



Multivariate characterization of the adaptive profile in Brazilian and Italian goat population



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ABSTRACT

The aim of this study was to characterize the adaptive profile and identify variables with great discriminatory power of the Brazilian *Azul* goat population and Italian Garfagnina population, through the use of principal component and canonical discriminant analysis. A total of 110 Garfagnina milking females (60 in winter and 50 in summer) and 80 Brazilian *Azul* (40 in winter and 40 in summer) were considered. Air temperature (°C), black globe temperature (BGT) and relative humidity (%) were measured with the aid of an automatic weather station. Some physiological parameters (rectal temperature – RT, respiratory rate – RR, skin temperature – ST and heart rate – HR), some anatomical parameters (hair diameter – HD and hair length – HL), some hematological parameters (erythrocyte – RBCs, packed cell volume – PCV and mean corpuscular volume – MCV), some blood biochemical parameters (glucose – GLI, cholesterol – COL, triglycerides – TRI, creatinine – CRE, urea – URE, total protein – PRT, albumin – ALB, globulin – GLO, albumin and globulin ratio – A/G, gamma – glutamyl transferase – GGT and aspartate aminotransferase – AST) and some stressed hormones (thyroxine – T4, triiodothyronine – T3 and cortisol – COR) were measured. The variables with greater discriminant power were T3, ST, COR, T4, GGT, HD, GLO, HL and PCV to Garfagnina population and PRT, MCV, PCV, ALB, T4, ST, HL, RBCs, TRI and GGT in the *Azul* Brazilian population. Classification of the animals was more accurate when considering morphological, physiological, hematological, biochemical and hormonal variables jointly.

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1. Introduction

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 population were not

adequately characterized in this aspect. Many studies have performed molecular and phenotypic characterization of local population, especially small ruminants (Martini et al., 2010; Hoffman, 2011; Francesch et al., 2011; Yakubu and Ibrahim, 2011). Few have been conducted that considered the adaptive profile with a multivariate approach. In Brazil, Bianchini et al. (2006), McManus et al. (2009), Castanheira et al. (2010a, 2010b), McManus et al. (2011a, 2011b), Correa et al. (2013) and in other countries as Yakubu et al. (2012) have considered this approach.

The Brazilian *Azul* and the Italian Garfagnina populations have great adaptive potential. The *Azul* ecotype

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is widespread throughout the Northeast of Brazil with higher concentrations in Paraíba, Pernambuco, Rio Grande do Norte and Piauí States (Ribeiro et al., 2004). Predominates in family production systems having key role in the development of rural marginal areas. They contribute to improving the quality of life, to fight poverty and hunger of hundreds of families in the region. Despite its economic and social importance, the Brazilian *Azul* population is classified as endangered (FAO, 2007) and may disappear even before it was officially recognized as a national population. The Garfagnina, originating in the Garfagnana, in the Tuscany region of Italy, is also an endangered population, with greatly reduced herd numbers despite of the important role in the maintenance of several families of small holders in the region. This breed is very important to the local production system, which generally aims to produce high quality foods such as cheese and other dairy products.

Goats, like other mammals have different ways of dealing with climate change and they use several mechanisms to cope with climate stress. Physiologically, animals react differently to drastic temperature changes by changing behavior and production. Endothermic animals maintain their body temperature within a thermal comfort zone, maintaining core body temperature with thermoregulatory mechanisms. Several physiological variables are used to evaluate the suitability of an animal to adverse weather. It helps to select animals capable of producing satisfactorily in harsh environments and outside the thermal comfort zone (Starling et al., 2002). There are numerous statistical tools available to study the adaptive profile of domestic animals. Individual analysis allows for the description of single variables and their variation, which enables the definition of maximum and minimum general standards. However, individual analysis does not describe a phenomenon on a global level. A better comprehension may be obtained by the multivariate approach, which help define the adaptive profile of a population by considering all variables simultaneously. Multivariate methods are based on correlations between variables and allows for simultaneous analysis, enabling more consistent and useful interpretations (Ferreira et al., 2009). Thus, multivariate analysis techniques may improve the interpretation of a large set of variables (Zepeda et al., 2002). Principal component analysis has been used to define those variables that explain better the total variation (Hair et al., 2006). This technique permits evaluation of interrelationships between variables, reducing an original set of variables to a smaller number of independent factors, facilitating interpretation (Cruz and Regazzi, 2003). A large group of qualitative and quantitative variables of different populations may be analyzed using a group of multivariate techniques simultaneously (Gould and Johnston, 1972; Dossa et al., 2007). The objectives of this research were determine the adaptive profile (biochemical, hormonal, physiological and morphological) for two local populations in their environments and determine which variables have the greatest discriminating power to the adaptive profile, through the use of principal component and canonical discriminant analysis.

2. Materials and methods

2.1. Local and experimental animals

One hundred and ten lactating Italian Garfagnina (60 in winter and 50 in summer) and 80 Brazilian *Azul* females (40 in winter and 40 in summer) were used in this study. Measurements were made in the morning (9 am) and afternoon (3 pm). Due to lack of control data, the age of the animals was obtained indirectly by analysis of dental chronology according to Quittet (1978) and all were classified as adult (over 2 years old).

Data from the Garfagnina population was collected at a farm located 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 according to Table 1.

Data from the Brazilian *Azul* population was collected in a farm located in Caiçara do Rio do Vento, Rio Grande do Norte (latitude 5° 45' 36" south and longitude 35° 59' 52" west), 175 m altitude. The climate is tropical with a summer and winter season (Koppen climate classification; type A), with a maximum of 33.0 °C minimum of 21.0 °C; average annual temperature of 27.2 °C (Table 1).

2.2. Data measurement

Phenotypic characterization of the animals was performed and data compiled into individual spreadsheets. Each animal was evaluated for the presence or absence of small ears, horns, earrings, long hair, beard, roan color (presence of white, black and red colors in the same coat), brown eumelanin and eumelanin pigmentation pattern. Hair samples were collected from animals in both seasons; the sample was obtained manually from a lateral region beneath the spinal column of the animals. The same region was used for surface temperature measurements.

The hair samples were cataloged and stored in paper envelopes. Morphological variables such as hair diameter (HD) and hair length (HL) were measured using a digital micrometer attached to a microscope; the longest hair in each sample was used to measure the HL (Kassab, 1964).

A digital thermometer with a scale from 32 to 43.9 °C was used to measure the rectal temperature (RT). The respiratory (RR) and heart rate (HR) was measured by indirect auscultation of the heart sounds in the laryngotracheal region using a flexible stethoscope; the numbers of movements and beats in a 20-second period were recorded, and the results were multiplied by 3 to obtain the rates per minute. A digital infrared thermometer was used to measure skin temperature of the animals (ST).

Blood samples were collected from each animal during the afternoon in both seasons (winter and summer) by puncturing the jugular vein after disinfection with iodine alcohol. For erythrocyte analysis, blood was collected in 5 ml vacuum tubes containing the anticoagulant sodium ethylene diamine tetraacetic acid (EDTA) at a final concentration of 10%. The number of erythrocytes (RBCs) was measured in a modified Neubauer-type chamber by diluting 20 microliters of the cells using a semi-automatic pipette (Vallada, 1999). The packed cell volume (PCV in %) was determined using capillary tubes in a micro-centrifuge as described by Ayres et al. (2001). The hematological index, mean corpuscular volume (MCV) was calculated as described by Ferreira Neto et al. (1977).

For analysis of blood biochemical and hormonal parameters, blood was collected in 7-ml vacuum tubes containing separating gel. The glucose analysis was made with a vacuum tubes containing sodium fluoride. The blood samples were immediately transported to the laboratory in isothermal boxes and centrifuged in a digital centrifuge at 4 °C and 3000 rpm for 15 min. After centrifugation, the supernatant was separated into 1.5-ml aliquots for biochemical tests. The following tests were used to measure the serum concentration of different compounds: the biuret colorimetric test for total protein (PRT), bromocresol colorimetric test for albumin (ALB), enzymatic colorimetric tests for glucose (GLU) and triglycerides (TRI), the enzymatic CHOD-POD test for cholesterol (COL), an enzymatic kinetic test for urea (URE), the Jaffe kinetic test for creatinine (CRE), the Szasz-Tris kinetic test for gamma glutamyl transferase SL (GGT), and a UV kinetic test for aspartate aminotransferase (AST). All tests were performed using commercial kits (ASSEL S.r.l) and data collected using biochemical-analysis apparatus with multiple wave length photometer.

Plasma concentrations of cortisol (COR), thyroxine (T4) and tri-iodothyronine (T3) were measured in duplicate by ELISA (Enzyme-Linked Immunosorbent Assay) using commercially available laboratory kits (Invitro).

Table 1

Climatic variables; air temperature (AT), relative humidity (RH), black globe temperature (BGT) and temperature and Index Unit (IBGT) for the two areas.

Season		TA (°C)	RU (%)	BGT (°C)	IBGT
<i>Orrido di Botri – Bagni di Lucca – Tuscany – Italy</i>					
Winter	Mean	20.1	36.5	26.7	68.8
	Maximum	28.9	83.0	39.6	81.2
	Minimun	9.4	13.7	10.1	53.0
Summer	Mean	29.5	33.6	37.3	81.8
	Maximun	38.0	61.5	54.0	97.4
	Minimun	21.1	13.4	20.2	65.5
<i>Caiçara do Rio do Vento – Rio Grande do Norte – Brazil</i>					
Winter	Mean	36.8	34.6	40.8	93.5
	Maximun	39.8	76.7	41.3	93.2
	Minimun	29.8	30.0	32.0	75.6
Summer	Mean	41.6	33.3	46.8	100.1
	Maximun	44.5	51.2	52.6	105.8
	Minimun	35.9	29.8	38.5	95.2

2.3. Statistical analysis

Data was analyzed using the Statistical Analysis System, SAS® ([SAS, 2001](#)). A general linear model was used with a model that included the season as a fixed effect (summer and winter) and a mean test (*t* 5%) for significant variables. After standardization, multivariate analyses were carried out in accordance to [Sneath and Sokal \(1973\)](#), to allocate animals in groups according to similarity and verify discriminatory capacity of the original variables. Multivariate procedures included principal component analysis to. Understand the sources of variation in the data; distances between groups (population within season); use the characteristics to predict the group to which a given animal belongs; select a subset of the quantitative variables for use in discriminating among the groups and; summarize between-class variation in the same way that principal components summarize total variation.

The principal component analysis (PCA) allows assessment of overall variance; on the other hand, discriminant analysis describes the variation among groups and identify variables with greater discriminatory power between groups. The PCA was performed by PRINCOMP procedure, separately for each population. Relative importance was assessed by eigenvalues (variances), thereby defining the factors to be extracted by the method of varimax rotation for better interpretability. Discriminant analysis was performed with support from STEPDISC procedures to determine which variables had greater power to discriminate groups. Relative importance of variables (for the discrimination of population within a season) was based on the significance level, partial R^2 and *F* statistic. The CANDISC procedure was used to perform multivariate one-way analysis of variance and Mahalanobis distance was calculated between the two populations in the two studied seasons.

3. Results and discussion

Averages, standard deviations and coefficients of variation of the adaptive profile of animals are presented in [Table 2](#). It was verified significant difference on the biochemical variables of the populations in two seasons (winter and summer), with considerable coefficient of variation. Principal component analysis reduced sample space, with ~70% of overall variability concentrated in the first seven components in the two populations.

Variables located far from zero point ([Fig. 1](#)) concentrate most of the total variation and are the most important to explain the total variance of the data. In the Brazilian Azul population, the variables that showed a great association with the first components contributed most to the total variation of the data and were in order of importance:

RBCs, PCV, HR, RR, GLO, AG, T4, URE, HD and TRI ([Fig. 1](#)). In Garfagnina population, the best contribution to the total variation was determined by T4, T3, COR, ST, HR, PRT, GLU, AG, RBCs and MCV variables. The other variables were either redundant or contributed little to the total variation as shown by their coefficients of variation ([Table 2](#)).

The most important variables to discriminate population were T3, ST, COR, T4, GGT, HD, GLO, HL and PCV to Garfagnina and PRO, MCV, PCV, ALB, T4, ST, HL, RBCs, TRI, GGT to the Azul Brazilian population. The cold stress in Garfagnina populations could be an important factor in this situation once the thyroid is stimulated to produce T4 and T3 to accelerate cell metabolism and maintain temperature ([Starling et al., 2005](#)). When air temperature increases, the animal uses the surface temperature to dissipate heat. When the temperature increases, the production of thyroid hormones falls, while cortisol synthesis increases to control heat production.

HL and HD are adaptive characteristic products of behavioral and physiological changes acquired to adjust to environmental changes. However, when the temperature exceeds the tolerance limits (minimum or maximum), animals use other mechanisms already described to control body temperature.

Surface temperature plays a key role in heat dissipation. When the air temperature is higher than the surface (skin) temperature, the animal reduces the loss of sensible heat by using and enjoying form and becomes insensitive manner, in this case, the respiratory rate to dissipate heat. The short hair and larger diameter of the Brazilian Azul population facilitates the renewal of the air close to the skin. This helps removal and replacement of hot heat for cooler air, contributing to great heat dissipation ([Table 2](#)).

The enzyme GGT is an important indicator of liver function. Levels observed in this study did not compromise the production of globulin. Hematocrit in turn, is important for the adaptation process at the moment to increase in air temperature. At this moment, the animal would lose fluid through the respiratory tract in addition to the dehydration that also contributes to increasing hematocrit level ([Guyton and Hall, 2011](#)).

Table 2

Means (μ), standard error (S.E.) and coefficient of variation (C.V.) of the adaptive variables of female Garfagnina Italian breed and Azul Brazilian population.

Variables	Azul population				Garfagnina breed			
	Summer		Winter		Summer		Winter	
	$\mu \pm S.E.$	C.V.	$\mu \pm S.E.$	C.V.	$\mu \pm S.E.$	C.V.	$\mu \pm S.E.$	C.V.
Glucose (mg/dl)	99.8 ± 4.6 ^a	4.6	100.8 ± 6.2 ^a	6.1	50.0 ± 5.9 ^c	11.7	56.6 ± 7.1 ^b	12.6
Cholesterol (mg/dl)	164.6 ± 36.2 ^b	22.0	204.4 ± 14.3 ^a	7.0	69.6 ± 14.8 ^c	21.2	71.3 ± 12.8 ^c	17.9
Triglycerides (mg/dl)	17.0 ± 3.9 ^b	23.0	13.7 ± 2.1 ^c	15.0	25.6 ± 9.4 ^a	36.7	15.6 ± 6.4 ^{bc}	41.1
Urea (mg/dl)	75.6 ± 14.1 ^a	18.7	75.6 ± 12.4 ^a	16.5	42.0 ± 5.7 ^c	13.5	46.4 ± 7.5 ^b	16.3
Creatinine (mg/dl)	1.3 ± 0.4 ^a	26.4	1.1 ± 0.4 ^b	31.1	0.8 ± 0.08 ^c	10.0	0.9 ± 0.1 ^c	13.4
Total protein (g/dl)	7.2 ± 0.7 ^b	10.1	3.8 ± 0.4 ^c	10.3	8.4 ± 0.8 ^a	9.8	7.5 ± 1.0 ^b	13.8
Albumin (g/dl)	2.8 ± 0.4 ^b	12.8	1.7 ± 0.5 ^c	27.8	3.5 ± 0.3 ^a	8.8	3.4 ± 0.4 ^a	11.2
Globulin (g/dl)	4.4 ± 0.8 ^b	19.0	2.1 ± 0.6 ^c	29.4	4.8 ± 0.8 ^a	17.4	4.1 ± 1.0 ^b	25.0
A/G	0.7 ± 0.2 ^c	26.3	1.0 ± 0.8 ^a	77.2	0.8 ± 0.2 ^{bc}	24.6	0.9 ± 0.3 ^{ab}	31.4
Gamma glutamyl transferase (U/l)	38.7 ± 8.8 ^b	22.7	40.9 ± 8.5 ^b	20.9	50.6 ± 9.5 ^a	18.8	41.0 ± 8.4 ^b	20.5
Aspartate aminotransferase (U/l)	72.6 ± 13.3 ^d	18.4	81.2 ± 15.1 ^c	18.5	132.8 ± 19.0 ^a	14.3	115.2 ± 23.8 ^b	20.6
Eritrócitos ($\times 10^6$ /ml)	14.6 ± 4.1 ^b	28.0	17.8 ± 2.1 ^a	11.5	15.2 ± 3.4 ^b	22.5	16.0 ± 3.9 ^b	24.1
PCV (%)	22.9 ± 6.4 ^c	28.0	38.2 ± 3.6 ^a	9.4	29.9 ± 4.6 ^b	15.3	29.1 ± 5.3 ^b	18.2
MCV (f/l)	16.1 ± 3.4 ^c	20.9	21.6 ± 2.7 ^a	12.7	20.4 ± 4.9 ^b	24.0	19.2 ± 5.4 ^b	28.4
T4 ($\mu\text{g}/\text{dL}$)	1.0 ± 0.4 ^c	18.8	1.4 ± 0.2 ^a	15.2	0.8 ± 0.02 ^d	2.8	1.2 ± 0.3 ^a	27.5
T3 (ng/mL)	0.9 ± 0.2 ^b	22.6	1.2 ± 0.3 ^a	21.6	0.7 ± 0.1 ^c	21.4	1.1 ± 0.2 ^a	16.6
Cortisol (ng/mL)	6.6 ± 1.4 ^c	21.3	4.8 ± 1.1 ^d	23.4	10.3 ± 0.8 ^a	7.6	7.5 ± 1.4 ^b	19.2
Hair length (cm)	3.0 ± 0.5 ^c	15.6	4.1 ± 1.1 ^c	27.2	10.5 ± 3.7 ^b	35.0	12.0 ± 3.2 ^a	26.3
Hair diameter (mm)	0.08 ± 0.01 ^b	10.2	0.08b ± 0.01 ^b	13.4	0.09 ± 0.01 ^a	13.2	0.07 ± 0.01 ^b	15.9
Rectal temperature (°C)	39.5 ± 0.6 ^b	1.6	39.7 ± 0.4 ^a	1.1	39.5 ± 0.5 ^b	1.4	39.6 ± 0.4 ^{ab}	1.0
Heart rate (bat./min)	88.5 ± 22.5 ^b	25.4	85.5 ± 16.3 ^b	19.04	100.7 ± 15.7 ^a	15.6	103.3 ± 15.7 ^a	15.2
Respiratory rate (mov./min.)	36.3 ± 12.5 ^b	34.5	34.2 ± 6.7 ^b	19.5	41.9 ± 14.7 ^a	35.1	37.7 ± 14.2 ^{ab}	37.6
Surface temperature (°C)	41.1 ± 2.7 ^a	6.6	37.8 ± 1.4 ^b	3.6	33.1 ± 2.2 ^c	6.7	26.5 ± 2.9 ^d	11.3

* Means in rows followed by the same letters are statistically different by *t* test ($p < 0.01$).

The Brazilian *Azul* population responded quickly to environmental changes, achieving physiological adjustments, better use of adaptive mechanisms, as were under heat stress throughout the experiment. To maintain homeostasis, animals used T4 hormone, which has a long half-life and is more potent than T3, and its concentration in blood was higher. Together with T4, T3 is important in the differentiation, growth and metabolism in several vertebrate tissues. These hormones are also important in reproduction and as indicators of nutritional and metabolic

status of the animals (Sejian et al., 2008). For this reason, they are always found in the blood stream even at low concentrations.

Table 3 contains classifications of the animals by canonical discriminant analysis according to the studied variables. Classification errors based on all variables were minimal and 95% of the animals were classified into their corresponding group. Only 9 Garfagnina animals of the summer group were classified in the winter group, likely due to their similar mechanisms in both seasons. All animals of the

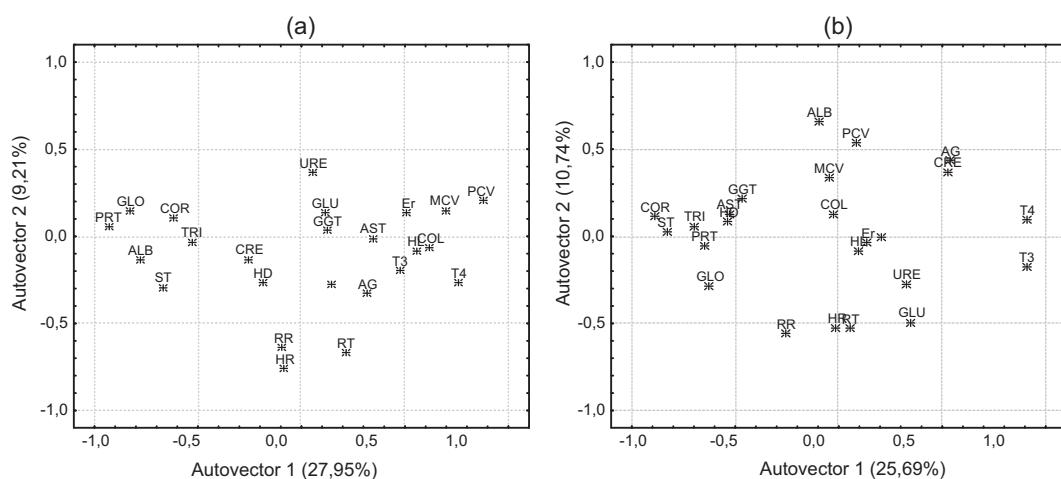


Fig. 1. Bi-dimensional plot of the studied variables in the Brazilian *Azul* population (a) and the Italian Garfagnina population (b). Glucose (GLO), cholesterol (COL), triglycerides (TRI), urea (URE), creatinine (CRE), total protein (PRT), albumin (ALB), globulin (GLO), albumin/globulin (AG), gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), Eritrócitos (Er), cortisol (COR), hair length (HL), hair diameter (HD), rectal temperature (RT), heart rate (HR), respiratory rate (RR), surface temperature (ST), mean corpuscular volume (MCV), packed cell volume (PCV).

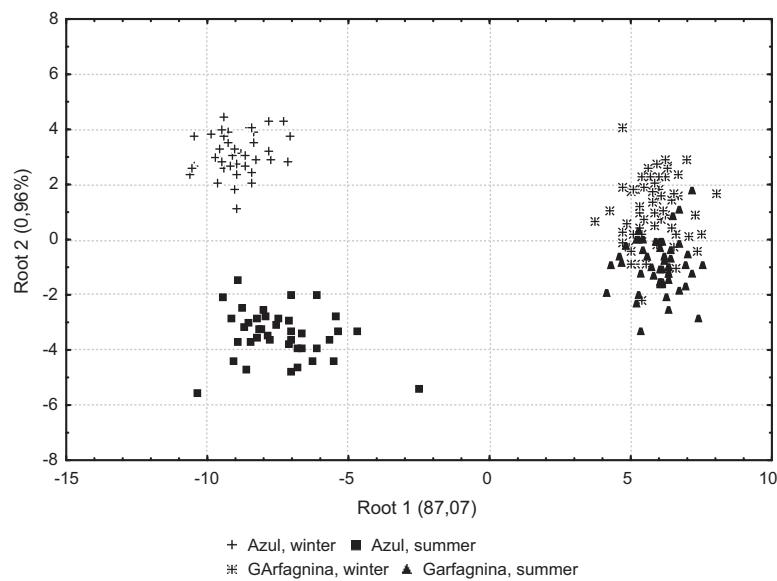


Fig. 2. Bi-dimensional plot for discriminant canonical analysis of the breeds in the two seasons based on all variables.

Table 3

Percentage of animals classified in each group according to the studied mechanisms.

Breed/season	Azul		Garfagnina	
	Winter	Summer	Winter	Summer
<i>Anatomical and physiological, blood biochemical, hormonal and erythrocyte mechanisms</i>				
Azul-winter	40	0	0	0
Azul-summer	0	40	0	0
Garfagnina-winter	0	0	51	9
Garfagnina-summer	0	0	0	50
Error (%)	0	0	5	0
<i>Blood biochemical, hormonal and erythrocyte mechanisms</i>				
Azul-winter	40	0	0	0
Azul-summer	0	40	0	0
Garfagnina-winter	0	0	56	4
Garfagnina-summer	0	0	1	49
Error (%)	0	0	6.6	2.0
<i>Anatomical and physiological mechanisms</i>				
Azul-winter	39	1	0	0
Azul-summer	7	33	0	0
Garfagnina-winter	0	0	52	8
Garfagnina-summer	3	0	1	46
Error (%)	2.5	17.5	13.33	8.0

Brazilian Azul population were correctly classified to their group of origin, because they presented different adaptive mechanisms in the two seasons. The classification errors depend on the characteristics used in the analysis (Yakubu et al., 2012; Correa et al., 2013). Higher misclassification was observed when considering only anatomical and physiological characteristics, which was also reported by Correa et al. (2013). According to Castanheira et al. (2010a), the physiological variables are good indicators of animal health but must be properly interpreted, since it can be influenced by species, age, exercise and stress level.

The error rate within population was high when only morphological and physiological characteristics were analyzed. The increase of percentage of misclassification was observed in the Garfagnina population, which showed little differentiation among the variables of the two seasons. The higher percentage of misclassification observed in the Garfagnina population are due to the little differentiation among the variables of the two seasons. This suggests that the morphological and physiological variables alone are not good parameters to discriminate the adaptive profile of a population. These should be associated with biochemical parameters.

The bi-dimensional plot for discriminant canonical analysis of the populations studied in the two seasons based on all variables are showed in Fig. 2. In the Garfagnina population, the adaptive mechanisms were similar in both periods, perhaps because they are raised in an environment with lower temperature values, but higher temperature oscillation. In the Brazilian Azul population, the adaptive was distinct in each season because these animals were subjected to prolonged heat. Additionally, the relative humidity increased and food shortage increased due to the arrival of the rain, changing the adaptive behavior of animals.

4. Conclusion

The most discriminatory variables in the Garfagnina population were (in order of importance): T3, ST, COR, T4, GGT, HD, GLO, HL and PCV while in the Azul Brazilian population: PRO, MCV, PCV, ALB, T4, ST, HL, Er, TRI, GGT.

Despite some more discriminant variables being the same in both population, they shown distinct adaptive mechanisms.

Variables that represent the biochemical mechanism should always be considered in future studies to minimize misclassification.

Conflict of interest statement

None declared.

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