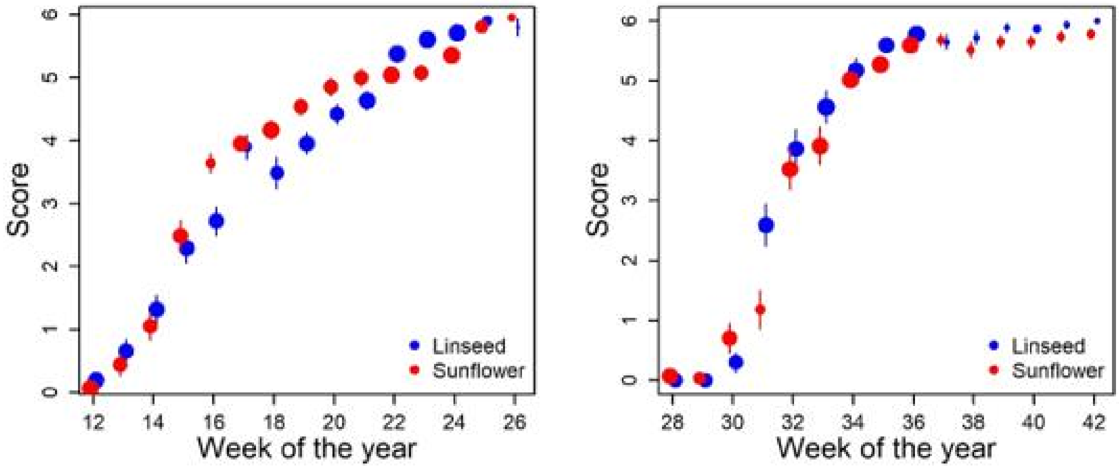


Fig. 7: Course of coat changes in 2019. Left panel: coat change summer 2019 (winter to summer fur before melatonin treatment). Right panel: Coat change of melatonin group. Plotted are means calculated over all body compartments and individuals ± s.e.m.. Symbol size increases proportionally with sample size.

In the control group, the change from winter to summer coat in spring occurred slightly faster in animals supplemented with linseed oil, after simultaneously considering later change in heavier, younger and reproducing individuals, and not significant effects of daily pellet intake (Table 13).

Table 13: Result of cumulative link mixed modeling of coat change scores in spring 2019 (Model: Influence of the factors "added feed oil" (supplementation), "body mass", "pellet-intake", "reproduction" and "age" on coat change score (Score-week), whereby the interaction with CW and the Individual animal is taken in account)

Interaction effect	Coefficient	Standard error	z-value	p-value
Week:oil supplementation	0.239	0.061	3.904	<0.001
Week:body mass	-0.009	0.002	-4.721	<0.001
Week:daily pellet intake	0.014	0.034	0.420	n.s.
Week:age	0.036	0.015	2.408	0.016
Week:reproduction	-0.206	0.081	-2.539	0.011

The same model was made for the change from summer into winter coat after melatonin treatment. This model failed to define the variance-covariance matrix for these parameters. However, a more simple model of these scores, with only the type of oil supplementation as fixed effect, revealed a slightly, but significantly later end of coat change in the sunflower supplemented group (interaction week:oil supplementation "sunflower seed oil", coefficient -0.496, standard error 0.075, z-value -6.640, p<0.0001).

5. Discussion

Some of our results are consistent with previous research on red deer shedding. For example, the temporal sequence in which the different body areas of red deer change their fur is partially coextensive with previous results identifying the sequence of coat shedding as follows: “the neck and outer part of the upper limbs first, along the backbone next, followed by flank and belly”(Ryder 1977). In our hinds we could only find significant differences in the shedding periods between molting head and flank, as well as neck and flank, but these results affirm our classification of the body compartments being useful as a basis for evaluation. Striking macroscopic changes of the coat regarding color and texture due to melatonin input weren’t analyzed during image analysis but would certainly be an interesting point to consider in further research.

We could observe an earlier end of the coat change under impact of exogenous melatonin administration in our test animals during the change from summer to winter coat. Not only was the molt finished about 7 weeks earlier, but also the amount of changed fur in CW 37 was significantly higher. CW 37 was chosen for this calculation, because a lot of data was available for this week in both years. These results are in line with prior findings, showing exogenous melatonin input advancing molting and therefore showing, coat change is a process being definitely controlled by the photoperiod and therefore melatonin (Fisher et al. 1988, Heydon et al. 1995, Lincoln et al. 2003, Milne et al. 1990). What was striking about our animals, however, was the fact that they changed their coat even earlier (6,72 weeks averagely) than in previous studies, in which it could be shown that melatonin advances coat change by about one month (Fisher et al. 1988, Heydon et al. 1995). The exact reason for this earlier end of coat change cannot be worked out from our results. However, on one hand the animals in our study received constant release melatonin implants with a much higher, but one-time dose of melatonin (1g), in contrast to much lower concentrated implants with 18 mg every 30 days, or 10 mg melatonin daily administrated orally (Fisher et al. 1988, Milne et al. 1990). On the other hand with 2 subcutaneous melatonin implants of 0.5 g each, which would be the equivalent dose to our dosage, only an advantage of shedding by an average of 35 days was achieved (Heydon et al. 1995). However, the animals from the previous study were all lactating and kept with low forage availability, whereas our experimental animals were only temporarily restricted in pellet availability but always had access to all pastures and only some lactated.

The transformation of the organism in winter can, as already mentioned earlier, be summarized in a simplified way under several energy-saving measures. This includes not only the conversion to better thermal insulation by changing summer to winter coat, but also the adaptation of the metabolism and digestive tract to lower feed availability. However, both processes take time to adapt, which is why it is so essential for animals to prepare before the onset of winter (Arnold 2020, Arnold, Beiglböck et al. 2015, Turbill et al. 2011). In addition, both shedding and lactation are physiological processes that cost additional energy and therefore, at best, should not occur at times of absolute energy conservation (Arnold 2020, Buttemer et al. 2020, Vetharaniam et al. 2009, Vries and Veerkamp 2000). Previously it could be shown, food intake of lactating red deer treated with melatonin on scares pasture being significantly higher than in the non-lactating group (Heydon et al. 1995). VFI decreases in principle in red deer during winter, modulated by photoperiod, and this decrease can be advanced in time by administrating melatonin (Milne et al. 1990). It has also been demonstrated that animals receiving only a meager food supply do not show that reduction in VFI, even under exogenous melatonin. Thus, appetite changes are not only dependent on photoperiod, but also on supply (Heydon et al. 1993, Milne et al. 1990). Along with that, metabolism and heart rate can also be manipulated (lowered) by restricting food availability, showing, animals can be brought to an energetic balance limit by food regime (Heydon et al. 1993, Turbill et al. 2011). Thus, in contrast to the other studies, the significantly earlier time of coat change in our animals under melatonin could be influenced by a better nutritional energy availability in our study animals, when cycle was manipulated. Animals starting coat change on sparse food available are already dealing with energy problems before having enough time to adjust their physiological requirements (Arnold 2020, Arnold, Beiglböck et al. 2015, Heydon et al. 1993, Turbill et al. 2011).

A third approach to explain the significantly premature coat change in autumn 2019 would be the simultaneously supplemented feed with either LA or ALA along with melatonin implants being responsible. This assumption would support previous researches, that have shown close links between PUFA and melatonin, like the hormone's positive effect on the n-6 content of phospholipids and the fact that n-3 PUFA are essential for the secretion of melatonin and thus the annual rhythm (Lavialle et al. 2008, Pita et al. 2002).This leads us directly to discussing differences of molting in the two food supply groups.

In this study, we were able to identify a tendency in all examined coat change periods, showing hinds being supplemented with linseed oil (n-3) changed their coat somewhat earlier and faster. The slightly shorter time for the coat change under linseed supplementation could be explained by several mechanisms. It could be that the faster coat change in linseed supplemented food group could come from an improved skin and hair quality and thus a promoted growth of coat could be linked to feeding n-3 PUFA, as positive effects on skin and hair for ALA have already be described several times in human and veterinary medicine (Scardino et al. 1999, Simopoulos 2002, Simopoulos 2009). Even an increased occurrence of skin diseases such as seborrhea with a n-3 deficiency in small animals could be shown (Scardino et al. 1999).

Low n-3 levels are known to negatively affect circadian rhythms by altering both the membrane composition of phospholipids in the pituitary gland and thus the level of melatonin in the body (Lavialle et al. 2008). Conversely, one could therefore assume that a good ALA supply optimizes the work of the pineal gland and thus the coat change. On the other hand, the positive effects of melatonin on the content of long-chain n-6 PUFA in phospholipids have been shown in previous studies (Pita et al. 2002). These findings are not directly linked to hibernation or pineal gland function, but one could imagine, under exogenous melatonin and n-6 supplementation even better adaptations of the body to hibernation and torpor could be possible (Arnold et al. 2004, Arnold 2020, Turbill et al. 2011).

In humans, it has been shown that not only supplementation with one of the two FAs, LA or ALA, is important for the body, but a balanced ratio (~1:1) of the two FAs is more important, than only supplying either one of them (McCusker and Grant-Kels 2010, Simopoulos 2002, Simopoulos 2009). Normally, the ratio is approximately balanced in wild animals and disproportions are more a problem in human western diets (Simopoulos 2002). However, the diet of red deer in this study was not only manipulated by supplemental pellet food and the addition of different FAs, which changed annually, but also the selection of pasture was limited by husbandry, and therefore the ratio of n-3 to n-6 could be even more drastic, than caused by oil supplementation alone. If we did not change the food oil addition in our deer from LA to ALA annually, but instead fed a group unilaterally over a longer timeperiod, we might be able to get even more prominent results.

In addition to the above discussed results, it was shown that reproducing animals changed their coats slightly slower in both spring and summer molt, a result that we expected due to the high energy expenditure for pregnancy and subsequent lactation. This was also previously shown in other studies (Kalucka et al. 2015, Sguizzato et al. 2020, Vries and Veerkamp 2000). Furthermore, also other unsaturated FA, which are synthetized from ALA, are additionally excreted by milk and must be provided for this purpose by the body in addition to its own needs (Simopoulos 2002).

In this study, deer with higher body weight needed longer for shedding their coat in both spring and autumn. Previous studies have shown that general weight gain in the fall was lower in melatonin-treated animals than in the control group (Milne et al. 1990) and that there was no effect of melatonin on body weight (Heydon et al. 1995).

Our results do not confirm or refute any of the above-mentioned effects of melatonin on coat change, but the results seem to show that increasing weight has a delayed effect on coat change. A possible reason for this could be that the metabolism is generally negatively affected with a high weight and our animals were on average rather in the upper range of the body condition score / body weight (Audige et al. 1998).

Interestingly, age had no effect at all in the change to winter coat, but it took longer for younger animals to shed their coat in spring.

The net pellet intake of the animals could not show any effect on the coat change. These findings are partly in line with previous studies, which showed clearly a restrictive diet could not prevent anticipated molting under exogen melatonin influence (Heydon et al. 1995).

6. Summary

Adaptations of animals in a seasonal changing environment are challenging for the organism. Apart from being modulated by melatonin the necessary seasonal adaptations have been shown to be slightly influenced by either n-3 or n-6 supplementation. Higher n-6 levels have a positive effect on the length of torpor or hibernation, body temperature, heart rate and can be further reduced, and thus the animal can conserve energy better. N-3 supplementation has mostly opposite effects than n-6, but adequate levels are essential for diurnal and seasonal rhythms, as well as skin and hair health. These findings on the physiological relationships under n-3 / n-6 supplementation corroborates our results regarding molt. We were able to show that n-3 supplementation slightly anticipates and shortens the shedding process.

Due to the body's adaptation to changing diets in different seasons seems to be very constant and not exogenously influenced, which is why we did not detect any effects of pellet intake on coat change. However, other parameters, such as high body weight, which may have other negative effects on metabolism, or energy-depleting physiological processes, such as reproduction and lactation, also seem to delay coat turnover.

The administration of exogenous melatonin made the deer in our experimental group "blind" to the actual length of the day. The animals of the melatonin treatment group changed their coats significantly earlier.

The results of this study show that the coat change of red deer is closely linked to the physical constitution and energetic, metabolic situation of the animals and that the coat change could be slightly influenced exogenously, not only by melatonin, and could even be further optimized. n-3 and n-6 supplementation could have valuable qualities for seasonal adaptation of red deer.

7. Zusammenfassung

Die Anpassung von Tieren an eine sich jahreszeitlich verändernde Umwelt ist eine Herausforderung für den Organismus. Es hat sich gezeigt, dass die notwendigen saisonalen Anpassungen nicht nur durch Melatonin, sondern auch durch eine n-3- oder n-6-Supplementierung in geringem Maß beeinflusst werden können. Höhere n-6-Spiegel wirken sich positiv auf die Dauer des Winterschlafs aus, denn die Körpertemperatur und die Herzfrequenz und können weiter gesenkt werden, sodass das Tier besser Energie sparen kann. Eine N-3-Supplementierung hat meist gegenteilige Auswirkungen als eine N-6-Supplementierung, doch ist ein ausreichender Gehalt für den Tages- und Jahreszeitenrhythmus sowie für die Gesundheit von Haut und Haaren unerlässlich. Diese Erkenntnisse über die physiologischen Zusammenhänge unter n-3 / n-6-Supplementierung bestätigen unsere Ergebnisse zum Fellwechsel. Wir konnten zeigen, dass eine n-3-Supplementierung den Fellwechsel leicht antizipiert und verkürzt.

Die Anpassung des Körpers an die wechselnde Ernährung in den verschiedenen Jahreszeiten scheint sehr konstant zu sein und nicht von außen beeinflusst zu werden, weshalb wir auch keine Auswirkungen der Pelletaufnahme auf den Fellwechsel feststellen konnten. Andere Parameter, wie ein hohes Körpergewicht, das negative Auswirkungen auf den Stoffwechsel haben kann, oder energieaufwendige physiologische Prozesse wie Fortpflanzung und Laktation scheinen den Fellwechsel jedoch ebenfalls zu verzögern.

Durch die Gabe von exogenem Melatonin wurden die Hirsche unserer Versuchsgruppe „blind“ für die eigentliche Tageslänge. Die Tiere der Melatonin-behandelten Gruppe wechselten ihr Fell deutlich früher.

Die Ergebnisse dieser Studie zeigen, dass der Fellwechsel von Rothirschen eng mit der körperlichen Konstitution und der energetischen, metabolischen Situation der Tiere verknüpft ist und dass der Fellwechsel exogen, nicht nur durch Melatonin, leicht beeinflusst und sogar weiter optimiert werden könnte. N-3 und n-6 Supplementierung könnten wertvolle Eigenschaften für die saisonale Anpassung von Rothirschen haben.

8. References

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