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Abstract: The present study was carried out in order to monitor daily and seasonal variations of rectal temperature in response to different environmental temperatures in alpacas bred in the Italian Apennines at 300 m a.s.l.. In each season, the rectal temperature of 33 clinically healthy alpacas was measured three times/day (morning, midday, afternoon). Ambient temperatures were also recorded. Rectal temperatures ranged from a minimum value of 35.1 to a maximum of 39.4 °C, with a maximum daily thermal excursion of 3.2 °C. They increased throughout the day, with highly significant differences recorded in both young and adult animals between all the time bands ($P < 0.001$). These differences were particularly dramatic for adults in summer, when the mean rectal temperature in the morning was 36.3 ± 0.13 °C, probably as a consequence of recent shearing. Significant differences in daily thermal excursion were recorded depending on the season in both young and adult animals ($P < 0.001$), with the highest excursions values recorded in summer (although the highest daily ambient excursion value was recorded in winter). In conclusion, similarly to alpacas bred in their natural environment, alpacas bred in Italy show a wide thermal neutrality zone, that is probably an adaptive response, which allows the animals to save energy. In the Italian Apennines, shearing should be carried out only in warm seasons, in order to prevent situations of hypothermia, with possible detrimental effects on alpacas' health and welfare.

We answered to all the reviewer's comments and changed the text accordingly. Here are all the specific changes.

Page 2, Line 22-24: Can you reference or provide details to support the statement: "Camelids can show daily variations which are considerably higher than those of other domestic mammals"?

These information were already present in the text; however, following the reviewer's suggestion, we made them clearer to the reader by rephrasing this sentence (p 2, l 22 - p 3, l 1).

Page 3, Line 1 - 13: Are you arguing that alpacas are less able to control it's thermal neutrality relative to ambient temperatures?

No, we are not arguing this. Actually we are only remarking that "Alpacas are able to modify some physiological values, including body temperature, in order to adapt to environmental changes" (p 3, l 11-12), which is an adaptive advantage in their home environment (i.e. with wide excursions of ambient temperature).

Page 4, What was yoru definition of "cria"? Normally, the term cria is used for pre-weaned young stock that are still nursing on the dam. The term "weanling" or "tui" has been used to refer to young stock no longer nursing. From you study pen description, I am not sure if these were very young (< 4 months) or older (> 6 months). Can you provide an age range? There may be dramatic differences between neonates (< 1 months), crias (1 to 6 months), tuis (6 to 12 months) etc.

The reviewer is right and the term "crìa" is not appropriated in this study. We replaced it throughout the text (and in tables and figure too) with the term "young" animals and included a definition for this term, specifying the age (6 months) at the beginning of the trial (p 4, l 8-9).

Page 4, line 12-13: Where the thermometers validated? We have seen up to 1 degree variation between rectal thermometers. What methods where used to ensure accuracy and precision?

We added specific information about thermometer calibration and accuracy of the measurement procedures (p 4, l 16-23)

Page 4, Line 14-16: I am very concerned with the practice of removing feces from the rectum prior to obtaining rectal temperature. Although feces can cause alteration in the rectal temperature recorded, the introduction of air and disruption of the anal sphincter barrier can cause marked changes in rectal temperature, especially during the winter. I am concerned that this could be a critical flaw especially in crias and the method in which this is done. Please provide detailed explanation as to why you feel the rectal temperature data obtained should be accepted as true and accurate. We have seen rectal temperature variation of > 1 degree by cleaning out feces.

The procedure of cleaning the rectum before measuring the temperature was done for practical reasons. We found no reference about significant differences in rectal temperature before and after defecation; on the contrary, there is evidence of consistency of temperatures (< 0.1 °C) before and after defecation (e.g. Burfeind O. et al., 2010. J. Dairy Sci., 93: 624-627). In any case, this procedure was simply achieved by inserting fingers in the distal part of the rectum, and the introduction of air was extremely limited;

furthermore, it was done in all animals and in all seasons, therefore we believe that it is not going to affect our results.

Page 4, line 17 - Did you record humidity in addition to ambient temperature? Can you add this into the model to analyse the relationship between ambient temperature and rectal temperature?

Unfortunately we were not able to record humidity. We agree it would have been useful, but we do not have those data and now there is nothing we can do about it...

Page 4, line 21-23: Did you generate correlation coefficients in the statistical model?

No, we did not. We performed an analysis of variance (ANOVA) and not a correlation analysis, as already stated in the description of statistical analysis (p 5, l 2-5)

Page 5, line 17-20: So this data suggests that alpacas are best able to deal with cold and least able to deal with heat?

No, we are not suggesting this. In this part of the paper, we are only describing our results, and we are not trying to suggest anything. The comments about these results can be found in the discussion (p 7, l 7 - p 8, l 12), where we explain in detail that this effect is due to the lack of fleece during the summer, that reduces the thermoregulation ability of the animals (and not to the fact that alpacas are best able to deal with cold than with heat).

Page 6, line 6-8: Can you be more specific RE what metabolic mechanisms may contribute to this? Also consider that crias have a very different body surface area relative to mass. What dietary effects may have influenced this study?

We added more comments and supporting literature to explain age effect on T_{rec} (p 6, l 13-16).

As to dietary effects, we believe that they can be neglected in this study, as young animals have already been weaned and their diet is similar to that of adult animals, based on concentrated feed and hay. We added some specific information on diet regimen in the materials and methods section (p 4 , l 10-11).

Page 6-7: You frequently refer to how ambient temperatures effect rectal temperature. Ambient temperatures effect core body temperature and rectal temperature is used to estimate core body temperature. Homeostasis centers around core body thermoneutrality, not around rectal thermoneutrality. You might re-consider how you are phrasing this.

We reconsidered and rephrased the text, where necessary. In detail, we made the following changes:

p 7, l 5: we changed “rectal” to “core body”

p 7, l 26: we changed “rectal” to “core body”

p 8, l 13: we changed “rectal” to “core body” p 8, l 16: we added “core”

p 8, l 23: we changed “their rectal temperature” to ‘‘their core body temperature (and as a consequence their rectal temperature)”

p 9, l 5: we changed “rectal” to “core body”.

Table 2-4 could be combined to allow the reader easier review of data.

Following the reviewer’s suggestion, we tried to combine Table 2 and Table 4. However, the results were really confounding, because there were too many data in only one table. Therefore, instead of improving the readability, the review of data was even more difficult than before. This is why we decided to leave them as they are. However, if the Editor considers that it would be better to merge them, we can easily do it.

Thermoregulation of alpacas bred in Italy

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Abstract

The present study was carried out in order to monitor daily and seasonal variations of rectal temperature in response to different environmental temperatures in alpacas bred in the Italian Apennines at 300 m a.s.l.. In each season, the rectal temperature of 33 clinically healthy alpacas was measured three times/day (morning, midday, afternoon). Ambient temperatures were also recorded. Rectal temperatures ranged from a minimum value of 35.1 to a maximum of 39.4 °C, with a maximum daily thermal excursion (ΔT_{rec}) of 3.2 °C. They increased throughout the day, with highly significant differences recorded in both young and adult animals between all the time bands ($P < 0.001$). These differences were particularly dramatic for adults in summer, when the mean rectal temperature in the morning was 36.3 ± 0.13 °C, probably as a consequence of recent shearing. Significant ΔT_{rec} differences were recorded depending on the season in both young and adult animals ($P < 0.001$), with the highest ΔT_{rec} values recorded in summer (although the highest daily ambient excursion value was recorded in winter). In conclusion, similarly to alpacas bred in their natural environment, alpacas bred in Italy show a wide thermal neutrality zone, that is probably an adaptive response, which allows the animals to save energy. In the Italian Apennines, shearing

should be carried out only in warm seasons, in order to prevent situations of hypothermia, with possible detrimental effects on alpacas' health and welfare.

Keywords *Lama pacos*, Body temperature, Thermal homeostasis, Rectal temperature, Shearing.

Introduction

Thermoregulation is a physiological process that helps organisms to maintain the homeostasis, which means that the animal is able to regulate its internal environment and to maintain it in a relatively stable, constant condition (Piccione and Refinetti 2003). This process plays a fundamental role in the response to a pathological state or to systemic inflammation, producing the arousal alternatively of fever or hypothermia (Fowler 1994; Romanovsky and Székely 1998).

Although homeothermic mammals can be exposed to environmental temperatures very different from their normal body temperature, their daily thermal range is usually limited (Piccione and Refinetti 2003). Thermal balance is maintained by producing metabolic heat and by the thermal exchanges through the mechanisms of evaporation, convection and radiation, in a system where there is a balance between heat losses and heat production (Bruné 2001). In extreme climatic conditions, the maintenance of thermal homeostasis can require high energy expenses, especially in the presence of dramatic daily thermal excursions. Such an environmental condition can be found in the natural environment of both Old World (desert) and new World (Andean high plateau) Camelids. For this reason, the thermal neutrality zone of the species belonging to the Camelid family shows a wide temperature range. Within this range, the mechanisms of thermoregulation are not stimulated either by cold stress or heat stress (Bligh 1985). As a consequence, in the presence of low ambient temperatures, Camelids can show a moderate hypothermia that seems to allow the animals to save 5-15% of their energy (Raggi 2000). Core body temperature can show daily variations which are considerably higher than those of other domestic mammals: for example, for sheep and goats average daily excursions between 0.3 and 1.9 °C are reported by Piccione and

Refinetti (2003), whereas earlier research (Schmidt-Nielsen 1959) showed that extremely high daily thermal excursions of rectal temperature (up to 6 °C) may occur in the dromedary (*Camelus dromedarius*). In alpacas, marked daily excursions of rectal temperature (average excursions of 2.56°C) were recorded in the Andean high plateau of Chile, while less marked excursions (1.62°C) were found in the central valley of Chile, at lower altitudes and in milder environmental conditions (Parraguez et al. 1993). Environmental conditions seemed to affect also the average body temperature, that was almost 1°C lower in the high plateau than in the central valley: this might be explained by differences of ambient temperature, barometric pressure and O_2 pressure (Banchero and Grover 1972). In agreement with these findings, a variation of body temperature in response to environmental conditions was recorded also by Mattiello (1993) in four different localities in the Ecuadorian Andes. Alpacas are able to modify some physiological values, including body temperature, in order to adapt to environmental changes. This was observed by Crossley et al. (1994) in animals transported from the high plateau to a lower area: the average body temperature of those alpacas raised from 37.8°C to 39°C, probably mainly in response to higher environmental temperatures.

The presence of a daily rhythmicity of physiological variables was recorded by Morgante et al. (2007) in alpacas bred in Italy. In the case of body temperature, this rhythmicity was not synchronous with that of most of the other variables recorded in this study, and it was probably influenced not only by the light/dark phase, but also by ambient temperature and by metabolism, related to food intake. However, the circadian rhythmicity of body temperature can disappear in alpacas kept in cold environments, such as the high Andean plateau (Parraguez et al. 1993).

Aim of the study was to monitor daily and seasonal variations of rectal temperature in response to different environmental temperatures in alpacas bred in Italy - where this species has been farmed since the ‘90s (de Fidelibus et al. 1996) - under climatic conditions that are far from those of their original environment.

Materials and methods

The study was carried out in a commercial alpaca farm in the Province of Parma (44°41’20’’, 10°00’45’’), located at 300 metres a.s.l. in the Italian Apennines. The area is characterized by severe winter conditions, with average seasonal temperatures around 0°C, alternated to hot summers, with temperatures raising above 30°C.

The study was carried out on 33 clinically healthy alpacas (huacaya breed): 23 adults (7 males and 16 females) and 10 young animals (3 males and 7 females). The animals were kept in three separate paddocks: one for adult males, one for adult females and one for young animals. For the purpose of this study, we defined as “young” all weaned animals aged about 6 months at the start of the trial. Both young and adult animals were fed on pasture and *ad libitum* alfalfa hay, integrated with concentrated feed. All paddocks included an open area (pasture and woods) and a sheltered area, where feed troughs and water were available. All animals were sheared at the end of spring. Three adult females were eliminated from the farm during the experimental period (two at the beginning of spring and one at the beginning of summer): for these animals rectal temperatures could not be collected in all seasons.

Rectal temperature was measured by a single investigator, using a calibrated digital rectal thermometer, and it was compared to a certified mercury thermometer to test for accuracy, checked before and after the examination and maintained at ambient room temperature. The digital thermometer was inserted into the rectum at 8 cm and extracted when it emitted an acoustic signal. In order to achieve this measurement, the animals were manually restrained by the farmer and the rectum was cleaned out from feces before inserting the thermometer. Measures were taken three times/day (morning, midday, afternoon) for each animal, and this was repeated once in each season. In total, 12 rectal temperatures for each animal were recorded.

At the same time when rectal temperatures were collected, ambient temperatures were recorded using a common mercury thermometer for exterior environments, placed in a shaded area.

For each day of measurement, we calculated the daily thermal excursion of individual rectal temperature (ΔT_{rec}) and of ambient temperature (ΔT_{amb}). Rectal temperatures and ΔT_{rec} were submitted to Oneway ANOVA (SPSS Inc.) in order to test the effects of individual factors (sex and age class) and of environmental factors (season and time band). Bonferroni test was used for multiple comparisons.

Results

Ambient temperatures recorded during the experimental days are reported in Table 1. The highest temperatures were registered in summer, while the lowest value was found in winter, in the morning time band. Thermal excursions were particularly remarkable in winter and in summer, while they were low in spring and in autumn.

Rectal temperatures ranged from a minimum value of 35.1 to a maximum of 39.4 °C, with a maximum ΔT_{rec} of 3.2 °C. No significant differences were detected between sexes (males: 37.88 ± 0.06 °C; females: 37.80 ± 0.68 °C), therefore sex effect was not taken into account for subsequent analysis. Significantly higher rectal temperatures were recorded in young than in adult animals (Table 2; $P < 0.001$). Significant differences were found among seasons ($P < 0.001$ in both age classes; Table 2) both in young and in adult animals. In young animals, rectal temperatures were significantly lower in the colder seasons (Autumn and Winter), while in adults the lowest rectal temperatures were recorded in summer.

Rectal temperatures increased throughout the day, with highly significant differences recorded in both age classes between all the time bands (Table 3; $P < 0.001$). These differences were always highly significant also within each season in both age classes (Figure 1; $P < 0.001$ in all cases), and were particularly dramatic for adults in summer.

Significant ΔT_{rec} differences were recorded depending on the season both in young and in adult animals (Table 4; $P < 0.001$): lower ΔT_{rec} were observed in the seasons with lower ΔT_{amb} (autumn and spring), while the highest ΔT_{rec} values were recorded in summer, although the highest ΔT_{amb} values were recorded in winter.

Discussion

Mean rectal temperatures recorded in adult alpacas in this study were lower than those reported by Parraguez et al. (1993) in Chile, but still within the normal range (37.5 - 38.9 °C) reported for this species by Fowler (1998). Maximum recorded values are always below 41.1 - 42.2 °C, which would indicate a condition of heat stress (Fowler 1998); however, minimum values were sometimes well below the normal range, although animals showed no clinical pathological sign.

Mean values in young animals were significantly higher than in adults, in agreement with what reported for this species (Fowler 1998) and also for other species, such as cattle (Berbigier 1988). The physiological decline of metabolic rate that occurs in older age may be a possible explanation for this phenomenon (Hulbert and Else 2000), because higher metabolic rates are significantly correlated with body temperature, as recently summarized by Clarke et al. (2010). Furthermore, body surface area-to-mass ratio decreases with age and this ratio is directly correlated with core body temperature (Havenith 2001).

ΔT_{rec} were higher in young than in adult alpacas probably because thermoregulatory mechanisms in young animals are not completely developed yet (Fowler 1998). However, in general, the trend of rectal temperatures and ΔT_{rec} of young animals was analogous to that of adults, with similar variations in response to season and time band. The lack of significant differences between males and females is also in agreement with previous research (Mattiello 1993).

The increase of rectal temperatures throughout the day is consistent with the results registered by Morgante et al. (2007), that set the acrophase of alpacas bred in Italy in the evening, after 22:00 h.

This pattern is probably related to a metabolism increase in response to daily activity and food assumption during the day, while animals are at pasture (Hulbert and Else 2000; Morgante et al. 2007). However, it seems related also to the progressive raise of ambient temperature from morning to evening, bearing in mind that the body thermal inertia provokes a delay of maximum rectal temperature compared to the time of maximum ambient temperature (Berbigier 1988). A positive relationship between rectal temperatures and ambient temperatures was also found in llamas (*Lama glama*) in an experimentally controlled environment (Schwalm et al. 1996) and this is a further evidence to support the role of ambient temperature for determining core body temperature of South American camelids.

While rectal temperatures showed a trend in agreement with ambient temperatures within the time band, seasonal changes of rectal temperatures did not always match the changes of ambient temperatures. In fact, rectal temperatures were low in autumn and winter (seasons with the lowest ambient temperatures), but they were low even in summer. Actually, in adults the lowest rectal temperatures were found in summer, when ambient temperatures were higher. However, the lowest values were recorded in summer in the first time band, while during the day rectal temperatures show a marked increase, reaching values among the highest of the whole period (Figure 1). This is clearly confirmed by the significantly higher ΔT_{rec} recorded in this season. These extremely low rectal temperatures in summer mornings can be explained by the absence of fleece in this season, as all animals were sheared at the beginning of summer. The fiber coat of alpacas is a highly efficient insulating barrier (Fowler 1998) and its absence may have a profound impact on thermoregulation ability, as reported by Navarre et al. (2001) in a comparative study on sheared and non sheared alpacas in east central Alabama. Navarre et al. (2001) observed that in non sheared alpacas rectal temperatures often went beyond the normal range reported for this species by Fowler (1998), while this occurred very seldom in sheared animals. These results suggested that the presence of fleece influences thermoregulation and that shearing helps to prevent heat stress in alpacas in southeastern United States, as confirmed also by thermographic analysis of surface body temperatures (Heat et al. 2001). Similar results had previously been obtained by Schwalm et al. (1996) in llamas. On the other hand, our data related to the Apennine environment showed that, in the morning, sheared alpacas may face some difficulties for preventing their core body temperature to fall below the normal range: in fact, mean rectal temperatures of adult alpacas in summer are below this range, due to the consistently low values registered in the morning, that in one case even lowered down to 35.10 °C. Given the above mentioned body thermal inertia (Berbigier 1998), this is probably not an instantaneous effect of ambient temperature, but rather a consequence of the low temperatures reached during the night in the Apennines, at 300 m a.s.l.. As our animals were all healthy and they showed no clinical sign of hypothermia, we may suppose that, even in presence of ΔT_{amb} less marked than in their original environment (where ΔT_{amb} can be over 30 °C; e.g. Parraguez et al. 1993), non sheared alpacas show a wide thermal neutrality zone in the Italian Apennines, as well as in the Andes. It may be important to remark that this kind of thermoregulatory response is not obvious, and it differs from that of sheep. In this last species, Piccione and Caola (2003) recorded an increase of rectal temperature one month after shearing (shearing-induced hyperthermia), that was explained by the authors in terms of "cold habituation" (Slee 1987). The thermoregulatory response of our alpacas, that lowered their core body temperature instead of producing heat in order to compensate heat losses during the night, is therefore probably an adaptive response, which allows the animals to save energy and it also helps them to benefit of nocturnal low ambient temperatures in order to lower their core body temperatures for facing the daily increase that normally occurs in summer, without risking to undergo to heat stress. In fact, although the overall average ΔT_{rec} was not higher than the values recorded for most other domestic mammal species (Piccione and Refinetti 2003), significant differences among seasons were found: ΔT_{rec} were lower in Spring and Autumn (when lower ΔT_{amb} were recorded), but higher in winter and, above all, in summer, when a maximum ΔT_{rec} of 3.2 °C was recorded.

It may be concluded that alpacas bred in Italy, as well as those bred in their natural environment, show a wide thermal neutrality zone. This means that their core body temperature (and as a consequence their rectal temperature) may show considerable fluctuations throughout the day. In the light of this, the reference values reported by Fowler (1998) might be revisited and they should be adapted to specific environmental conditions. Rectal temperature is a key diagnostic parameter: it is important that veterinarians are aware of its possible variations related to ambient temperature, and that they use it always in association with other parameters for assessing the health status of alpacas.

Due to this wide thermal neutrality zone, shearing has a dramatic effect on alpacas' core body temperature. Our results provide no evidence of hyperthermia (that may eventually lead to arousal of heat stress) in sheared animals; however, it was not possible to compare these results in non sheared animals, as all our alpacas were sheared. In the Italian Apennines, shearing is probably useful in order to prevent heat stress in alpacas. However, it should be carried out only in warm seasons, as ambient temperatures lower than those recorded in summer might lead to situations of hypothermia, with detrimental effects on alpacas' health and welfare.

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Figure 1 Daily variations of rectal temperature of young and adult alpacas in the four seasons (means ± stderr).

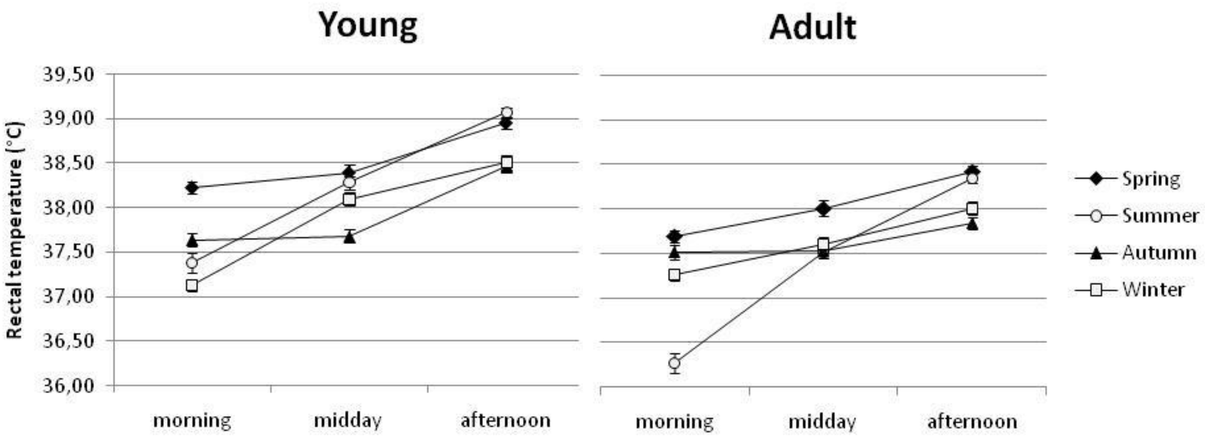

Table 1 Ambient temperatures and thermal excursions (ΔT_{amb}), expressed in °C, recorded during the experimental days in each season and in each time band.

Time band	Season			
	Autumn	Winter	Spring	Summer
Morning	5.0	-2.0	24.0	25.0
Midday	6.0	13.0	22.0	29.0
Afternoon	10.0	10.0	26.0	35.0
Mean	7.0	7.0	24.0	29.7
ΔT_{amb}	5	15	4	10

Table 2 Rectal temperatures, expressed in °C, recorded in the different seasons in young and adult animals.

	Young					Adult				
	N*	Mean	stderr	min	max	N*	Mean	stderr	min	max
Autumn	30	37.93[a]	0.08	37.30	38.80	69	37.63[a]	0.04	36.60	38.30
Winter	30	37.91[a]	0.11	36.90	38.80	69	37.63[a]	0.05	36.70	38.50
Spring	30	38.53[b]	0.07	38.00	39.40	63	38.05[b]	0.06	37.00	39.20
Summer	30	38.24[ab]	0.14	37.00	39.30	60	37.38[a]	0.12	35.10	38.90
Overall	120	38.15	0.06	36.90	39.40	261	37.68	0.04	35.10	39.20

*Three measurements for each animal in each season. Two missing adults in Spring, three in Summer.

[a, b]Means in a column with different superscript letters are significantly different ($P < 0.001$).

Table 3 Rectal temperatures, expressed in °C, recorded in the different time bands in young and adult animals.

	Young					Adult				
	N*	Mean	stderr	min	max	N*	Mean	stderr	min	max
Morning	40	37.60[a]	0.08	36.90	38.70	87	37.21[a]	0.07	35.10	38.60
Midday	40	38.12[b]	0.06	37.40	38.70	87	37.67[b]	0.04	36.60	38.90
Afternoon	40	38.75[c]	0.05	38.10	39.40	87	38.15[c]	0.04	37.20	39.20
Overall	120	38.15	0.06	36.90	39.40	261	37.68	0.04	35.10	39.20

*Four measurements for each animal in each time band. Three missing adults in each time band.

[a, b]Means in a column with different superscript letters are significantly different ($P < 0.001$).

Table 4

Table 4 Thermal excursions of rectal temperatures (ΔT_{rec}), expressed in °C, recorded in the different seasons in young and adult animals.

	Young					Adult				
	N	Mean	stderr	min	max	N	Mean	stderr	min	max
Autumn	10	0.84[a]	0.11	0.2	1.3	23	0.34[a]	0.05	0.0	0.7
Winter	10	1.38[b]	0.09	0.9	1.7	23	0.75[b]	0.08	0.2	1.4
Spring	10	0.77[a]	0.06	0.4	1.1	21	0.69[ab]	0.07	0.1	1.3
Summer	10	1.69[b]	0.15	0.9	2.2	20	2.08	0.16	1.0	3.2
Overall	40	1.17	0.08	0.2	2.2	87	0.93	0.08	0.0	3.2

[a, b]Means in a column with different superscript letters are significantly different ($P < 0.01$).