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Abstract: This study was conducted to investigate the adaptive profile of the Garfagnina goat breed in two different seasons (spring and summer) through physiological, biochemical, haematological, and hormonal parameters. Fifty adult lactating females were studied twice a day (morning and afternoon) in each season. The air temperature, black globe temperature and air relative humidity was recorded using an automatic weather station. Physiological parameters recorded were rectal temperature, respiratory rate, heart rate, skin temperature and rectal-skin temperature gradient. The results of this study showed that there was a significant effect of season and period on all environmental variables. Physiological variables, rectal temperature, respiratory rate, heart rate and skin temperature showed higher values in the afternoon in both seasons. Both biochemical and hormonal parameters were significantly affected by season of the year. Biochemical and hormonal characteristics undergo changes during different seasons such that metabolism is reduced during heat stress and accelerated during cold stress; these metabolic changes are controlled by the thyroid hormones and cortisol. It has been shown from this study that these hormones facilitates the physiological parameters involved with the adaptation process confirming that adaptive capacity of animals cannot be described solely by rectal temperature and respiratory rate.

Revision note

Ms. No. Rumin-D-15-7359R1

Title: Adaptive profile of Garfagnina goat breed assessed through physiological, haematological, biochemical and hormonal parameters

Responses to reviewer 2:

1. - Line 50: considerer removing the sentence "Seasonal weather change has adverse effects on livestock production" is repeated on line 52-53.

The sentence has been removed

2. - Lines 52-53: don't agree with the replacement of "Changes in environmental variables…" by "Changes in seasonal variables …"

The word “environmental” has been placed back

3. Line 231-232: don't agree with the replacement of "environmental" by "seasonal"

The word “environmental” has been placed back

Response to the Editor:

The reference style has been revised following the Guide for Authors

Highlights

- At high temperatures the thyroid hormones synthesis decreased while cortisol increased
- Physiological parameters alone are not good variables to discriminate adaptive profile
- Goats under heat stress maintained rectal temperature
- Thyroid hormones and cortisol facilitated the understanding of the adaptive process of the animals in the semiarid
- Seasonal changes affected blood biochemical parameters

Adaptive profile of Garfagnina goat breed assessed through physiological, haematological, biochemical and hormonal parameters

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Running head: Adaptive profile of Garfagnina goat

Abstract

This study was conducted to investigate the adaptive profile of the Garfagnina goat breed in two different seasons (spring and summer) through physiological, biochemical, haematological, and hormonal parameters. Fifty adult lactating females were studied twice a day (morning and afternoon) in each season. The air temperature, black globe temperature and air relative humidity was recorded using an automatic weather station. Physiological parameters recorded were rectal temperature, respiratory rate, heart rate, skin temperature and rectal-skin temperature gradient. The results of this study showed that there was a significant effect of season and period on all environmental variables. Physiological variables, rectal temperature, respiratory rate, heart rate and skin temperature showed higher values in the afternoon in both seasons. Both biochemical and hormonal parameters were significantly affected by season of the year. Biochemical and hormonal characteristics undergo changes during different seasons such that metabolism is reduced during heat stress and accelerated during cold stress; these metabolic changes are controlled by the thyroid hormones and cortisol. It has been shown from this study that these hormones facilitates the physiological parameters involved with the adaptation process confirming that adaptive capacity of animals cannot be described solely by rectal temperature and respiratory rate.

Keywords: climate, homeothermy, hormones, temperature, goat

1. Introduction

Over centuries natural selection has acted on local breeds resulting in improved animal fitness in different environmental conditions. Consequently, changes in environmental variables are recognized as potential hazard in livestock growth and production. In particular, seasonal variations are considered physiological stressors, which affect the animal's biological systems (Aengwanich et al., 2009; Al-Eissa et al., 2012). During this process, animals have acquired unique adaptive traits such as disease resistance and tolerance to heat and cold (Silanikove, 2000).

Goats are warm-blooded animals and as such are characterized by the ability to maintain body temperature within narrow limits, for this purpose, in addition to the exchange with the environment, they need to alter their metabolism for internal heat production (Nardone et al., 2010). Few studies have been conducted on the adaptive profile of local goat population, especially in rural marginal areas. These studies are extremely important for the definition of management and conservation strategies. Even in some developed countries, many local populations were not adequately characterized in this aspect (Ribeiro et al., 2015). To improve farming efficiency, the interaction between animals and the environment must be considered; knowledge on climate variables and their effects on the physiological, haematological, blood biochemical, hormonal and genetic responses is critical for the optimization of livestock-raising systems.

Animals respond differently to drastic temperature changes by changing several aspects of their physiology and behaviour (Silva et al., 2010). These include alterations in physiological parameters (rectal temperature, heart rate, respiratory rate and surface temperature) (Marai et al., 2007), erythrogram (Al-Eissa et al., 2012), blood biochemical parameters (Abdelatif et al., 2009), cortisol (Sejian et al., 2010) and thyroid hormones (Helal et al., 2010). These parameters may vary within the same species due to factors such as diet, age, physiological status, breed, level of production, handling and especially climate stress. Therefore, the aim of this study was to investigate the adaptive profile of Garfagnina goat breed in two different seasons (spring and summer) by means of physiological, biochemical, haematological, and hormonal parameters.

2. Materials and Methods

2.1 Experimental site and animals

The study was performed in Bagni di Lucca, Italy (44’01° latitude and 10’58° longitude) at an altitude of approximately 634 m. According to Thornthwaite’s classification, the climate at this location is type A with average annual temperatures ranging from 2.0 to 29.0°C, average relative humidity of 68% and average annual rainfall of 1851-2050 mm.

The study was carried out with 100 lactating (30 primiparous and 70 multiparous) females of the Garfagnina goat breed: 50 animals were evaluated during spring (two days in March) and 50 were evaluated during summer (two days in July) (Mattiello et al., 2011; Srikandakumar et al., 2003). Measurements were made twice a day at morning (9:00h) and at afternoon (15:00h). The study was performed in one family farm using semi-extensive farming practices. The key part of the goat diet was natural pasture and water *ad libitum*. The goats were on pasture between morning and evening milking while they were confined overnight. Few feed integrations with mixed hay were given during the unfavourable periods of the year. The lambing period (beginning of the lactation) took place generally in January and February. The age of the animals was estimated indirectly by dental chronology, and all animals were classified as adult (two - five years old). Females with body condition score of 2.0 in the spring and summer 2.25 and average weight of 45.5 - 50.0 Kg.

2.2 Climatological data and thermal comfort index

On the days of data collection climatological data were also recorded using an automated meteorological station installed at the location where the animals spent the day. Dry-bulb temperature (DBT), wet-bulb temperature (WBT), black globe temperature (BGT) and relative humidity (RH) were recorded every 15 seconds. The black globe humidity index (BGHI) was then derived using black globe temperature (BGT) and dew point temperature (DPT), according to Buffington et al. (1981). The thermal radiation load (TRL) was calculated according to Esmay (1969) where the mean radiant temperature (MRT) is the temperature of a surrounding region considered uniformly black capable of eliminating the effect of reflection, with which the body (black globe) exchanges so much energy as the environment under consideration (Bond et al., 1954). The time at the beginning and at the end of each animal's sampling were registered and the average values of the environmental variables registered between these periods were assigned to the corresponding animal. The values obtained were then used to estimate the differences between season and period of the day for the environmental parameters.

2.3 Physiological parameters

Rectal temperature (RT) of animals was measured with a digital clinical thermometer with range 32.0ºC to 43.9°C. The thermometer was inserted into the rectum of each animal, with the bulb in contact with the mucosa, remaining in the rectum until the thermometer made a beep, which was indicative of temperature stabilization. The respiratory rate (RR) and heart rate (HR) were measured through auscultation of the heart sounds with the aid of a flexible stethoscope at the level of the laryngo tracheal region by counting the number of movements and beats for 20 seconds, and the results multiplied by 3 to report at a minute time scale. The skin temperature (ST) was recorded using a digital infrared thermometer Minipar MT-350 at a distance within 10 to 50 cm from the body (there is no difference in measurements between those distances), measured in left flank. The RT – ST gradient was calculated.

2.4 Haematological, blood biochemical and hormonal parameters

Blood samples were collected from each animal only during the afternoon sampling (15:00h) by puncturing the jugular vein after disinfection with iodine alcohol, the analysis of the samples was performed on the day following collection. The animals were also evaluated for the presence of ectoparasites, lymphadenitis or other types of skin problems just after the blood collection. For haematological analysis, blood was collected in 5 ml vacuum tubes containing 10% anticoagulant ethylene diamine tetra acetic acid (EDTA). The haematological studies were carried out according to Jain (1993).

For the analysis of biochemical and hormonal parameters, blood was collected in vacuum tubes of 7ml containing separating gel and sodium fluoride (used for glucose analysis), and then centrifuged in a digital centrifuge at 4°C at 3000 rpm (1100XG) for 15 minutes. After centrifugation, the supernatant was separated into 1.5-ml aliquots for biochemical and hormonal tests; the analysis was performed on the day following collection. Analyses were carried out using a biochemical-analysis apparatus (VegaSys) with a multiple wave length photometer, on the following biochemical parameters: total protein (PRT), albumin (ALB), glucose (GLU), triglycerides (TRI), cholesterol (CHO), urea (URE), creatinine (CRE), gamma glutamyl transferase SL (GGT), and aspartate aminotransferase (AST). All tests were performed using commercial kits (ASSEL S.r.l.). Intra- and interassay coefficients of variation (CV) were 1.35% and 2.39%, 0.79% and 1.78%, 1.59% and 4.54%, 2.08% and 2.00%, 1.86% and 2.76%, 3.3% and 3.8%, 1.07% and 2.15%, 1.5% and 3.0%, 2.9% and 3.1% for PRT, ALB, GLU, TRI, CHO, URE, CRE, GGT and AST respectively. The analytical sensitivity of the assays were 1 g/dL for PRT and ALB, 1 mg/dL for GLU, TRI, CHO, URE, CRE and 1 UI/I for GGT and AST respectively. The plasma concentrations of cortisol (COR), total thyroxine (T4) and total triiodothyronine (T3) were measured in duplicate and quantified by the method Enzyme Linked ImmunoSorbent Assay (ELISA by competition) using kits (In Vitro diagnostic Ltda.) developed for quantitative evaluation of hormones (Uribe-Velasquez et al., 1998). The sensitivity is reported to be lower than 0.05 ng/dL, 0.22 g/dL and 1.1 ng/dL for T3, T4 and COR respectively. Intra and inter-assays coefficients of variation are 2.3-7.7%, 1.6-5.0% and 4.58-6.33% for T3, T4 and COR respectively.

2.5 Statistical analyses

All the data were analyzed with the Statistical Analysis System (SAS, 2004) using GLM procedures, and the Tukey test for significant variables was applied. The following mathematical model was used: $y_{ijk} = \mu + S_i + P_j + (SP)_{ij} + e_{ijk}$ in which: y_{ijk} is the dependent variable; μ is the overall mean; S_i is the fixed effect of season (summer and spring), P_j is the fixed effect of period (morning and afternoon), $(SP)_{ij}$ the interaction between i^{th} season and j^{th} period and e_{ijk} is the random error with mean 0 and variance σ^2. The haematological, blood biochemical and hormonal parameters were analysed using a similar model including season as the only fixed effect. Pearson correlations among all variables were estimated using the CORR procedure of the Statistical Analysis System (SAS, 2004). Relationship between thermal gradient (RT-ST), cortisol, T3 and T4 with air temperature was analysed through a linear regression analysis using the REG procedure (SAS, 2004).

3. Results and Discussion

3.1Climatological data and thermal comfort index

Average air temperature (AT), relative humidity (RH), black globe humidity index (BGHI) and thermal radiation load (TRL) recorded both in the morning and in the afternoon for the two seasons considered are shown in Table 1. The different AT values observed between seasons and periods considered resulted in significant changes in both physiological and blood profile parameters measured in these animals. Regarding the RH, significant difference was observed between seasons and periods of the day. The data on RH shows higher values in the morning due to the lower AT values observed in this period.

The BGHI were higher both in the afternoon and during the summer with a significant interaction between season and period. Despite the absence of reference values for goat, these values cannot be considered dangerous, since RT values are normal, demonstrating the absence of heat storage. TRL was statistically higher at afternoon and during the summer and also for this parameter a significant interaction between season and period of the day was observed. The higher TRL value in the afternoon was due to the high AT and black globe temperature and low RH.

3.2 Physiological parameters

As shown in Table 2 respiratory rate (RR), heart rate (HR), skin temperature (ST) and thermal gradient (RT-ST) were significantly different between periods and seasons, whereas RT showed differences only between periods of the day (higher in the afternoon). Goats are active during the day, which causes alterations in their physiological parameters. The maximum daily variation for RT was 1.2°C in spring, value within the limit, according to Piccione and Refinetti (2003), should be range from 0.3ºC to 1.9ºC.

In this study the RR was significantly higher in the afternoon and during the summer. RR is influenced by the time of day because AT is higher in the afternoon than in the morning, which increases RR (Sejian et al., 2010; Silva et al., 2010). At morning, RR values were within the limit considered normal (below 40 mov/min) for goats (Silanikove, 2000) whereas in the afternoon RR was 40.0 mov/min, indicating that the animals could potentially be subjected to high stress in this period.

Heart rate was higher in the afternoon with 102.0 beats/min and during the spring with 96 beats/min with a significant interaction between season and period. Normally the HR increases with air temperature, which is always higher in the afternoon (Silva et al., 2010). ST was significantly higher in the afternoon and in the summer season (Table 2) and the animal responded to this heat stress dissipating heat by increasing the RR. When the AT increased the gradient decreased because these parameters are inversely correlated (Figure 1) and the body heat is dissipated primarily because of the temperature gradient. There was a strong correlation between AT and ST (87%) and a weak correlation between AT and RT (3%), indicating the independence of the latter variables, which suggested that these animals are well adapted to the local climate conditions where they are raised.

3.3 Haematological and blood biochemical parameters

The mean values of haematological and blood biochemical parameters assessed in Garfagnina goats are shown in Table 3. There was no effect of season on haematocrit (Hct) and mean corpuscular volume (MCV) whereas erythrocytes (RBC) were significantly higher in spring ($P<0.05$). The values obtained in this study are in agreement with those reported in previous studies (Kaneko et al., 2008; Piccione et al., 2010) and the changes are likely to be adaptive or acquired by the breed as a response to the local climate over several generations. The blood biochemical parameters varied significantly between the seasons with glucose and cholesterol levels significantly higher in spring. During stress, the cholesterol level decreased in response to the demand for the synthesis of cortisol. The cortisol limits the use of glucose by mobilizing other energy reserves such as triglycerides and proteins (Sejian et al., 2010).

The creatinine level was significantly lower in summer, most likely due to the increased air temperature, which promotes increased respiratory rate. The creatinine level was positively correlated with RR (0.33 $P<0.05$) and negatively correlated with AT (-0.46 $P<0.05$); these observations were consistent with the results of Piccione et al. (2010) who studied native Italian Girgentana goats. The total protein and globulin levels were significantly higher in the summer and they were positively correlated with AT (0.43 $P<0.05$). Abdelatif et al. (2009) and Al-Eissa et al. (2012) related higher levels of globulin in the summer, resulting in a lower level of the albumin/globulin ratio during the spring which was not observed in the present work. In both seasons GGT and AST values remained within the optimal limit for this species, as already stated by Kaneko et al. (2008). However the two mentioned variables had higher values in summer.

3.4 Hormonal parameters

The hormone levels varied between the two seasons, which revealed the combined action of meteorological and physiological parameters of the animals (Table 4). The thyroid and adrenal glands play key roles in the adaptation mechanism, and well-adapted animals respond quickly to environmental changes with necessary physiological adjustments (Coelho et al., 2008; Sejian, 2013; Uribe-Velasquez et al., 1998). Plasma levels of T3 and T4 were lower in the summer and the result is consistent with other studies in which an inverse correlation was observed between the concentration of thyroid hormones and the air temperature (-0.93 $P<0.05$) in goats, suggesting that this decrease is an adaptive parameter to reduce heat stress (Costa et al., 2015; Helal et al., 2010; McNabb, 1995). RT of the animals remained within the optimal limits for goats. In spring, with lower temperatures and relative humidity (Table 1), T3 and T4 concentrations were higher.

The highest levels of cortisol was observed in summer when the AT is higher. High air temperature influenced the concentration of cortisol in the blood because it negatively affects thermolysis in animals (Yousef et al., 1967). The level of cortisol found in this study corresponds to the values reported in other papers in different goat breeds mostly ranging from 3 to 15 ng/mL (Al-Busaidi et al., 2008; Aoyama et al., 2008; Ortiz-de-Montellano et al., 2007). The cortisol concentration was positively correlated with air temperature (0.91 $P<0.05$), whereas thyroid hormone levels were both (T3 and T4) inversely correlated with air temperature (-0.93 $P<0.05$). Cortisol concentration resulted positively associated with thermal load (Figure 2) whereas the increase of AT, as already stated by other authors (McFarlane et al., 1995; Vasquez and Herrera, 2003; Yousef et al., 1967), negatively affects thyroid activity.

In the environment studied, Garfagnina goats showed adaptation to cold and heat stress. During the hot periods, the animals have a higher ability to maintain rectal temperature increasing the respiratory rate with a great capacity for heat dissipation whereas in the colder periods the animals showed an increased heat-production capacity. Physiological (RR, HR, ST, RT-ST), biochemical (glucose, urea, cholesterol, triglycerides, creatinine, total protein, globulin, A/G, GGT and AST) and hormonal (T3, T4 and cortisol) characteristics undergo changes during different seasons. In particular, metabolism is reduced during heat stress and accelerated during cold stress and these changes are controlled by the thyroid hormones and cortisol (Helal et al., 2010). These hormones facilitate the physiological changes involved in adaptation. Endocrine and physiological changes occurred in Garfagnina goat reflecting the endogenous adaptive processes reacting in advance to the environmental changes associated with the change of seasons (Piccione et al., 2009).

4. Conclusions

In the environment studied the Garfagnina goat showed adaptation to seasonal weather ~~climate~~ changes. In the hottest period (i.e. afternoon summer) the animals showed high ability to maintain a constant rectal temperature slightly increasing the respiratory rate and they also showed a great capacity for heat dissipation. On the other hand, they easily react to colder temperature increasing the heat-production capacity. Biochemical and hormonal characteristics undergo changes during different seasons such that metabolism is reduced during heat stress and accelerated during cold stress; these changes are controlled by the thyroid hormones and cortisol. The study of these hormones facilitates the understanding of the physiological parameters involved in the adaptation process. In fact, the adaptive capacity of animals cannot be described solely by rectal temperature and respiratory rate. Therefore, proper assessment of the adaptive profile requires consideration of the physiological, hormonal and behavioural responses of animals to environmental conditions.

Conflict of interest statement

None declared.

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Table 1 *Effect of season and period of the day on environmental parameters registered during sampling of Garfagnina goats (least squares means)*

	Season		Period			P-value		
Items	Spring	Summer	Morning	Afternoon	RSD	S	P	SxP
AT (°C)	20.3	29.1	18.6	30.8	3.1	<0.001	<0.001	<0.001
RH (%)	37.3	33.5	50.5	20.3	11.8	0.02	<0.001	0.05
BGHI	68.5	81.8	64.1	86.2	4.2	<0.001	<0.001	<0.001
TRL (W/m^2)	560	680	449	790	46	<0.001	<0.001	<0.001

AT = Air temperature; RH = Relative humidity; BGHI = Black globe temperature and humidity index; TRL = Thermal radiation load; RSD = residual standard deviation; P = period of the day; S = season; ns= not significant.

Table 2 *Effect of season and period of the day on physiological parameters of Garfagnina goats (least squares means)*

	Season		Period			P-value		
Items	Spring	Summer	Morning	Afternoon	RSD	S	P	SxP
RT (ºC)	38.9	38.9	38.3	39.5	0.5	ns	<0.001	ns
RR (mov/min)	30	39	29	40	10	<0.001	<0.001	0.02
HR (beat/min)	96	87	81	102	14	<0.001	<0.001	<0.001
ST (ºC)	27	31	27	30	3	<0.001	<0.001	<0.001
RT-ST (ºC)	12	8	11	9	3	<0.001	<0.001	<0.001

RT = Rectal temperature; RR = respiratory rate; HR = heart rate; ST = skin temperature; RT-ST = skin temperature and rectal temperature thermal gradient; RSD = residual standard deviation; P = period of the day; S = season; ns= not significant.

Table 3 *Effect of season on blood biochemical parameters of Garfagnina goats (least squares means)*

	Season			
Items	Spring	Summer	RSD	P-value
RBC (x10^6/ml)	15.5	13.6	4.2	0.02
Hct (%)	29.7	29.0	5.0	ns
MCV (f/l)	20.8	23.1	7.0	ns
Glucose (mg/dl)	57.3	51.1	7.5	<0.001
Urea (mg/dl)	46.0	42.8	7.1	0.02
Cholesterol (mg/dl)	73.1	67.3	13.4	0.03
Triglycerides (mg/dl)	14.2	24.4	7.6	<0.001
Creatinine (mg/dl)	0.9	0.8	0.1	<0.001
Total Protein (g/dl)	7.5	8.2	1.0	<0.001
Albumin (g/dl)	3.4	3.5	0.3	ns
Globulin (g/dl)	4.0	4.7	1.0	<0.001
A/G	0.9	0.8	0.3	0.02
GGT (U/l)	39.9	49.7	8.9	<0.001
AST (U/l)	111.1	132.9	20.6	<0.001

RBC = Erythrocytes; Hct = Haematocrit; MCV = Mean Corpuscular Volume; A/G = Albumin and Globulin ratio; GGT = Gamma Glutamyltransferase; AST = Aspartate Aminotransferase; RSD = residual standard deviation; ns = not significant.

Table 4 *Effect of season on hormonal parameters of Garfagnina goats (least squares means)*

Items	Season		RSD	P-value
	Spring	Summer		
T3 (ng/mL)	1.2	0.7	0.2	<0.001
T4 (µg/dL)	1.4	0.7	0.3	<0.001
Cortisol (ng/mL)	6.9	9.0	1.8	<0.001

T3 = total triiodothyronine; T4 = total thyroxin; RSD = residual standard deviation.

Fig. 1 Thermal gradient (RT-ST) according to air temperature in Garfagnina goats. Linear regression equation and coefficient of determination are shown.

Fig. 2 Concentrations of thyroid hormones and cortisol in Garfagnina goats according to air temperature. Linear regression equation and coefficient of determination are shown.

*Manuscript (latest version with changes highlighted)

Adaptive profile of Garfagnina goat breed assessed through physiological, haematological, biochemical and hormonal parameters

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Running head: Adaptive profile of Garfagnina goat

Abstract

This study was conducted to investigate the adaptive profile of the Garfagnina goat breed in two different seasons (spring and summer) through physiological, biochemical, haematological, and hormonal parameters. Fifty adult lactating females were studied twice a day (morning and afternoon) in each season. The air temperature, black globe temperature and air relative humidity was recorded using an automatic weather station. Physiological parameters recorded were rectal temperature, respiratory rate, heart rate, skin temperature and rectal-skin temperature gradient. The results of this study showed that there was a significant effect of season and period on all environmental variables. Physiological variables, rectal temperature, respiratory rate, heart rate and skin temperature showed higher values in the afternoon in both seasons. Both biochemical and hormonal parameters were significantly affected by season of the year. Biochemical and hormonal characteristics undergo changes during different seasons such that metabolism is reduced during heat stress and accelerated during cold stress; these metabolic changes are controlled by the thyroid hormones and cortisol. It has been shown from this study that these hormones facilitates the physiological parameters involved with the adaptation process confirming that adaptive capacity of animals cannot be described solely by rectal temperature and respiratory rate.

Keywords: climate, homeothermy, hormones, temperature, goat

1. Introduction

~~Seasonal weather change has adverse effects on livestock production. However, o~~Over centuries natural selection has acted on local breeds resulting in improved animal fitness in different environmental conditions. Consequently, changes in ~~seasonal~~ environmental variables are recognized as potential hazard in livestock growth and production. In particular, seasonal variations are considered physiological stressors which affect the animal's biological systems (Aengwanich et al., 2009; Al-Eissa et al., 2012). During this process, animals have acquired unique adaptive traits such as disease resistance and tolerance to heat and cold (Silanikove et al., 2000).

Goats are warm-blooded animals and as such are characterized by the ability to maintain body temperature within narrow limits, for this purpose, in addition to the exchange with the environment, they need to alter their metabolism for internal heat production (Nardone et al., 2010). Few studies have been conducted on the adaptive profile of local goat population, especially in rural marginal areas. These studies are extremely important for the definition of management and conservation strategies. Even in some developed countries, many local populations were not adequately characterized in this aspect (Ribeiro et al., 2015). To improve farming efficiency, the interaction between animals and the environment must be considered; knowledge on climate variables and their effects on the physiological, haematological, blood biochemical, hormonal and genetic responses is critical for the optimization of livestock-raising systems.

Animals respond differently to drastic temperature changes by changing several aspects of their physiology and behaviour (Silva et al., 2010). These include alterations in physiological parameters (rectal temperature, heart rate, respiratory rate and surface temperature) (Marai et al., 2007), erythrogram (Al-Eissa et al., 2012), blood biochemical parameters (Abdelatif et al., 2009), cortisol (Sejian et al., 2010) and thyroid hormones (Helal et al., 2010). These parameters may vary within the same species due to factors such as diet, age, physiological status, breed, level of production, handling and especially climate stress. Therefore, the aim of this study was to investigate the adaptive profile of Garfagnina goat breed in two different seasons (spring and summer) by means of physiological, biochemical, haematological, and hormonal parameters.

2. Materials and Methods

2.1 Experimental site and animals

The study was performed in Bagni di Lucca, Italy (44’01° latitude and 10’58° longitude) at an altitude of approximately 634 m. According to Thornthwaite’s classification, the climate at this location is type

A with average annual temperatures ranging from 2.0 to 29.0°C, average relative humidity of 68% and average annual rainfall of 1851-2050 mm.

The study was carried out with 100 lactating (30 primiparous and 70 multiparous) females of the Garfagnina goat breed: 50 animals were evaluated during spring (two days in March) and 50 were evaluated during summer (two days in July) (Srikandakumar et al., 2003; Mattiello et al., 2011). Measurements were made twice a day at morning (9:00h) and at afternoon (15:00h). The study was performed in one family farm using semi-extensive farming practices. The key part of the goat diet was natural pasture and water *ad libitum*. The goats were on pasture between morning and evening milking while they were confined overnight. Few feed integrations with mixed hay were given during the unfavourable periods of the year. The lambing period (beginning of the lactation) took place generally in January and February. The age of the animals was estimated indirectly by dental chronology, and all animals were classified as adult (two - five years old). Females with body condition score of 2.0 in the spring and summer 2.25 and average weight of 45.5 - 50.0 Kg.

2.2 Climatological data and thermal comfort index

On the days of data collection climatological data were also recorded using an automated meteorological station installed at the location where the animals spent the day. Dry-bulb temperature (DBT), wet-bulb temperature (WBT), black globe temperature (BGT) and relative humidity (RH) were recorded every 15 seconds. The black globe humidity index (BGHI) was then derived using black globe temperature (BGT) and dew point temperature (DPT), according to Buffington et al. (1981). The thermal radiation load (TRL) was calculated according to Esmay (1969) where the mean radiant temperature (MRT) is the temperature of a surrounding region considered uniformly black capable of eliminating the effect of reflection, with which the body (black globe) exchanges so much energy as the environment under consideration (Bond et al., 1954). The time at the beginning and at the end of each animal's sampling were registered and the average values of the environmental variables registered between these periods were assigned to the corresponding animal. The values obtained were then used to estimate the differences between season and period of the day for the environmental parameters.

2.3 Physiological parameters

Rectal temperature (RT) of animals was measured with a digital clinical thermometer with range 32.0ºC to 43.9°C. The thermometer was inserted into the rectum of each animal, with the bulb in contact with the mucosa, remaining in the rectum until the thermometer made a beep, which was indicative of temperature stabilization. The respiratory rate (RR) and heart rate (HR) were measured through auscultation of the heart sounds with the aid of a flexible stethoscope at the level of the laryngo tracheal region by counting the number of movements and beats for 20 seconds, and the results multiplied by 3 to report at a minute time scale. The skin temperature (ST) was recorded using a digital infrared thermometer Minipar MT-350 at a distance within 10 to 50 cm from the body (there is no difference in measurements between those distances), measured in left flank. The RT – ST gradient was calculated.

2.4 Haematological, blood biochemical and hormonal parameters

Blood samples were collected from each animal only during the afternoon sampling (15:00h) by puncturing the jugular vein after disinfection with iodine alcohol, the analysis of the samples was performed on the day following collection. The animals were also evaluated for the presence of ectoparasites, lymphadenitis or other types of skin problems just after the blood collection. For haematological analysis, blood was collected in 5 ml vacuum tubes containing 10% anticoagulant ethylene diamine tetra acetic acid (EDTA). The haematological studies were carried out according to Jain (1993).

For the analysis of biochemical and hormonal parameters, blood was collected in vacuum tubes of 7ml containing separating gel and sodium fluoride (used for glucose analysis), and then centrifuged in a digital centrifuge at 4°C at 3000 rpm (1100XG) for 15 minutes. After centrifugation, the supernatant was separated into 1.5-ml aliquots for biochemical and hormonal tests; the analysis was performed on the day following collection. Analyses were carried out using a biochemical-analysis apparatus

(VegaSys) with a multiple wave length photometer, on the following biochemical parameters: total protein (PRT), albumin (ALB), glucose (GLU), triglycerides (TRI), cholesterol (CHO), urea (URE), creatinine (CRE), gamma glutamyl transferase SL (GGT), and aspartate aminotransferase (AST). All tests were performed using commercial kits (ASSEL S.r.l.). Intra- and interassay coefficients of variation (CV) were 1.35% and 2.39%, 0.79% and 1.78%, 1.59% and 4.54%, 2.08% and 2.00%, 1.86% and 2.76%, 3.3% and 3.8%, 1.07% and 2.15%, 1.5% and 3.0%, 2.9% and 3.1% for PRT, ALB, GLU, TRI, CHO, URE, CRE, GGT and AST respectively. The analytical sensitivity of the assays were 1 g/dL for PRT and ALB, 1 mg/dL for GLU, TRI, CHO, URE, CRE and 1 UI/I for GGT and AST respectively. The plasma concentrations of cortisol (COR), total thyroxine (T4) and total triiodothyronine (T3) were measured in duplicate and quantified by the method Enzyme Linked ImmunoSorbent Assay (ELISA by competition) using kits (In Vitro diagnostic Ltda.) developed for quantitative evaluation of hormones (Uribe-Velasquez et al., 1998). The sensitivity is reported to be lower than 0.05 ng/dL, 0.22 g/dL and 1.1 ng/dL for T3, T4 and COR respectively. Intra and inter-assays coefficients of variation are 2.3-7.7%, 1.6-5.0% and 4.58-6.33% for T3, T4 and COR respectively.

2.5 Statistical analyses

All the data were analyzed with the Statistical Analysis System (SAS, 2004) using GLM procedures, and the Tukey test for significant variables was applied. The following mathematical model was used: $y_{ijk} = \mu + S_i + P_j + (SP)_{ij} + e_{ijk}$ in which: y_{ijk} is the dependent variable; μ is the overall mean; S_i is the fixed effect of season (summer and spring), P_j is the fixed effect of period (morning and afternoon), $(SP)_{ij}$ the interaction between i^{th} season and j^{th} period and e_{ijk} is the random error with mean 0 and variance σ^2. The haematological, blood biochemical and hormonal parameters were analysed using a similar model including season as the only fixed effect. Pearson correlations among all variables were estimated using the CORR procedure of the Statistical Analysis System (SAS, 2004). Relationship between thermal gradient (RT-ST), cortisol, T3 and T4 with air temperature was analysed through a linear regression analysis using the REG procedure (SAS, 2004).

3. Results and Discussion

3.1Climatological data and thermal comfort index

Average air temperature (AT), relative humidity (RH), black globe humidity index (BGHI) and thermal radiation load (TRL) recorded both in the morning and in the afternoon for the two seasons considered are shown in Table 1. The different AT values observed between seasons and periods considered resulted in significant changes in both physiological and blood profile parameters measured in these animals. Regarding the RH, significant difference was observed between seasons and periods of the day. The data on RH shows higher values in the morning due to the lower AT values observed in this period.

The BGHI were higher both in the afternoon and during the summer with a significant interaction between season and period. Despite the absence of reference values for goat, these values cannot be considered dangerous, since RT values are normal, demonstrating the absence of heat storage. TRL was statistically higher at afternoon and during the summer and also for this parameter a significant interaction between season and period of the day was observed. The higher TRL value in the afternoon was due to the high AT and black globe temperature and low RH.

3.2 Physiological parameters

As shown in Table 2 respiratory rate (RR), heart rate (HR), skin temperature (ST) and thermal gradient (RT-ST) were significantly different between periods and seasons, whereas RT showed differences only between periods of the day (higher in the afternoon). Goats are active during the day, which causes alterations in their physiological parameters. The maximum daily variation for RT was 1.2°C in spring, value within the limit, according to Piccione and Refinetti (2003), should be range from 0.3ºC to 1.9ºC.

In this study the RR was significantly higher in the afternoon and during the summer. RR is influenced by the time of day because AT is higher in the afternoon than in the morning, which increases RR (Silva et al., 2010; Sejian et al., 2010). At morning, RR values were within the limit considered normal (below 40 mov/min) for goats (Silanikove, 2000) whereas in the afternoon RR was 40.0 mov/min, indicating that the animals could potentially be subjected to high stress in this period.

Heart rate was higher in the afternoon with 102.0 beats/min and during the spring with 96 beats/min with a significant interaction between season and period. Normally the HR increases with air temperature, which is always higher in the afternoon (Silva et al., 2010). ST was significantly higher in the afternoon and in the summer season (Table 2) and the animal responded to this heat stress dissipating heat by increasing the RR. When the AT increased the gradient decreased because these parameters are inversely correlated (Figure 1) and the body heat is dissipated primarily because of the temperature gradient. There was a strong correlation between AT and ST (87%) and a weak correlation between AT and RT (3%), indicating the independence of the latter variables, which suggested that these animals are well adapted to the local climate conditions where they are raised.

3.3 Haematological and blood biochemical parameters

The mean values of haematological and blood biochemical parameters assessed in Garfagnina goats are shown in Table 3. There was no effect of season on haematocrit (Hct) and mean corpuscular volume (MCV) whereas erythrocytes (RBC) were significantly higher in spring ($P<0.05$). The values obtained in this study are in agreement with those reported in previous studies (Kaneko et al., 2008; Piccione et al., 2010) and the changes are likely to be adaptive or acquired by the breed as a response to the local climate over several generations. The blood biochemical parameters varied significantly between the seasons with glucose and cholesterol levels significantly higher in spring. During stress, the cholesterol level decreased in response to the demand for the synthesis of cortisol. The cortisol limits the use of glucose by mobilizing other energy reserves such as triglycerides and proteins (Sejian et al., 2010).

The creatinine level was significantly lower in summer, most likely due to the increased air temperature, which promotes increased respiratory rate. The creatinine level was positively correlated with RR (0.33 $P<0.05$) and negatively correlated with AT (-0.46 $P<0.05$); these observations were consistent with the results of Piccione et al. (2010) who studied native Italian Girgentana goats. The total protein and globulin levels were significantly higher in the summer and they were positively correlated with AT (0.43 $P<0.05$). Abdelatif et al. (2009) and Al-Eissa et al. (2012) related higher levels of globulin in the summer, resulting in a lower level of the albumin/globulin ratio during the spring which was not observed in the present work. In both seasons GGT and AST values remained within the optimal limit for this species, as already stated by Kaneko et al. (2008). However the two mentioned variables had higher values in summer.

3.4 Hormonal parameters

The hormone levels varied between the two seasons, which revealed the combined action of meteorological and physiological parameters of the animals (Table 4). The thyroid and adrenal glands play key roles in the adaptation mechanism, and well-adapted animals respond quickly to ~~seasonal~~ environmental changes with necessary physiological adjustments (Uribe-Velasquez et al., 1998; Coelho et al., 2008; Sejian, et al., 2013). Plasma levels of T3 and T4 were lower in the summer and the result is consistent with other studies in which an inverse correlation was observed between the concentration of thyroid hormones and the air temperature (-0.93 $P<0.05$) in goats, suggesting that this decrease is an adaptive parameter to reduce heat stress (McNabb, 1995; Helal et al., 2010; Costa et al., 2015). RT of the animals remained within the optimal limits for goats. In spring, with lower temperatures and relative humidity (Table 1), T3 and T4 concentrations were higher.

The highest levels of cortisol was observed in summer when the AT is higher. High air temperature influenced the concentration of cortisol in the blood because it negatively affects thermolysis in animals (Yousef et al., 1967). The level of cortisol found in this study corresponds to the values reported in other papers in different goat breeds mostly ranging from 3 to 15 ng/mL (Ortiz-de-Montellano et al., 2007; Al-Busaidi et al., 2008; Aoyama et al., 2008). The cortisol concentration was positively correlated with air temperature (0.91 $P<0.05$), whereas thyroid hormone levels were both (T3 and T4) inversely correlated with air temperature (-0.93 $P<0.05$). Cortisol concentration resulted positively associated with thermal load (Figure 2) whereas thyroid activity is negatively affected by the Increase of AT as already stated by other authors (Yousef et al., 1967; McFarlane et al., 1995; Vasquez and Herrera, 2003).

In the environment studied, Garfagnina goats showed adaptation to cold and heat stress. During the hot periods, the animals have a higher ability to maintain rectal temperature increasing the respiratory rate with a great capacity for heat dissipation whereas in the colder periods the animals showed an increased heat-production capacity. Physiological (RR, HR, ST, RT-ST), biochemical (glucose, urea, cholesterol, triglycerides, creatinine, total protein, globulin, A/G, GGT and AST) and hormonal (T3, T4 and cortisol) characteristics undergo changes during different seasons. In particular metabolism is reduced during heat stress and accelerated during cold stress and these changes are controlled by the thyroid hormones and cortisol (Helal et al., 2010). These hormones facilitate the physiological changes involved in adaptation. Endocrine and physiological changes occurred in Garfagnina goat reflecting the endogenous adaptive processes reacting in advance to the environmental changes associated with the change of seasons (Piccione et al., 2009).

4. Conclusions

In the environment studied the Garfagnina goat showed adaptation to seasonal weather ~~climate~~ changes. In the hottest period (i.e. afternoon summer) the animals showed high ability to maintain a constant rectal temperature slightly increasing the respiratory rate and they also showed a great capacity for heat dissipation. On the other hand they easily react to colder temperature increasing the heat-production capacity. Biochemical and hormonal characteristics undergo changes during different seasons such that metabolism is reduced during heat stress and accelerated during cold stress; these changes are controlled by the thyroid hormones and cortisol. The study of these hormones facilitates the understanding of the physiological parameters involved in the adaptation process. In fact the adaptive capacity of animals cannot be described solely by rectal temperature and respiratory rate. Therefore proper assessment of the adaptive profile requires consideration of the physiological, hormonal and behavioural responses of animals to environmental conditions.

Conflict of interest statement

None declared.

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Table 1 *Effect of season and period of the day on environmental parameters registered during sampling of Garfagnina goats (least squares means)*

	Season		Period			P-value		
Items	Spring	Summer	Morning	Afternoon	RSD	S	P	SxP
AT (°C)	20.3	29.1	18.6	30.8	3.1	<0.001	<0.001	<0.001
RH (%)	37.3	33.5	50.5	20.3	11.8	0.02	<0.001	0.05
BGHI	68.5	81.8	64.1	86.2	4.2	<0.001	<0.001	<0.001
TRL (W/m^2)	560	680	449	790	46	<0.001	<0.001	<0.001

AT = Air temperature; RH = Relative humidity; BGHI = Black globe temperature and humidity index; TRL = Thermal radiation load; RSD = residual standard deviation; P = period of the day; S = season; ns= not significant.

Table 2 *Effect of season and period of the day on physiological parameters of Garfagnina goats (least squares means)*

	Season		Period			P-value		
Items	Spring	Summer	Morning	Afternoon	RSD	S	P	SxP
RT (ºC)	38.9	38.9	38.3	39.5	0.5	ns	<0.001	ns
RR (mov/min)	30	39	29	40	10	<0.001	<0.001	0.02
HR (beat/min)	96	87	81	102	14	<0.001	<0.001	<0.001
ST (ºC)	27	31	27	30	3	<0.001	<0.001	<0.001
RT-ST (ºC)	12	8	11	9	3	<0.001	<0.001	<0.001

RT = Rectal temperature; RR = respiratory rate; HR = heart rate; ST = skin temperature; RT-ST = skin temperature and rectal temperature thermal gradient; RSD = residual standard deviation; P = period of the day; S = season; ns= not significant.

Table 3 *Effect of season on blood biochemical parameters of Garfagnina goats (least squares means)*

	Season			
Items	Spring	Summer	RSD	P-value
RBC ($x10^6$/ml)	15.5	13.6	4.2	0.02
Hct (%)	29.7	29.0	5.0	ns
MCV (f/l)	20.8	23.1	7.0	ns
Glucose (mg/dl)	57.3	51.1	7.5	<0.001
Urea (mg/dl)	46.0	42.8	7.1	0.02
Cholesterol (mg/dl)	73.1	67.3	13.4	0.03
Triglycerides (mg/dl)	14.2	24.4	7.6	<0.001
Creatinine (mg/dl)	0.9	0.8	0.1	<0.001
Total Protein (g/dl)	7.5	8.2	1.0	<0.001
Albumin (g/dl)	3.4	3.5	0.3	ns
Globulin (g/dl)	4.0	4.7	1.0	<0.001
A/G	0.9	0.8	0.3	0.02
GGT (U/l)	39.9	49.7	8.9	<0.001
AST (U/l)	111.1	132.9	20.6	<0.001

RBC = Erythrocytes; Hct = Haematocrit; MCV = Mean Corpuscular Volume; A/G = Albumin and Globulin ratio; GGT = Gamma Glutamyltransferase; AST = Aspartate Aminotransferase; RSD = residual standard deviation; ns = not significant.

Table 4 *Effect of season on hormonal parameters of Garfagnina goats (least squares means)*

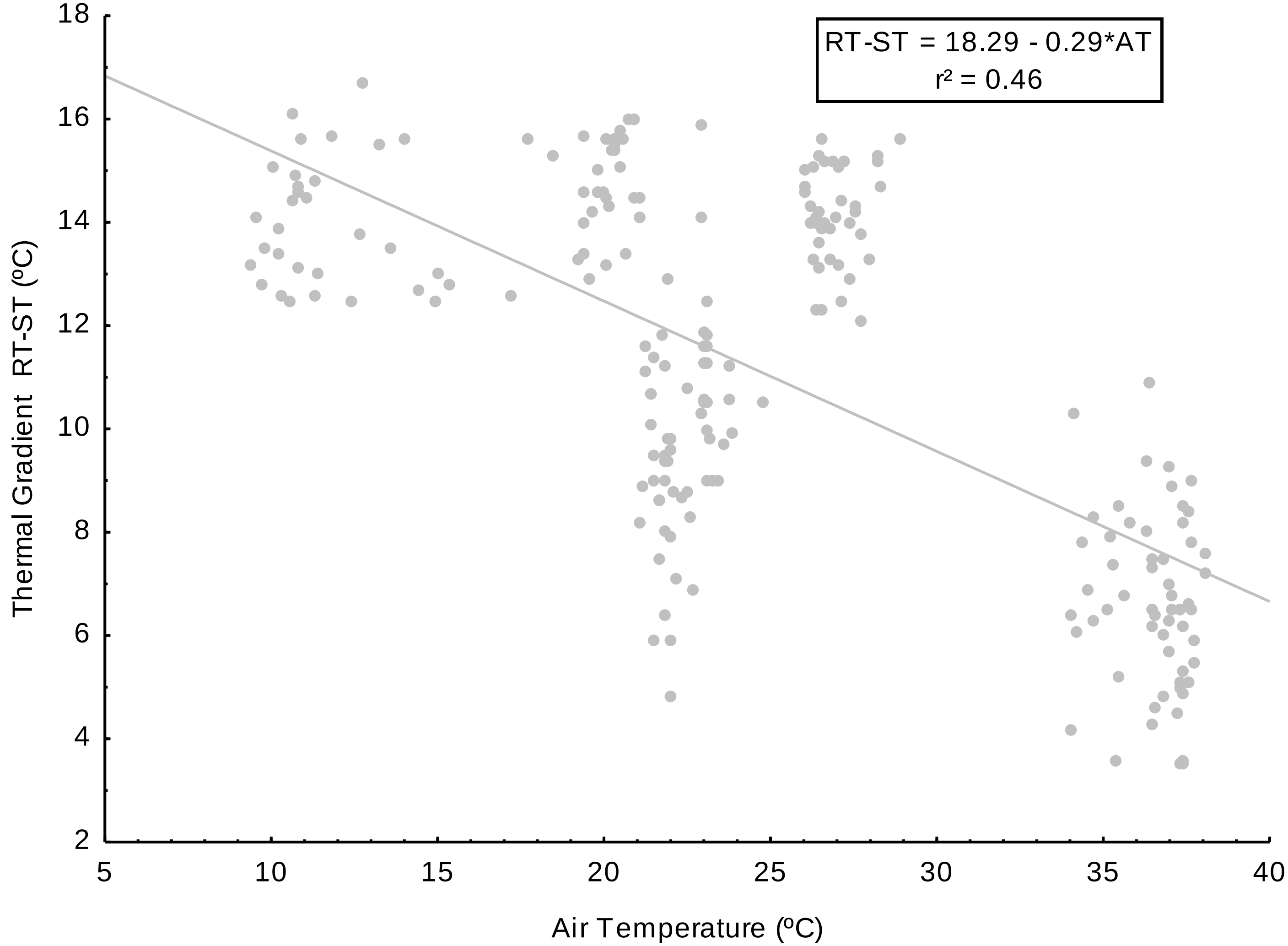
	Season			
Items	Spring	Summer	RSD	P-value
T3 (ng/mL)	1.2	0.7	0.2	<0.001
T4 (µg/dL)	1.4	0.7	0.3	<0.001
Cortisol (ng/mL)	6.9	9.0	1.8	<0.001

T3 = total triiodothyronine; T4 = total thyroxin; RSD = residual standard deviation.

Fig. 1 Thermal gradient (RT-ST) according to air temperature in Garfagnina goats. Linear regression equation and coefficient of determination are shown.

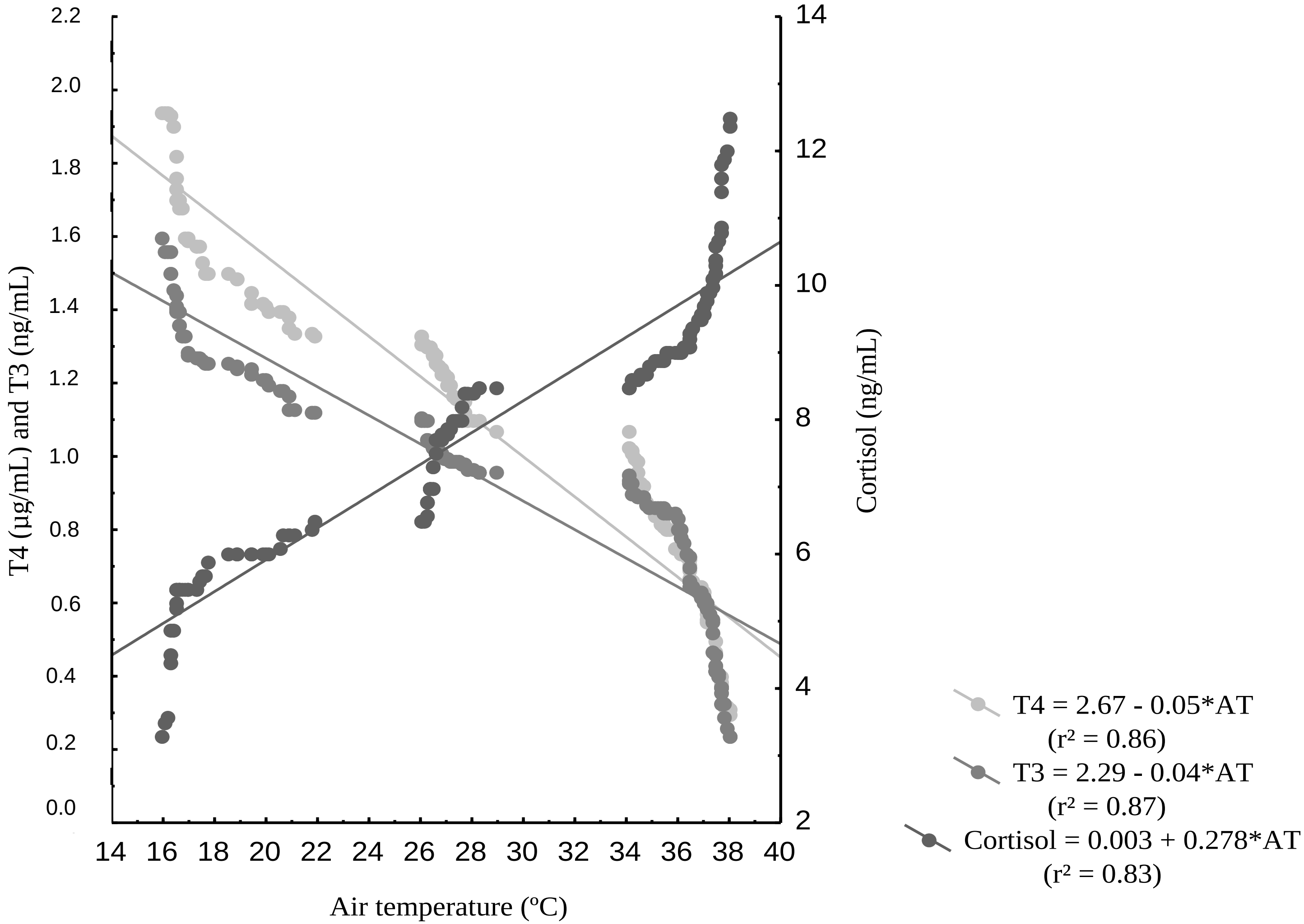
Fig. 2 Concentrations of thyroid hormones and cortisol in Garfagnina goats according to air temperature. Linear regression equation and coefficient of determination are shown.

Figure 1



RT-ST = 18.29 - 0.29*AT
r² = 0.46
Thermal Gradient RT-ST (ºC)
Air Temperature (ºC)
18
16
14
12
10
8
6
4
2
5
10
15
20
25
30
35
40

Figure 2



2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
T4 (µg/mL) and T3 (ng/mL)
14
12
10
8
6
4
2
Cortisol (ng/mL)
14 16 18 20 22 24 26 28 30 32 34 36 38 40
Air temperature (ºC)
T4 = 2.67 - 0.05*AT
(r² = 0.86)
T3 = 2.29 - 0.04*AT
(r² = 0.87)
Cortisol = 0.003 + 0.278*AT
(r² = 0.83)

***Conflict of Interest Statement**

Firenze, 23/11/2015

Conflict of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.
We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.
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