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Oscillatory thermo-regulatory behavior of fecundity-gene-introgressed sheep in the hot semi-arid region

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ABSTRACT

Fecundity gene (*FecB*) introgression improves the prolificacy of sheep. However, it may adversely affect the adaptability of thermoregulatory behavior when it is introgressed in other than hot-humid regions because it is found in the sheep of the hot-humid region. Therefore, the present study was initiated to assess the oscillatory thermoregulatory behavior of *FecB*-introgressed sheep in a hot semi-arid region. The thermoregulatory behavior parameters (i.e., respiration rate [RT], heart rate [HR], rectal temperature [RT], and skin temperature [ST]) were recorded at a three-hour interval for continuous 24 hours in well-adapted sheep of the semi-arid region and in *FecB*-introgressed sheep of the same region during extreme climatic conditions (summer and winter). The cosinor analysis showed a daily circadian rhythm ($P < 0.01$) in RR, HR, and ST values, as well as similar significant ($P < 0.01$) differences in thermoregulatory behavior in both the sheep during the summer and winter. The rhythmic change of ambient temperature throughout the day influenced the thermoregulatory physiological behaviors of both well-adapted and *FecB*-introgressed sheep under semi-arid conditions. The nonsignificant ($P > 0.05$) difference in mesor, amplitude, and acrophase in RR, PR, RT, and ST among the breeds during summer and winter indicated that the *FecB*-introgressed sheep also use a similar adaptive strategy to cope with environmental extremes of the hot, semi-arid region like well-adapted sheep.

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Introduction

In the hot-arid and semi-arid region where high temperature and solar radiation severely affect animal production, sheep husbandry has been a sustainable livelihood since ancient times (Naqvi et al., 2013). The survival and performance of a breed depends on heat tolerance and adaptive capability in that environment (Silva et al., 2016). The regulation of body temperature in homeotherms is ensured by mechanisms of thermolysis and thermogenesis. Thermoregulatory adjustments can be induced not only by changes in environmental temperature but also by a variety of physiological situations, including age, fasting, and food intake, inducing changes in internal temperature (Arfuso et al., 2016a, b). The evaluation of body temperature represents a valuable tool to monitor the physiologic status, welfare, and the stress responses of animals (Piccione et al., 2002; Piccione et al., 2012; Rizzo et al., 2017).

The Malpura sheep is one of the heaviest sheep breeds of India where it is widely distributed in the semi-arid region and is well adapted to this environment/agroclimatic region for the production of mutton and wool (Gowane et al., 2014). However, the explosive growth in the human population with an associated increasing purchasing capability has increased the demand for the livestock product (Rojas-Downing et al., 2017). To meet this huge demand, a high-performing prolific three-breed cross has been developed in the semi-arid region by the introgression of the fecundity gene (*FecB*) from Garole (hot-humid region breed) to native Malpura breed. The *FecB* gene is a single autosomal gene located on ovine chromosome 6 that has an additive effect on ovulation rate. The *FecB* gene was introduced in Booroola sheep flocks via importation of Garole sheep in the late 19th century from its natural habitat located in the low-lying, hot, and humid Sunderban region in the West Bengal state of India (Kumar et al., 2012).

The *FecB*-introgressed breed has the potential to increase the farmer's income one and a half times. The native breeds of the hot

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semi-arid region are adapted to the local environments, but the *FecB*-introgressed sheep might suffer from the stressful environmental conditions during extreme temperatures in the fluctuating part of the day during the extreme seasons. The adapted native breeds have their own management strategies to cope with these environmental challenges. Along with environmental management, selection of much-adapted animals may reduce the deleterious effect of thermal stress and retain maximum productive and reproductive performance (Da Silva et al., 2017).

The circadian rhythmicity is a regular endogenously developed physiological property. Sheep also show nycthemeral variations of deep body temperature (De et al., 2017a). This process involves temperature regulation along with that regulation of respiration rate (RR), heart rate (HR), and cutaneous temperature variations. All these processes probably coevolved together. The circadian rhythmicity is presumably an adaptive mechanism of an animal with the change of environmental conditions in day-night and season (Pittendrigh, 1993). In animals, skin temperature (ST) also changes in the day-night cycle as core temperature changes in the cycle and temperature passes from the core to the periphery. Variation of ST is also influenced by the oscillation of ambient temperature (Piccione et al., 2013). However, the control in this process is affected by both genetic inheritance and environmental factors. Although the regulation of core temperature is controlled by the thermoregulatory centers in the brain, sheep under different ambient temperature activate a series of compensatory mechanisms to control heat exchange and maintain homeothermy.

The *FecB*-introgressed sheep is a cross of native sheep breeds of the semi-arid region and hot-humid region breeds. Thermoregulatory ability of this introgressed crossbred sheep in the semi-arid region is unknown. If this crossbreed shows similar thermoregulatory ability as well-adapted Malpura sheep in terms of body temperature, RR, and ST with a similar oscillatory pattern, then this sheep can be adaptable to this region and may contribute potentially in the socioeconomic condition of this region. Therefore, the present study was initiated to evaluate the oscillatory thermoregulatory behavior of *FecB*-introgressed sheep in a hot semi-arid region.

Materials and methods

Site of study

The experiment was carried out at the experimental animal farm of the Indian Council of Agricultural Research—Central Sheep and Wool Research Institute, Avikanagar, located in the semi-arid region of India (Longitude 75°28′E, Latitude 26°26′N, and Altitude 320 m above mean sea level). The annual ambient temperature ranges between 3°C and 46°C, and annual relative humidity (RH) ranges between 10% and 85%. The rainfall in this area is erratic and distributed throughout the year. Annual precipitation ranges from 200 to 500 mm.

Animals, handling, and feeding

The present experiment was carried out on seven well-adapted sheep of the native Malpura breed of the semi-arid region and seven crossbred sheep of a prolific three-breed cross Avishaan ram. Malpura is a triple purpose, hardy sheep breed that originated in the arid and semi-arid areas of western tropical India. They are well known for their hardiness and adaptability to the local environment. Avishaan is a prolific crossbred sheep evolved at the Indian Council of Agricultural Research—Central Sheep and Wool Research Institute, Avikanagar, by introgression of *FecB*. Prophylactic measures were taken against sheep diseases from the Garole to Malpura breeds. The prolific cross is a composite cross, having 12.5% Garole, 37.5% Malpura, and 50% Patanwadi blood. All Avishaan rams were confirmed to be *FecB* carrier by forced restriction fragment length polymorphism-polymerase chain reaction technique using the protocol of Wilson et al. (2001) as described by Kumar et al. (2006). Both Malpura and Avishaan rams were selected from the experimental animal flock institute, so that both the groups should have almost similar body weight. The selected Malpura and Avishaan rams were of 748.7 ± 119.5 days and 814.3 ± 93.3 days of age with an average body weight of 37.6 ± 2.1 kg and 36.6 ± 1.4 kg, respectively. The animals were housed in well-ventilated sheds made up of asbestos roofing and open from the sides and were maintained under proper hygienic conditions. All the animals were fed individually for a maintenance requirement (ME: 1.81 ± 0.04 Mcal and digestible crude protein: 49.1 ± 1.33 g) on the body weight basis as per Indian Council of Agricultural Research recommendations (ICAR, 2013). The animals were stall-fed with a diet consisting of 70% roughage (*Cenchrus ciliaris* hay) and 30% concentrate feed (barley 650 g/kg, groundnut cake 320 g/kg, minerals 30 g/kg including 10 g/kg NaCl, with crude protein = 180 g/kg and total digestible nutrients = 650 g/kg). All the animals were kept inside the same experimental shed and the same feeding regime in similar condition 15 days before interventions for sheep pox, peste des petits ruminants, enterotoxaemia, and endoparasitic and ectoparasitic infestations were carried out as prescribed by the health division of the institute to ensure that the animals were in a healthy condition throughout the study.

Data collection/experimental procedure

During the summer and winter, the RR, HR, rectal temperature (RT), and ST were measured at 3-h intervals for 24 consecutive hours from each native-breed and native crossbred animal on two occasions inside the shed. All the recording was taken at 0600h, 0900h, 1200h, 1500h, 1800h, 2100h, 2400h, and 0300h of the day by the trained veterinarian. The animals were habituated to the new experimental shed for a period of 15 days before the start of the experiment. During this 15-day period, the animals were accustomed daily to handling and recording procedure to reduce their stressful impact (Guesdon et al. 2012, 2015). The dry bulb temperature, wet bulb temperature, and RH were also recorded inside the shed at the time of physiological parameter study (3 hours interval). The temperature-humidity index was calculated by the formula as described by Marai et al. (2007). The variation in ambient temperature and temperature-humidity index at the different time

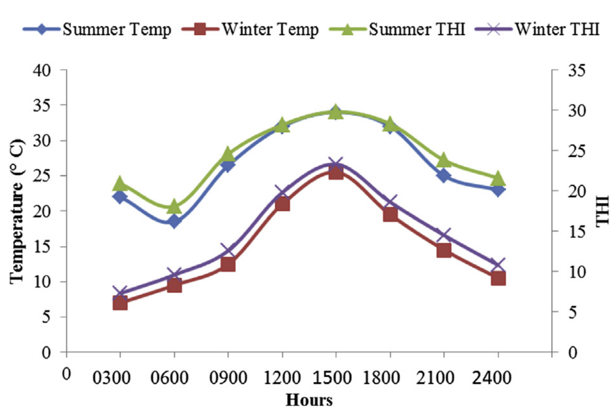

Figure 1. Temperature and temperature-humidity index (THI) in summer and winter during different hours of the day in the semi-arid tropical environment. THI were calculated using the formula THI = db °C − {(0.31−0.31 RH) (db °C − 14.4)} given by Marai et al. (2007). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

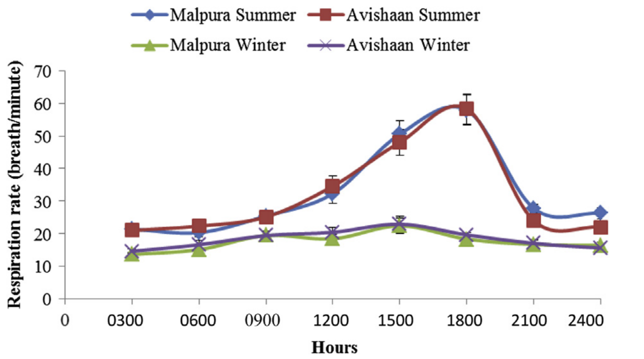

Figure 2. Oscillation of respiration rate during summer and winter in fecundity gene (*FecB*)-introgressed sheep in the semi-arid region. Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed. Mean values of the respiration rate maintained during summer and winter measured every 3 h for 24 h in sheep. Each data point is the mean (±SE) of 7 sheep. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

point of the day during summer and winter is depicted in Figure 1. A complete inward and outward movement of the flank of the animal was counted as single respiration, and the total number of movement in one minute, RR. Counting of the number of pulses (beats) in one minute in the femoral artery using the middle finger indicated the HR. Rectal temperature was recorded using a digital clinical thermometer (Model No KFT-04; Microgene Diagnostic Systems Pvt Ltd, India; accuracy: ±0.1°C) that was gently inserted about 3 cm deep into the rectum, so that probe remained in contact until the reading becomes stabilized. The ST was taken by using an infrared laser thermometer (DT-8550 Handheld Non-Contact Laser Infrared Thermometer, France; Accuracy± 2%) in the prescapular region. Before taking a reading of ST, 1 cm^2 area of the prescapular region was clean shaved.

Data analysis

The data of RT, RR, HR, and ST were collected at eight data points from each animal. The rhythmicity of all the parameters was evaluated through repeated-measures analysis of variance and by the cosinor procedure (Refinetti et al., 2007). Three rhythmic parameters were determined for each time series: mesor (mean level), amplitude (half the range of excursion), and acrophase (time of peak). The cosinor procedure uses an F test to evaluate whether the amplitude of a cosine wave fitted to the data is significantly greater than zero (Refinetti et al., 2007). An amplitude not significantly greater than zero implies the absence of rhythmicity. The effects of the season and breed on circadian rhythmicity of RR, HR, and RT were analyzed by comparing the data of mesor, amplitude, and acrophase of individual animals across seasons using general linear model in SPSS 14.0.

Results

The mean values of RR at eight time points of summer and winter of native-breed and crossbred sheep are described in Figure 2. In both breeds, RR during summer was significantly ($P < 0.01$) higher than winter in all the time points. The cosinor analysis demonstrated significant ($P < 0.01$) rhythmicity of RR of both native-breed and crossbred sheep during summer and winter is described in Table 1 and Table 2, respectively. The mesor of RR during summer and winter did not differ ($P > 0.01$) between native-breed and crossbred sheep (Tables 3 and 4, respectively). However, in both the breeds, the mesor of RR differs significantly ($P < 0.01$) between the seasons (Tables 1 and 2). The amplitude of RR during summer and winter did not differ between native-breed and crossbred sheep. However, the amplitude of RR differs significantly ($P < 0.01$) between the seasons in both the breeds (Tables 3 and 4). The acrophase of the native breed did not differ significantly ($P > 0.05$) from crossbred sheep both in summer (Table 1) and winter (Table 2). However, the acrophase of RR in crossbred sheep differ significantly ($P < 0.05$) in winter (Table 4).

The mean value of HR of native-breed and crossbred sheep during summer and winter at eight time points of the day is depicted in Figure 3. The HR of both breeds at all time points of the day was significantly ($P < 0.01$) higher during summer than winter. The daily rhythmic oscillation of HR during summer and winter in both native-breed and crossbred sheep was confirmed by the cosinor analysis in Tables 1 and 2, respectively. The mesor of HR both during summer (Table 1) and winter did not differ ($P > 0.05$) significantly between native-breed and crossbred sheep (Table 2). However, the mesor of HR in Malpura was significantly ($P < 0.01$) higher in summer than winter (Table 3). No significant ($P > 0.05$) difference was found in mesor of HR in crossbred sheep between summer and winter (Table 4). The amplitude of HR did not differ significantly ($P > 0.05$) between the breeds in both summer (Table 1) and winter (Table 2), as well as between the seasons in both the breeds (Tables 3 and 4). The acrophase of HR also did not show any significant ($P > 0.05$) variation between the breeds (Tables 1 and 2) and seasons (Tables 3 and 4).

The mean value of RT of native-breed and crossbred sheep during summer and winter at eight different time points of the day is depicted in Figure 4. The RT of both breeds was significantly ($P < 0.05$) higher in summer than winter at 1200h and 0300h of the day. At 1500 h of the day in Malpura sheep, the RR differs ($P < 0.05$) between the seasons; however, no difference was found in crossbred sheep at the same time point between the seasons. At 1800 h and 2100h of the day, the RT did not differ ($P > 0.05$) between the seasons in crossbred sheep at the same points. The cosinor analysis

Table 1
Comparison of mesor, amplitude, and acrophase of thermoregulatory responses between well-adapted and *FecB*-introgressed sheep during summer in the semi-arid tropical environment

Parameters	Mesor				Amplitude				Acrophase				*P*-value of rhythmicity			
	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST
Malpura	32.7	64.6	38.7	36.9	16.4	6.7	0.4	1.2	16.6	13.4	17.6	17.4	<0.01	<0.01	<0.01	<0.01
Avishaan	31.9	61.0	38.8	37.1	15.9	4.4	0.4	1.1	16.0	15.3	16.4	17.4	<0.01	<0.01	<0.01	<0.01
SE	1.0	1.5	0.1	0.2	1.4	0.8	0.1	0.2	0.5	1.4	1.3	0.6				
P value	0.61	0.11	0.61	0.33	0.87	0.10	0.84	0.74	0.06	0.44	0.34	0.46				

FecB, fecundity gene; RR, respiration rate; HR, heart rate; RT, rectal temperature; SE, standard error of the mean; ST, skin temperature.
Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed.
Cosinor analysis was conducted for each individual time series (7 animals), and group differences in the three parameters (mesor, amplitude, and acrophase) were compared with GLM using SPSS 14.0.

Table 2
Comparison of mesor, amplitude, and acrophase of thermoregulatory responses between native-breed and *FecB*-introgressed sheep during winter in the semi-arid tropical environment

Parameters	Mesor				Amplitude				Acrophase				*P*-value of rhythmicity			
	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST
Malpura	17.6	52.3	38.2	34.2	3.4	5.1	0.3	2.9	13.9	11.8	18.2	18.2	<0.01	<0.01	0.1	<0.01
Avishaan	18.3	56.1	38.2	33.7	3.7	4.5	0.3	2.8	14.2	12.8	17.2	19.4	<0.01	<0.01	0.3	<0.01
SE	1.0	1.5	0.1	0.2	1.4	0.8	0.1	0.2	0.5	1.4	1.3	0.6				
P value	0.53	0.11	0.99	0.07	0.76	0.66	0.82	0.97	0.76	0.47	0.66	0.06				

FecB, fecundity gene; RR, respiration rate; HR, heart rate; RT, rectal temperature; SE, standard error of the mean; ST, skin temperature.
Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed.
Cosinor analysis was conducted for each individual time series (7 animals), and group differences in the three parameters (mesor, amplitude, and acrophase) were compared with GLM using SPSS 14.0.

suggests that the changes in RT at different time points of the day were rhythmic ($P < 0.01$) in both the breeds during summer and winter (Tables 1 and 2, respectively). The mesor of RT during summer (Table 1) and winter (Table 2) did not differ significantly ($P > 0.05$) between native-breed and crossbred sheep, whereas the mesor of RT was significantly ($P < 0.05$) higher during summer than winter on both the breeds (Tables 3 and 4). The amplitude and acrophase of RT did not differ significantly ($P > 0.05$) between the breeds in both the seasons (Tables 1 and 2), as well as between the seasons in both the breeds (Tables 3 and 4).

The mean values of ST of native-breed and crossbred sheep during summer and winter at eight different time prints of the day are depicted in Figure 5. In both the sheep breeds, the ST during summer was significantly ($P < 0.05$) higher than that during winter in all the time points of the day. The rhythmic changes of ST in different time points during summer and winter in both the breeds were established ($P < 0.01$) by the cosinor analysis (Tables 1 and 2, respectively). The mesor of ST during summer (Table 1) and winter (Table 2) did not differ ($P > 0.01$) between the breeds. However, the mesor of ST of both native-breed and crossbred sheep differed significantly ($P < 0.01$) between summer (Table 3) and winter (Table 4). The amplitude of ST for summer (Table 1) and winter (Table 2) did not differ significantly ($P > 0.05$) between the breeds, but the amplitude of ST in both native-breed (Table 3) and crossbred (Table 4) sheep differed significantly ($P < 0.01$) between summer and winter. The acrophase of ST for summer (Table 1) and winter (Table 2) did not vary ($P > 0.01$) between native-breed and crossbred sheep. The amplitude of ST of Malpura sheep showed no significant ($P > 0.01$) variation between the seasons (Table 3), whereas it varied significantly ($P < 0.01$) in crossbred sheep between summer and winter (Table 4).

Discussion

The environmental influence on livestock is through environmental temperature and humidity or a combination of both (Da Silva et al., 2017). Extreme climatic variable fluctuations may hamper the homeothermy of sheep, and the ability to regulate body temperature depends on the breed (Sejian et al., 2017). However, the native sheep breeds of arid and semi-arid regions are well adapted to such extreme environmental conditions (Naqvi et al., 2013). Therefore, appropriate breed selection is a foremost criterion for sustainable production in the semi-arid region, although sheep show higher adaptability to the harsh environment (Sejian et al., 2017).

The present study showed that the changes of RR at different time points of the day in both seasons was rhythmic ($P < 0.01$) in both native-breed and crossbred sheep. In accordance with previous studies (Fahmy and thesis, 1994), the RR was significantly higher in summer than winter in both sheep breeds. The higher ambient temperature in summer may causes a reduction in the thermal gradient between the animal and the surrounding environment that leads to failure of sensible heat loss. This will force the animals to resort evaporative respiratory cooling, ultimately resulting in higher RR in summer in both the breeds (Al-Haidary, 2004). The significantly higher mesor of RR and the higher RR at the different time points of the day during summer in both the breeds indicated that both sheep breeds take necessary thermolysis action to maintain the homeothermy in their body to avoid an unusual increase in body temperature (McManus et al., 2009). A similar finding was also reported (De et al., 2017a, b) in their previous study. The significantly higher amplitude of RR during summer in both the breeds might be due to a higher ambient temperature in summer, which was beyond the upper critical limits at some time points of the day. The similar acrophase of RR in native-breed sheep during summer and winter and the significant difference in acrophase of RR in crossbred sheep during summer and winter reflect the difference in the pattern of RR with the change of ambient temperature during summer and winter in crossbred sheep, although the values were similar with the native breed. Furthermore, the resemblance of mesor, amplitude, and acrophase of RR in both the breed during summer and winter

Table 3
Comparison of mesor, amplitude, and acrophase of thermoregulatory responses between seasons in native-breed sheep in the semi-arid tropical environment

Parameters	Mesor				Amplitude				Acrophase				*P*-value of rhythmicity			
	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST
Summer	32.7	64.6	38.7	36.9	16.3	6.7	0.4	1.2	16.6	13.4	17.6	17.4	<0.01	<0.01	<0.01	<0.01
Winter	17.6	52.2	38.2	34.2	3.4	5.1	0.3	2.9	13.9	11.8	18.2	18.2	<0.01	<0.01	0.1	<0.01
SE	1.0	1.5	0.1	0.2	1.4	0.8	0.1	0.2	0.5	1.4	1.2	0.6				
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.40	0.10	<0.01	0.10	0.50	0.70	0.40				

FecB, fecundity gene; RR, respiration rate; HR, heart rate; RT, rectal temperature; SE, standard error of the mean; ST, skin temperature.
Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed.
Cosinor analysis was conducted for each individual time series (7 animals), and group differences in the three parameters (mesor, amplitude, and acrophase) were compared with GLM using SPSS 14.0.

Table 4
Comparison of mesor, amplitude, and acrophase of thermoregulatory responses between seasons in *FecB*-introgressed sheep in the semi-arid tropical environment

Parameters	Mesor				Amplitude				Acrophase				*P*-value of rhythmicity			
	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST	RR	PR	RT	ST
Summer	31.9	61.0	38.8	37.1	15.9	4.4	0.4	1.1	16.0	15.3	16.4	16.6	<0.01	0.02	<0.01	<0.01
Winter	18.2	56.1	38.2	33.7	3.7	4.5	0.3	2.8	14.2	12.8	17.2	19.4	<0.01	0.02	0.3	<0.01
SE	1.0	1.5	0.1	0.2	1.4	0.8	0.1	0.2	0.5	1.4	1.2	0.6				
P value	<0.01	0.10	<0.01	<0.01	<0.01	0.90	0.20	<0.01	0.01	0.10	0.70	<0.01				

FecB, fecundity gene; RR, respiration rate; HR, heart rate; RT, rectal temperature; SE, standard error of the mean; ST, skin temperature.
Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed.
Cosinor analysis was conducted for each individual time series (7 animals), and group differences in the three parameters (mesor, amplitude, and acrophase) were compared with GLM using SPSS 14.0.

reflects that the crossbred sheep also take the similar favorable adaptive strategy of heat loss during summer and winter, like the native sheep breed, to maintain the core body temperature.

In the present study, the HR showed circadian rhythmicity during summer and winter in both native-breed and crossbred sheep. The significantly higher HR at all the time points of the day during summer in both the sheep breeds might be attributed to a higher temperature during summer, which compelled the animals to peripherally vasodilate and thereby increased cardiac output and HR (Silanikove, 2000). The higher mesor of HR during summer in both breeds was due to higher HR during different time points of the day. This pattern represents an effort to increase blood flow from the core to the surface to facilitate better heat loss by sensible and insensible means (Sejian et al., 2010). The nonsignificant difference of amplitude and acrophase of HR between summer and winter in both the breeds suggests that they have similar metabolic activity and rate during summer and winter. The similar mesor, amplitude, and acrophase of HR of native-breed and crossbred sheep during summer and winter also reflect the similar metabolic activity of both the breeds during summer and winter.

Rectal temperature is an indicator of core body temperature (Nieslon, 1995). In the present study, the RT showed circadian rhythmicity ($P < 0.01$) in both native-breed and crossbred sheep both during summer and winter. The mesor of RT was significantly ($P < 0.01$) higher during summer in both the breeds, which might be attributed to higher ambient temperature during summer than winter. The similar amplitude and acrophase in both native-breed and crossbred sheep suggests that they have the ability to maintain their homeothermy within a narrow range during summer and winter. Furthermore, the resemblance for mesor, amplitude, and acrophase among native-breed and crossbred sheep during summer and winter indicated a similar oscillatory pattern and a similar thermoregulatory adaptability in both native-breed and crossbred sheep. These physiological adjustments are essential for newly developed *FecB*-introgressed sheep in the arid and semi-arid region to maintain normal body temperature where environmental temperature varies significantly between day and night time.

In the present study, a significant ($P < 0.01$) oscillation of ST was observed in both native-breed and crossbred sheep during summer and winter. Piccione et al. (2013) also reported a similar rhythmic pattern in ST of sheep. The significantly higher ST during summer in both the breeds might be attributed to higher ambient temperature during summer. Owing to vasodilatation of skin at higher ambient temperature, the capillary bed increases the blood flow to the skin surface for heat dissipation (De et al., 2017a, b), which leads to significantly higher ST during summer. Solar radiation at the high ambient temperature might be another possible reason for increasing ST. During winter, higher fluctuation of environmental temperature than summer leads to higher variation in ST in both the breeds. This pattern resulted in significantly ($P < 0.05$) higher amplitude in ST during winter in both native-breed and crossbred sheep. The acrophase in native-breed sheep did not differ between summer and winter, but it differed in crossbred sheep indicating a slight difference in strategy in crossbred sheep to combat low ambient temperature. However, the nonsignificant difference in mesor, amplitude, and acrophase of ST between native-breed and crossbred sheep during summer and winter indicated that the crossbred also followed a similar pattern of maintenance of ST like native well-adapted breeds. This finding reflects similar adaptability of crossbred and native-breed sheep during extreme

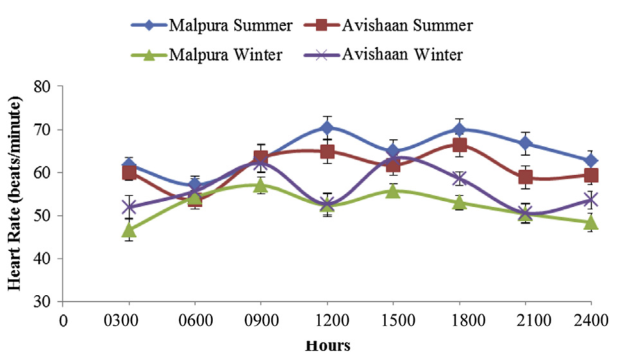

Figure 3. Oscillation of heart rate during summer and winter in fecundity gene (*FecB*)-introgressed sheep in the semi-arid region. Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed. Mean values of the heart rate maintained during summer and winter measured every 3 h for 24 h in sheep. Each data point is the mean (±SE) of 7 sheep. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

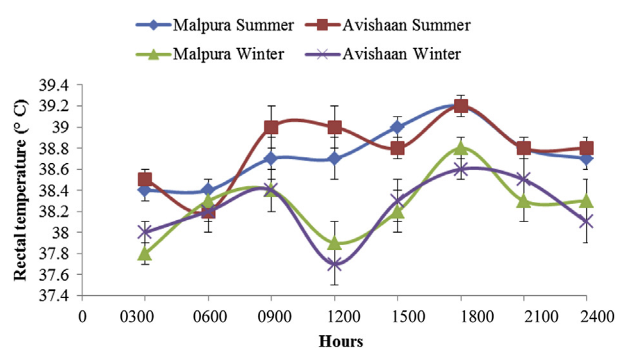

Figure 4. Oscillation of rectal temperature during summer and winter in fecundity gene (*FecB*)-introgressed sheep in the semi-arid region. Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed. Mean values of the rectal temperature maintained during summer and winter measured every 3 h for 24 h in sheep. Each data point is the mean (±SE) of 7 sheep. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

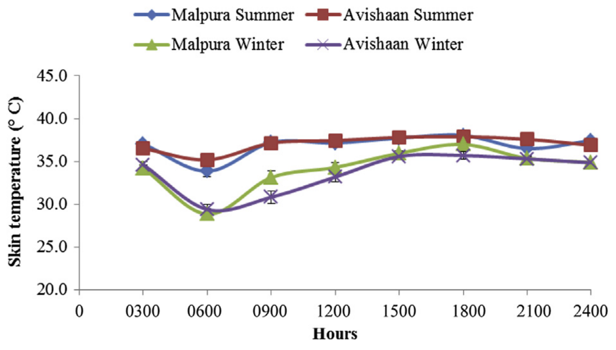

Figure 5. Oscillation rhythmicity of skin temperature during summer and winter in fecundity gene (*FecB*)-introgressed sheep in the semi-arid region. Malpura, a native well-adapted sheep breed of the semi-arid region; Avishaan, an *FecB*-introgressed sheep breed. Mean values of the skin temperature maintained during summer and winter measured every 3 h for 24 h in sheep. Each data point is the mean (±SE) of 7 sheep. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

environmental conditions like summer and winter in semi-arid tropical regions.

Conclusion

The present study shows that the rhythmic change of ambient temperature throughout the day both during summer and winter influences the thermoregulatory physiological behaviors of both native-breed and *FecB*-introgressed sheep. The variation in thermoregulatory behaviors between summer and winter in both the breeds indicated both native-breed and crossbred sheep to modify their thermoregulatory behaviors as per the environmental condition. Furthermore, in both seasons, the similar changing pattern and almost similar oscillation of thermoregulatory behaviors of *FecB*-introgressed sheep, like native well-adapted sheep breed, highlight the good thermoregulatory control of crossbred sheep under semi-arid environmental conditions.

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Conflict of interest

The authors hereby disclose that there is no any actual or potential conflict of interest including any financial, personal, or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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