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Intelligent methodologies: An integrated multi-modeling approach to predict adaptive mechanisms in farm animals

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A R T I C L E I N F O

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Path analysis

A B S T R A C T

"Welcome to the Machine" is the second song on Pink Floyd's 1975 album "Wish You Were Here". Today, 49 years later, machine learning is a field of artificial intelligence that has been gaining prominence, especially in order to solve multifactorial problems such as animal adaptation, which involves thermoregulatory, morphological, hematological and immunological responses. For the first time, we seek to trace the adaptive profile, identify biomarkers of animal adaptation to thermal stress and investigate the potential effects underlying the complex relationships of animal adaptation with climatic variables, morphological, thermoregulatory, hematological, immunological and productive responses in the animal model using an intelligent predictive modeling as an auxiliary method. The database contains 7608 pieces of information. All variables were measured simultaneously on the animal. Machine learning techniques and path analyses were used. Our main results were: (*i*) we identified patterns that hematological variables represent important biomarkers to study animal adaptation; (*ii*) there are three adaptive profiles with thermoregulatory, hematological and immunological mechanisms varying according to the thickness of the coat; (*iii*) age, body condition score and lactation order are factors that influence adaptive responses; and (*iv*) environmental variables and thermal comfort indices influence thermoregulatory responses (primary mechanism), but do not influence hematological and immunological responses, while thermoregulatory variables influence hematological and immunological responses (secondary mechanism). Most of the variables under study and other indicators such as behavioral, biochemical, metabolic and physical related to the adaptive physiology in production animals are already measured by sensors. These variables can be used in a machine intelligence approach for assertive decision-making by farmers, as technology based on sensors in production animals is an innovative approach to maximize animal welfare and provide better alternatives to evaluate the animal response.

1. Introduction

Future projections on global warming reveal an increase of up to 1.5 °C in Earth's temperature by the year 2050 (Lima et al., 2022), combined with the increased demand for food production due to the growing global population. This creates a paradox and reinforces the need for studies to understand the impact of climate on the thermoregulation physiology of production animals, as well as the impact of heat stress on productive performance (de Catro Júnior et al., 2023; Silveira et al., 2021).

The physiology of thermoregulation in farm animals is complex and systematic, involving body fluids, tissues and organs (Leite et al., 2021). Age, diet, production level, body score condition, sex and morphological aspects are the main factors studied in animal adaptation, as this set of factors determines the magnitude of thermal stress in mammals (de Castro Júnior e Silva, 2020). Animal adaptation is the phenotypic and

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genotypic ability of an animal to survive in a specific environment, even under adverse environmental conditions (de Vasconcelos et al., 2019a,b; Façanha et al., 2020; Vieira et al., 2022). Basically, animal adaptation involves morphological, physiological and behavioral capacity, associated or not with genetic variations of animals during the adaptive process. This process is observed throughout natural selection, i.e, morphological and physiological changes which are responsible for alternating the adaptive mechanisms of animals to adapt to environmental challenges. In short, the main changes that can occur alone or simultaneously to promote animal survival in a specific environment are morphophysiological, behavioral, hematological, neuroendocrine, serum biochemical, molecular and cellular responses (Sejian et al., 2018).

Recent studies have been carried out to define the adaptive profile in populations of production animals (de Vasconcelos et al., 2019a,b). For example, the adaptive profile of the Italian goat population (temperate climate) was explained by thyroid hormones, biochemical and hematological variables, while the Brazilian goat population (semi-arid climate) was related to the same indicators. However, although some more discriminating variables were the same in both populations, they showed different adaptive mechanisms (Ribeiro et al., 2015).

All these factors involved in animal adaptation to the environment require multifaceted and multidimensional approaches, as adaptation is multifactorial and complex (Façanha et al., 2019; Silveira et al., 2021, 2023). However, the general field of heat stress biology lacks an integrated theory and associated predictive models to allow quantitative and predictive research linking the multiple mechanisms and outcomes. The application of machine learning is one of the statistical tools available for assertive decision making (Silveira et al., 2023). This science has the ability to study large quantity of data and serve as a basis for analysis and prediction in decision-making tools as well as serve as data for learning in systems that acquire intelligence in the classification and analysis of information. So, the main question of this proposal is to answer *how the use of machine learning techniques can be used to investigate animal adaptation? What bioclimatic impact on adaptive and productive responses?*

These findings will help to understand adaptive responses in ruminants to climate change, in addition to identifying phenotypic markers that can be used to quantify responses to heat stress in different populations of production animals. These markers could be incorporated into breeding programs in an attempt to develop breeds with phenotypic plasticity for different agroecological zones (Sejian et al., 2018). Since the conservation of animal genetic resources is a strategic activity for the sustainability of the current animal production system, it prioritizes the use of breeds with maximum productive capacity. Several studies point out that the conservation of native genetic resources is a way to guarantee options to respond to current and future challenges, allowing food production in the face of future scenarios of sustainable animal production, due to global changes in environmental conditions (Façanha et al., 2021; Ferreira et al., 2021).

Our goals were to trace the adaptive profile, identify biomarkers of animal adaptation and investigate the potential effects underlying the complex relationships of animal adaptation with climatic variables, morphological, thermoregulatory, hematological, immunological and productive responses in the animal model using an intelligent predictive modeling as an auxiliary method.

2. Animal model

The choice to evaluate the goat species as an animal model is justified because goats are considered the ideal animal model for climate change, due to their phenotypic plasticity in scenarios of rapid changes in tolerance limits for temperature, food and water resources (Nair et al., 2021). This will be the first study that proposes to study the adaptive mechanisms of Spanish goats adapted in arid and temperate regions of the world in order to reveal the impact of the thermal environment on adaptation and animal production using different machine learning techniques.

The populations of Spanish goats studied were Majorera, Palmera and Tinerfeña Norte breeds. These Spanish goat populations are known to originate in different climates. For example, Majorera comes from the island of Fuerteventura, considered the most arid island in the archipelago (98 mm/year), while Palmera breed comes from the island of La Palma, which has a humid climate with milder temperatures (369 mm/year).

3. Hypothesis

- The use of machine learning techniques to allow to determine different adaptive mechanisms in the animal model
- Machine intelligence and path analysis will identify biomarkers to study animal adaptation and production
- The adaptive and productive dynamics of an animal model is partly determined by the thermal environment
- Productive factors influence adaptive responses

4. Material and methods

4.1. Local and environmental conditions

This study was performed in North of Tenerife Island, belonging to the archipelago in *Canary Institute of Agrarian Investigation* (28° 31′30″ N e 16° 22′08″ O and 246 m a.s.l.) in *Comunidad Autonoma de Canaria*, with *Consejería de Agricultura, Ganaderia, Pesca y Alimentación*, Canary Islands, Spain. The climate in the region is characterized by a high temperate with hot and dry summers, classified as *Csa* according to Köppen (1936) The territorial relief is mountainous with predominantly volcanic soil with strong erosion and impermeability, in addition to herbaceous and shrubby vegetation. The meteorological data and thermal comfort indices recorded during the study are presented in Table 1.

4.2. Animals and productions systems

Data was collected on three goat breeds (30 Majorera breed; 18 Palmera breed; 28 North Tinerfeña), all female, aged between 1 and 4 years, lactating, and non-pregnant. The average body weight (BW) of the three goats breed were 45.63 ± 9.54 kg, 38.37 ± 4.54 kg and 39.45 ± 3.58 kg, respectively.

The production systems was classified as intensive, where the animals are kept in installations covered with metal plates, open laterally (larger side, with 4 m) and cement floor. The animals are separated into random groups according to breed in stalls on 1 m of height, containing metal feeders and water *ad libitum.*

Concentrate feed based on corn and soy was provided in the first hours of the day (6 a.m.) and throughout the day the animals received chopped forage based on native plants.

Table 1
Meteorological data and thermal comfort indexes recorded during the experiment.

Variables	Minimum	Maximum	Mean	Std. Deviation
Air temperature (°C)	11.33	32.22	17.98	4.99
Humidty relative (%)	28.00	83.00	59.15	13.35
Black globe temperature (°C)	14.00	45.80	22.21	7.79
Radiant heat load (W.m^2)	375.92	767.72	475.62	87.24
Black Globe Temperature and Humidity Index	57.72	92.29	67.04	8.13

4.3. Data collection

4.3.1. Meteorological parameters and thermal comfort indices

Relative humidity (RH, %), dry point temperature (DPT), dew point temperature (DPT), black globe temperature (BGT) and wind speed (WS, m/s) were measured using a portable digital thermohygrometer (TGD-400, Instrutherm, São Paulo, Brazil), positioned approximately 0.5 m above the animals. Moreover, black globe temperature-humidity index (BGHI; Buffington et al., 1997) and radiant heat load (RHL, W m^{-2}; Esmay, 1969) were also calculated as:

$$BGHI = BGT + 0.36DPT + 41.5 \tag{1}$$

B_{GT} Black globe temperature (°C)
D_{PT} Dew point temperature (°C)

$$RHL = \sigma(MRT)^4 \tag{2}$$

σ Stefan − Boltzmann constant (5.67×10^{-8} W m^{-2} K^{-4});
MRT Mean radiant temperature (K).
MRT is expressed by Equation (3).

$$MRT = 100\sqrt[4]{\left(2.51\sqrt{WS}\,(BGT - DBT) + \left(\frac{BGT}{100}\right)^4\right)} \tag{3}$$

Where:
WS = wind speed, m s^{-1};
BGT = black globe temperature, K;
DBT = dry bulb temperature, K.

4.3.2. Thermoregulation patterns and coat thickness

Before data collection, the animals were kept in a single holding pen for 30́ so that there was no influence of handling on the responses of the animals. The variables were measured following a predetermined order so that there was no interference between them. First, respiratory rate (RR; breaths. min^{-1}), followed by rectal temperature (RT; °C); then blood collection and coat sample; and finally, weighing. Each goat was sampled on 3 days, in two periods (morning and afternoon), except body weight. The respiratory rate was recorded by counting the number of flank movements for one minute. The rectal temperature was measured with a digital thermometer (accuracy of ± 0.1 °C; Omron Flex Temp Digital Thermometer, China) which was inserted into the animal's rectum until it stabilized (about 1 min). The tidal respiratory volume (TRV) was calculated according to Silva (2008).

$$TRV = 0.0496 + RR - 1.557 \tag{4}$$

Where:
RR = respiratory rate (breaths. min^{-1}).

Coat thickness (CT, cm) was determined *in situ* in the middle of the thorax of each animal, about 20 cm below the dorsal line, with a thin metal rule according to Silva (2008).

4.3.3. Blood examinations

The blood collection was performed using vacuum tubes of 10 mL with anticoagulant ethylenediaminetetraacetic acid. The red blood cell count (RBC; $\times 10^6$/mL), haemoglobin concentration (HC; g/dL^{-1}), packed cell volume (PCV; %), mean corpuscular volume (MCV; fl), mean corpuscular haemoglobin (MCH; pg/hm), mean corpuscular haemoglobin concentration (MCHC; g/dL^{-1}), segmented cells (SEG; cells/mL^{-1}), lymphocyte (LYM; cells/mL^{-1}), monocytes (MON; cells/mL^{-1}), eosinophils (EOS; cells/mL^{-1}), platelets (PAL; cells/mL^{-1}) and white blood cell (WBC, cells/mL^{-1}) values were measured using an automatic analyzer (Labtest Diagnostica SA – Lagoa Santa, Minas Gerais, Brazil).

4.4. Statistical methods

Framework of the methodological proposal of the systematic approach for construction and validation of typological analysis and path analysis is presented in Fig. 1. Data were analyzed using SPSS® (version 26, Chicago, USA) (SPPS, 2019) and Weka® in seven successive steps:

Step 1 [*Number of typologies*] The optimal number of clusters was identified using the hierarchical cluster analysis (HCA). The HCA aimed to group adaptive profiles according to their degree of similarity. The morphophysiological and hematological responses were used in the analysis. The model used for hierarchical clustering is described in Eq. (5).

$$d[k,(i,j)] = max[d[k,i), d(k,j)] \tag{5}$$

This agglomerative algorithm calculates the shortest distance between elements i and j using the distance matrix dij (Hair et al., 2009). The elbow rule based on Ward (Ward, 1963) was used to decide the most appropriate number of clusters. In order to assess the optimal number of clusters, it was necessary to plot the number of clusters against the change in the merge coefficient for each stage (each stage reflects a combination between 2 clusters) and find the 2 stages with the biggest jump in the difference between their distance coefficients (Gelasakis et al., 2012). This was rather obvious for stages 73 and 74. Then, the number of steps (n = 73) was subtracted from the number of observations (n = 76); the result indicated the ideal number of clusters for the successive analysis of k-means clusters (n = 3). The Euclidean distance was used as a measure of dissimilarity.

Step 2. [*Classification of animals*] The classification of animals in their group was defined using K-Means, an unsupervised learning algorithm. The same variables used in the HCA were used in this step. This procedure follows a simple way to classify a given set of data through a certain number of clusters (assume k clusters) fixed *a priori*.

$$W(S,C) = \sum_{k=1}^{k}\sum_{i\in S_k}\|y_i - c_k\|^2 \tag{6}$$

Where S is a K-cluster partition of the entity set represented by vectors y_1 ($i \in I$) in the M−dimensional feature space, consisting of non-empty non-overlapping clusters S_k, each with a centroid C_k (k = 1,2,…K).

Step 3. [*Comparisons between typologies*] The normality of the residuals and the homogeneity of the variances were previously confirmed by the Shapiro-Wilk and Bartlett tests, respectively. Clusters were canalysed using an analysis of variance (ANOVA) and means compared by Tukey's test. The three adaptive profiles revealed by k-means were used as a fixed effect. Significant differences were accepted if $P < 0.05$ in all analyses.

The general linear model used was:

$$Y_{ij} = \mu + F_i + \varepsilon_{ij} \tag{7}$$

where: Y_{ij} = experimental response measured in treatment i and repetition j; μ = average population; F_i = adaptive profiles i (i = Clusters 1, 2 and 3); and ε_{ij} = residual error.

Step 4. [*Validation*] For validating the cluster analysis, a two-step cluster was performed. The TwoStep cluster analysis procedure is an exploratory tool designed to reveal natural groupings (or clusters) within a dataset that would otherwise not be apparent. The TwoStep clustering procedure involves two stages: (*1*) the original cases are grouped into preclusters (Okazaki, 2007) with the aim of reducing the size of the matrix containing distances between all possible pairs of cases (Norusis, 2011) and (*2*) pre-clusters are clustered using hierarchical clustering algorithms, which yields a range of solutions which is then reduced to the best number of clusters based on Schwarz's Bayesian information criterion (Norusis, 2011). The Euclidean measure was used, since it is indicated for quantitative variables. The cluster number

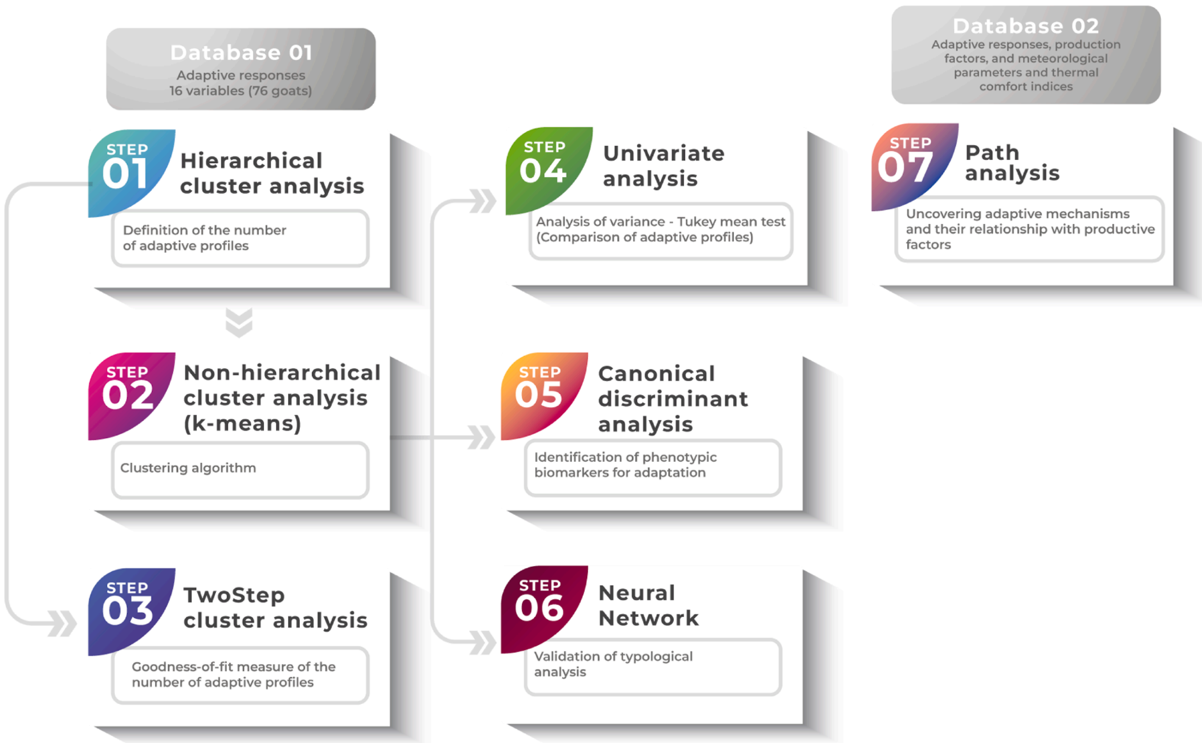

Fig. 1. Framework of the methodological proposal of the systematic approach.

included in the analysis was 3, as indicated in Step 1.

Step 5. [*Discriminatory power of indicators / variables*] Canonical discriminant analysis (CDA) was carried out to discriminate the main variables that differentiate the adaptive typologies and which indicators have discriminatory power. The general model of the CDA is described in the Eq (8)

$$Z_n = \propto + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_n X_n \tag{8}$$

Where: Z_n is the dependent variable (adaptive profile), $\propto$ is the intercept, X_i are the explanatory variables, and β_i is the discriminant coefficients for each explanatory variable. This step was carried out using adaptive profiles and breeds. This was used only to compare the dynamics of adaptive profiles with the breed of Spanish goats. For this, a stepwise method was used, which is indicated when there are a large number of variables for inclusion in the function (Silveira et al., 2021). The original variables were used in the CDA.The discriminant power was evaluated by % of variance, Wilks' Lambda statistic, and standardized coefficients.

Step 6. [Neural network] The neural network was used to perform pattern recognition of adaptive profiles. The neural network machine learning algorithm (multilayer perceptron) was used for three categories, with two layers of neurons (Hidden layers) and hyper parameters learning rate = 0.3 and momentum = 0.2.

Step 7 (*Path Analysis*) Paths were constructed from exogenous variables (morphological and climatic) to the effect variable (Rectal temperature).

For a correlative system with one dependent variable y and independent variables x_i ($i = 1, 2, \ldots, n$), the linear equation is:

$$y = b_0 + b_1 X_1 + b_2 X_2 + \cdots + b_n X_n \tag{9}$$

A regular matrix equation based on Eq. (10) can be established as:

$$\begin{pmatrix} 1 & r_{x_1x_n} & \cdots & r_{x_1x_n} \\ r_{x_2x_n} & 1 & \cdots & r_{x_1x_n} \\ \vdots & \vdots & \ddots & \vdots \\ r_{x_nx_1} & r_{x_nx_1} & \cdots & 1 \end{pmatrix} \hat{A}\cdot \begin{pmatrix} P_{yx_1} \\ P_{yx_2} \\ \vdots \\ P_{yx_n} \end{pmatrix} = \begin{pmatrix} r_{yx_1} \\ r_{yx_2} \\ \vdots \\ r_{yx_n} \end{pmatrix} \tag{10}$$

where $r_{x_ix_j}$ is the simple correlation coefficient of x_i and x_j, is P_{yx_1} the path coefficient.

$$P_{yx_1} = b_i \frac{\sigma_{x_1}}{\sigma_y}, \ (i = 1, 2, \cdots, n)$$

where b_i is the partial regression coefficient of y to x_i, σ_{x_1} and σ_y are the standard deviations of x_i and y, and $r_{x_i\ x_j}$ and P_{yx_1} may be used to express the indirect path coefficient of x_i through x_j to y.

5. Results

Hierarchical cluster analysis defined that there are three adaptive profiles for the animal model (Fig. 2 and Table 2). The k-mean cluster analysis algorithms classified the animals into their groups, with the main variables being morphophysiological responses and erythrocytes (Table 2). It was also noted that RT and the white blood series were not important in classifying the adaptive mechanisms. Based on the similarities and differences between the adaptive profiles (Table 3), the animal model was characterized as follows:

1) Adaptive profile 1 - Animal model with thin coats

Animals with low respiratory rate ($P < 0.05$). High hematological variables ($P < 0.05$), with the exception of RBC and HC, are characteristic in these animals. In addition, the immune system parameters include low numbers of platelets ($P < 0.05$).

2) Adaptive profile 2 - Animal model with medium thick coats

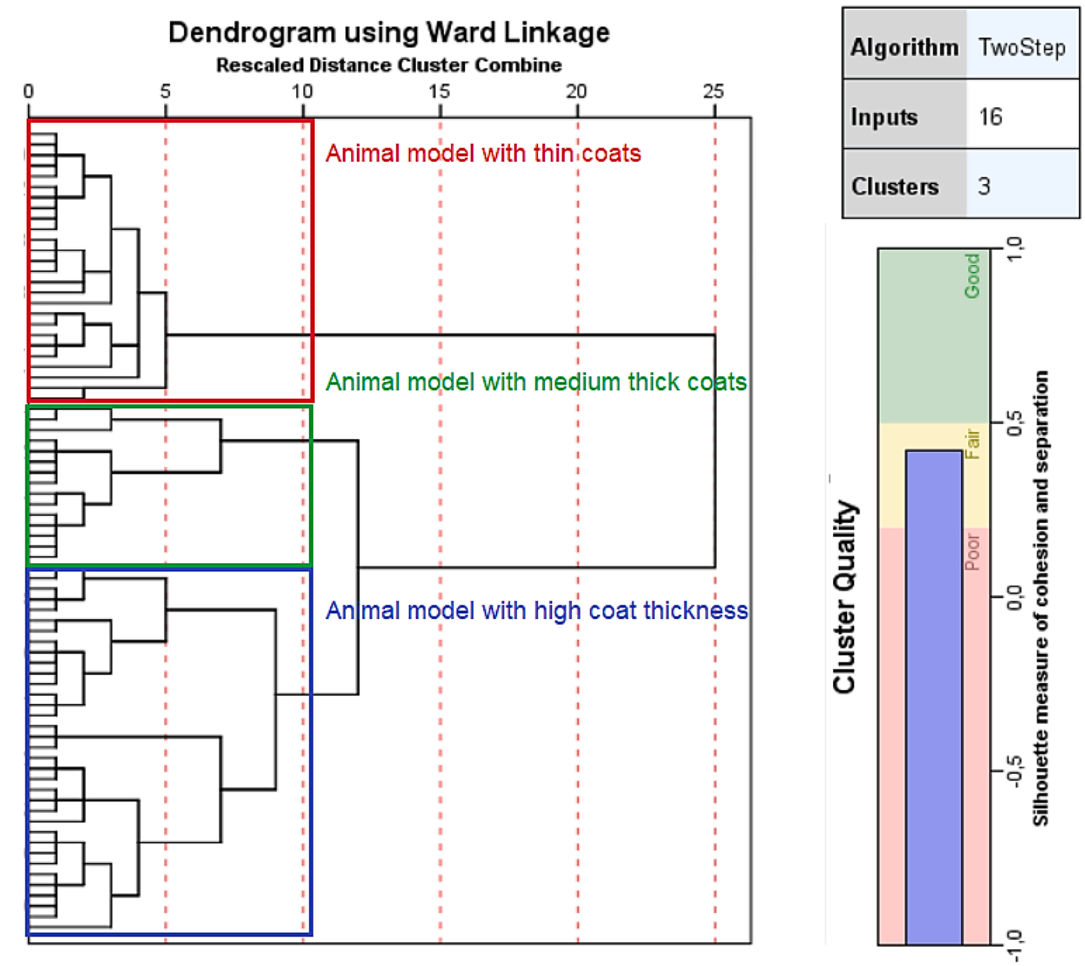

Fig. 2. Dendrogram of the HCA (A) and goodness-of-fit measure of the number of clusters through TwoStep cluster (B) for the three adaptive profiles.

Table 2
Agglomeration schedule of the hierarchical cluster analysis and variance analysis of the k-mean cluster analysis of the morphophysiological and hematological variables of the animal model.

Agglomeration Schedule - HCA				ANOVA – K means			
Stage	Cluster Combined		Coefficients		Cluster	Error	P – value
	Cluster 1	Cluster 2			Mean Square	Mean Square	
1	46	48	0.81	RT	0.01	0.12	= 0.96
2	64	65	1.85	RR	191.02	51.52	= 0.03
3	61	70	3.35	TRV	0.00	0.00	= 0.08
4	26	50	5.05	CT	261.44	26.19	< 0.001
5	27	53	6.85	RBC	108213.04	1444698.99	= 0.93
6	22	23	8.68	PCV	78.54	15.92	= 0.01
7	37	40	10.65	HC	3.08	1.22	= 0.09
8	24	44	12.71	MCV	47.11	7.45	< 0.001
–	–	–	–	MCH	2.12	0.49	= 0.02
69	8	31	643.24	MCHC	100.24	28.59	= 0.04
70	5	64	684.60	WBC	1517972.30	9733942.19	= 0.86
71	2	61	741.96	SEG	19.55	94.57	= 0.81
72	1	34	804.05	LYM	33.78	89.71	= 0.69
73	**2**	**5**	**875.27**	MON	0.53	1.15	= 0.63
74	1	2	979.27	EOS	4.56	5.73	= 0.45
75	1	8	1200.00	PLA	1046316261513.16	4757675018.02	< 0.001

RR – Respiratory rate (breaths/min^{-1}); TRV – Tidal respiratory volume; RT – Rectal temperature (°C); CT – Coat thickness (cm); RBC – Red blood cell ($\times 10^{6/}$mL); PCV – Packed cell volume (%); HC – haemoglobin concentration (g/dL^{-1}); MCV – Mean corpuscular volume (fl); MCH – Mean corpuscular haemoglobin (pg/hm); MCHC – Mean corpuscular haemoglobin concentration (g/dL^{-1}). WBC – White blood cell (cells/mL^{-1}); SEG – Segmented cells (cells/mL^{-1}); LYM – Lymphocytes (cells/mL^{-1}); MON - Monocytes (cells/mL^{-1}); EOS – Eosinophils (cells/mL^{-1}); PAL – Platelets (cells/mL^{-1}).

Table 3
Adaptive mechanisms of the animal model (n = 76).

Traits		Low coat thickness profile		Medium coat thickness profile		High coat thickness profile		P-value
		53.0 % Majorera breed		61.11 % Tinerfeña do Norte breed		71.42 % Palmera breed		
		Mean	± SEM	Mean	± SEM	Mean	± SEM	
Morphophysiological responses	RR	26.93b	0.75	28.45ab	1.00	32.06a	1.55	= 0.029
	TRV	0.07289	0.00068	0.07200	0.00095	0.07041	0.00074	= 0.084
	RT	39.32	0.05	39.34	0.05	39.31	0.07	= 0.958
	CT	3.74c	1.14	7.56b	1.30	10.18a	0.79	< 0.001
Hematology	RBC	13388.42	352.28	13441.50	264.65	13515.41	165.32	= 0.928
	PCV	23.58a	1.18	21.76ab	0.78	20.09b	0.58	= 0.010
	HC	9.34	0.31	8.77	0.28	8.65	0.14	= 0.088
	MCV	17.59a	0.73	16.21ab	0.53	14.89b	0.44	= 0.003
	MCH	6.98a	0.18	6.54ab	0.19	6.41b	0.09	= 0.017
	MCHC	40.49a	1.37	40.63a	1.09	43.81a	0.86	= 0.035
	WBC	13488.42	676.20	13879.50	634.84	13403.65	548.72	= 0.856
	SEG	53.26	2.56	54.70	2.40	55.00	1.36	= 0.814
	LYM	41.79	2.28	40.70	2.44	39.51	1.37	= 0.688

[a-c] Letters on the same row indicate difference by Tukey's test (P < 0.05); RR – Respiratory rate (breaths/min^{-1}); TRV – Tidal respiratory volume; RT – Rectal temperature (°C); CT – Coat thickness (cm); RBC – Red blood cell (×10$^{6/}$mL); PCV – Packed cell volume (%); HC – haemoglobin concentration (g/dL^{-1}); MCV – Mean corpuscular volume (fl); MCH – Mean corpuscular haemoglobin (pg/hm); MCHC – Mean corpuscular haemoglobin concentration (g/dL^{-1}). WBC – White blood cell (cells/mL^{-1}); SEG – Segmented cells (cells/mL^{-1}); LYM – Lymphocytes (cells/mL^{-1}); MON - Monocytes (cells/mL^{-1}); EOS – Eosinophils (cells/mL^{-1}); PAL – Platelets (10^3/mm^3).

Animals with respiratory rate and tidal respiratory volume were similar to animal model with thin coats (P < 0.05). The hematological components show intermediary PCV, MCV and MCH when compared to the other profiles. The number of platelets is greater than the other adaptive profiles.

3) Adaptive profile 3 - Animal model with high coat thickness

Animals that have higher respiratory rate (P < 0.001). Hematological responses are low for the other groups, not significantly different from the medium-coated group.

Differentiation was seen between adaptive profiles (Fig. 3-A) and the three goat populations (Fig. 3-B). Both canonical functions (P < 0.001) differentiated clusters and breed with 100 % discrimination. Morphology and hematology (red count) traits are the main traits that differentiate groups and breeds. (See the standardized coefficients of the canonical discriminant functions - Supplementary Table 1). Adaptive profile 1 was marked by different responses from profile adaptive 2 and 3. Adaptive profile 1 presented a greater distance from the centroid of adaptive profile 2 and 3, (Fig. 3A). It is also observed that the Majorera, Palmeira and Tinerna do Norte breeds have different adaptive mechanisms (Fig. 3B). Observing the classification rate of animals correctly designated in their group of origin according to the clusters and breeds of the animal model that discrimination using the hematological variables resulted a higher rate of success, while morphophysiological responses had a lower rate of success. When we used both indicators (morphophysiological and hematological responses), the rates in general reached almost 100 % classification (Table 4).

The neural network confusion matrix for classifying animals according to adaptive profiles determined that 90.79 % of observations were classified in their group of origin, which validates the construction of the typology of adaptive profiles by cluster analysis algorithms (Table 5). The best neural network model contained only one neuron layer (Fig. 4).

From the path analysis (Table 6), factors such as age, stage of lactation, and BCS did influence RR (P < 0.05). RBC and PCV positive effects on RR and vice versa (0.100** and 0.061*), respectively. There is a negative relationship between TV and RR (-0.701***) and PLA (-0.092***) but a positive effect with MON (0.091*) and HC (0.100**). As for RR, this showed a negative effect on WBC (-0.054***) and a positive on PLA (0.018***). PLA (0.021***), and RR (0.271***) had positive effect on RT but TV showed a negative effect (-0.248***).

AT, RH, BGT, RHL and BGTI influence thermoregulatory responses, especially in activating the RR for heat dissipation (Table 6). It was observed that no hematological variable was influenced by climate variables and thermal comfort index (P > 0.05). However, thermoregulatory variables (RR, RT and TV) influenced hematologic responses mainly through a two-dimensional relationship.

6. Discussion

Animal adaptation is complex due to the large number of variables needed to understand adaptive mechanisms, requiring a systematic approach to understanding the dynamics of changes and the global relationships between variables and effects. This complexity can be reduced by identifying groups of animals with common and distinct adaptive mechanisms in order to seek to understand the adaptive dynamics of these animals. This is one of the few studies carried out with farm animals adapted by natural selection in different thermal environments, which uses procedures that combine analytical methods in machine learning to reveal what these adaptive mechanisms are and what are the main biomarkers involved in animal adaptation.

The combination of different machine learning and path analysis techniques allowed us to detect large differences between adaptive profiles, helping to visualize different ways of adapting to the thermal environment according to thermoregulatory, morphological, hematological and immunological responses. Specifically, the cluster analysis algorithms identified the animals' three adaptive profiles, confirming the first hypothesis. Subsequently, the canonical discriminant analysis determined the main biomarkers of animal adaptation, besides showing that the formation of clusters is related to the breeds of the animal model. Next, the neural network analysis validated the typological construction with over 90 % accuracy according to the confusion matrix. Finally, the relationship between adaptive responses, climatic variables and productive factors was clarified by path analysis, visualizing the possible paths that this group of variables is related to. It is important to note that the use of this systematic approach to determine the mechanisms of farm animals was used for the first time to our knowledge, as other investigations use descriptive methods (De Pantoja et al., 2017; Yamin et al., 2022) or some of the analysis used here in isolation (de Vasconcelos et al., 2021a,b; Façanha et al., 2021).

Our main results were: (i) we identified patterns that hematological variables represent an important biomarker to study animal adaptation; (ii) there are three adaptive profiles with thermoregulatory, hematological and immunological mechanisms varying according to coat thickness; (iii) age, body condition score and lactation order are factors

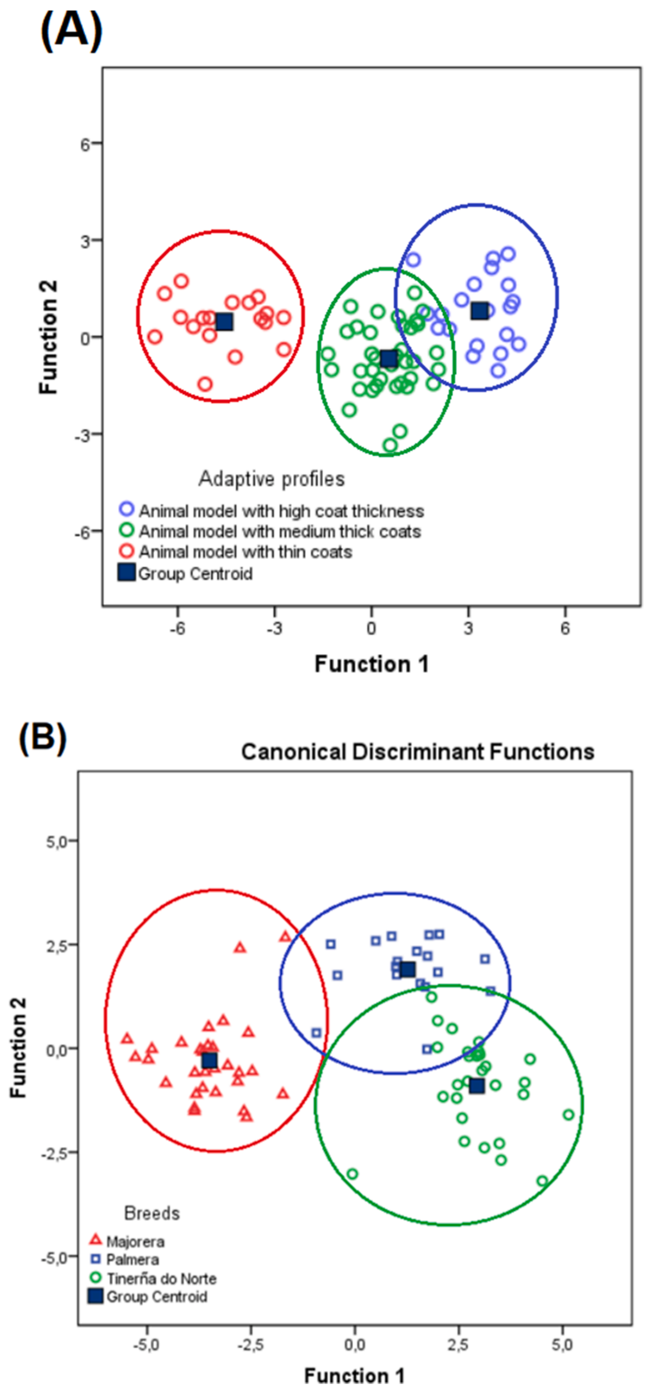

Fig. 3. Two-dimensional plot of canonical discriminant analysis referring to thermoregulation patterns. hair morphology. and haematological using three adaptive profiles (A) and three populations of Spanish goats (B).

that influence adaptive responses; and (iv) the climatic variables and the thermal comfort indices influence the thermoregulatory responses (primary mechanism), however, they do not influence the hematological and immunological responses, but the thermoregulatory variables influenced the hematological and immunological responses (secondary mechanism).

Although most studies evaluating animal adaptation result in the analysis of thermoregulatory responses, such as rectal temperature and respiratory rate, our results demonstrate that the inclusion of hematological variables can serve as a biomarker to assess heat stress in farm animals. Studies report the importance of including the hematological profile in the adaptive dynamics of cows (de Vasconcelos et al., 2019b) and goats (Ribeiro et al., 2015). Our results reinforce these findings and show the importance of including this group of variables, being justified because the blood system participates in thermoregulation. The blood is heated in the innermost regions of the body, and when it passes through the regions which are closest to the skin, it releases this heat to the environment. That is, in hot environments, we have vasodilation, and this favors the heat exchange between the body and the environment (Silveira et al., 2021; Gujar et al., 2022).

Our results also showed that the trait that was most notable among the three adaptive profiles was coat thickness, which is related to natural selection over the years in the animal model. Here, we use data from Spanish goat breeds, specifically from Tenerife, but these animals were developed in an arid climate, as is the example of the Majorera breed, which has a lower coat thickness. The Tinerfeña and Palmera breeds bred in rainy areas (intermediate coat thickness and thicker coat, respectively.). For the first time, this result of the adaptive dynamics of these Spanish goat breeds is presented.

The thicker coat is an adaptive mechanism of animals to low temperatures and rainy environments (15 to 21 °C), serving as a thermal insulator, as opposed to the thinner coat that was associated with animals that were developed in arid regions. The thickness of the coat acts as a physical barrier against the incidence of direct solar radiation on the animals, reducing the absorption of heat from the radiation. However, the opposite can also occur, functioning as a thermal insulator (Rashamol et al., 2018; Mascarenhas et al., 2022). For example, a thin coat facilitates especially the cutaneous evaporative heat exchange, as is the case for animals of the Majorera breed, as opposed to a thicker coat, which acts as a thermal insulator, in this case for the Palmeira and Tiñerna do Norte breeds (Table 2),

The lower values respiratory rate in adaptive profile 1 is justified by the lower thickness of the coat of these animals, which facilitated the thermal exchanges between the animal and the environment. The opposite was observed in animals with intermediate and higher coat thickness, because the greater the thickness of the coat, the greater the difficulty of sensitive losses, causing the animal to store more heat, which increases the rectal temperature, an important indicator of animal homeostasis, and dissipates by increasing respiratory rate (latent loss) changing thermoregulatory mechanisms.

The cause of the blood alterations are endogenous, being mainly related to the morphology of the coat and the respiratory rate as observed in the formation of clusters and in the path analysis, respectively. Erythrocyte parameters under conditions of thermal stress are influenced by endogenous (water and food intake; color, depth, density, diameter of coat; sweat gland volume; and regulation of epidermal and respiratory vascular supply) and exogenous (climatic variables) (Habibu et al., 2018) factors, *i.e.*, the exogenous factors were the same since the animals were in the same thermal environment at the time of data collection.

The animals with the lowest coat thickness present the highest values of the erythrocytes, being justified because these animals are more adapted to the environment compared to the other groups, since the high economy of body fluid is an indication of animal adaptation to the thermal environment. It is well established that animals with thin fur and short hair are efficient in dissipating heat to the environment (McManus et al., 2020; Leite et al., 2021). For example, Cardoso et al. (2015) identified that the most adapted bovine breed to the tropical conditions (lower rectal temperature) had higher values of PCV and HC, in relation to less adapted breeds.

The similarity of rectal temperature between adaptive profiles indicates that the adaptive mechanisms used were able to dissipate heat, although physiological changes and blood changes occurred, especially in red blood cells. It is also worth mentioning the importance of the morphological parameters of erythrocytes in an animal adaptation study, as these variables were the main biomarkers of the hematological indicator. The MCV, MCH and MCHC are calculated using HC, PCV and/

Table 4
Classification of the canonical discriminant analysis according to the adaptive mechanisms of the animal model.

	Adaptive profiles Predicted Group Membership (%)			Breeds	Predicted Group Membership (%)		
	CT Medium	CT High	CT Low		Majorera	Palmera	Tererfeña do Norte
(1) Morphophysiological responses (59.20 % %of original grouped cases correctly classified)				(1) Morphophysiological responses (73.70 % %of original grouped cases correctly classified)			
CT Medium	5.0	55.5	40.0	Majorera	93.3	0.0	6.7
CT High	0.0	81.1	18.9	Palmera	5.6	16.7	77.8
CT Low	0.0	26.3	73.7	Tererfeña	3.6	7.1	89.3
(2) Hematology (96.1 % of original grouped cases correctly classified)				(2) Hematology (73.7 % %of original grouped cases correctly classified)			
CT Medium	90.0	10.0	0.0	Majorera	76.7	20.0	3.3
CT High	0.0	100.0	0.0	Palmera	44.4	50.0	5.6
CT Low	0.0	5.3	94.7	Tererfeña	0.0	3.6	96.4
(3) All variables (98.70 %of original grouped cases correctly classified)				(3) All variables (93.40 % %of original grouped cases correctly classified)			
CT Medium	95.0	15.0	0.0	Majorera	96.7	3.3	0.0
CT High	0.0	100.0	0.0	Palmera	5.6	88.9	5.6
CT Low	0.0	5.3	100.0	Tererfeña	0.0	7.1	92.9

Table 5
Confusion matrix for classification of animal model adaptive profiles.

Correctly Classified Instances	69		90.78
Incorrectly classified instances	7		9.21
Kappa statistic	0.85		
Mean absolute error	0.07		
Confusion Matrix			
High coat thickness	Medium coat thickness	Low coat thickness	Profiles
17	3	0	High coat thickness
1	36	0	Medium coat thickness
0	3	16	Low coat thickness

or RBC, based on any alterations in hematological variables alternating in their erythrocyte morphology (Habibu et al., 2018). These parameters were also the main variables that showed discriminatory power between clusters in the canonical discriminant analysis (Fig. 3).

Path analysis also pointed out that the lactation order, animal age and body weight, are important factors to be considered in studies that evaluate animal adaptation, but what we find in most published studies is that these variables are ignored in the statistical models under study that evaluate animal adaptation (Façanha et al., 2018; de Vasconcelos et al., 2019b). Our results reinforce the need for these factors to be included as a covariate in statistical models to assess thermoregulatory, immunological and hematological responses (Vieira et al., 2022).

The influence of body size in general reflects the age and body weight of animals on animal adaptation it is justified because small animals have a proportionally larger specific surface area and, therefore, may be more vulnerable to environmental temperatures (Mcmanus et al., 2020). On the other hand, larger animals have a lower metabolic rate and, therefore, gain heat at a slower rate (Lima et al., 2022).

Another interesting factor in the path analysis is that the climatic variables and thermal comfort indices influenced the thermoregulatory responses (primary mechanism), but not the hematological and immunological responses, but the thermoregulatory variables influenced the hematological and immunological ones (secondary mechanism). Therefore, we can infer that the alteration of the hematological values of the animal's thermoregulatory homeostasis, and not a direct effect of the climate, as is discussed in some studies.

The relationship of RT and RR with hematological and immunological variables can be explained because hematological changes during heat stress they are modulated by are modulated by the hypothalamic–pituitary–adrenal axis. The central nervous system controls erythrocytes by influencing RR, circulation, sweating and water intake in order to increase heat loss during heat stress. Leukocyte parameters are modulated by the effects of neuroendocrine products of the hypothalamic–pituitary–adrenal axis, on the proliferative and cytolytic activities of leukocytes (Habibu et al., 2018; Abduch et al., 2022), so that more stressors can lead to increased synthesis of free radicals due to the imbalance between the generation of oxidizing compounds and the performance of antioxidant defense systems. The generation of free radicals and/or non-radical reactive species results from oxygen metabolism (increased respiratory rate).

The CDA validated the construction of the typology of the three adaptive profiles with 86.8 % (Fig. 3) of the cases correctly classified in their group of origin, demonstrating that the three different adaptive mechanisms of the animals already discussed above. In addition, it presented a higher percentage of cases correctly classified in their group of origin when compared to the same analysis, but evaluating the races. It is noteworthy that our aim is to discuss adaptive mechanisms based on machine learning algorithms, and not to compare races as in most studies (De Pantoja et al., 2017; Yamin et al., 2022).

7. Implications

A systematic assessment of the impacts of heat stress on animal production and adaptation is needed to identify appropriate biomarkers, as adaptive physiology is defined as the biological response of an individual organism to environmental stress. The search for this understanding of the biological mechanisms is the main implication of this study, being the thick coat thickness an important adaptive mechanism of animals adapted to the arid climate, and animals with intermediate and greater thickness to the temperate climate. These discoveries were only possible using a machine learning approach. Such an approach can also identify phenotypic traits, which can be useful indicators of susceptibility or tolerance to heat stress in different livestock species, such as, for example, the use of hematological variables as biomarkers in genetic improvement programs in an effort to develop thermotolerant breeds using selection assisted by markers. Such efforts can help to identify specific breeds according to a bioclimatic zoning (McManus et al., 2016a), providing a mechanism for sustainable livestock production in the scenario of climate change (Mcmanus et al., 2016b).

8. Limitation

The main limitation of this study is the use of only one morphological variable, coat thickness, to assess animal adaptation. We know that coat characteristics are an important phenotypic biomarker related to animal adaptation (Sejian et al., 2018). Therefore, we encourage future studies that carry out measurements on the set of skin characteristics such as pigmentation, dermal and epidermal thickness, blood capillaries and sweat glands to verify the relationship with the other indicators under

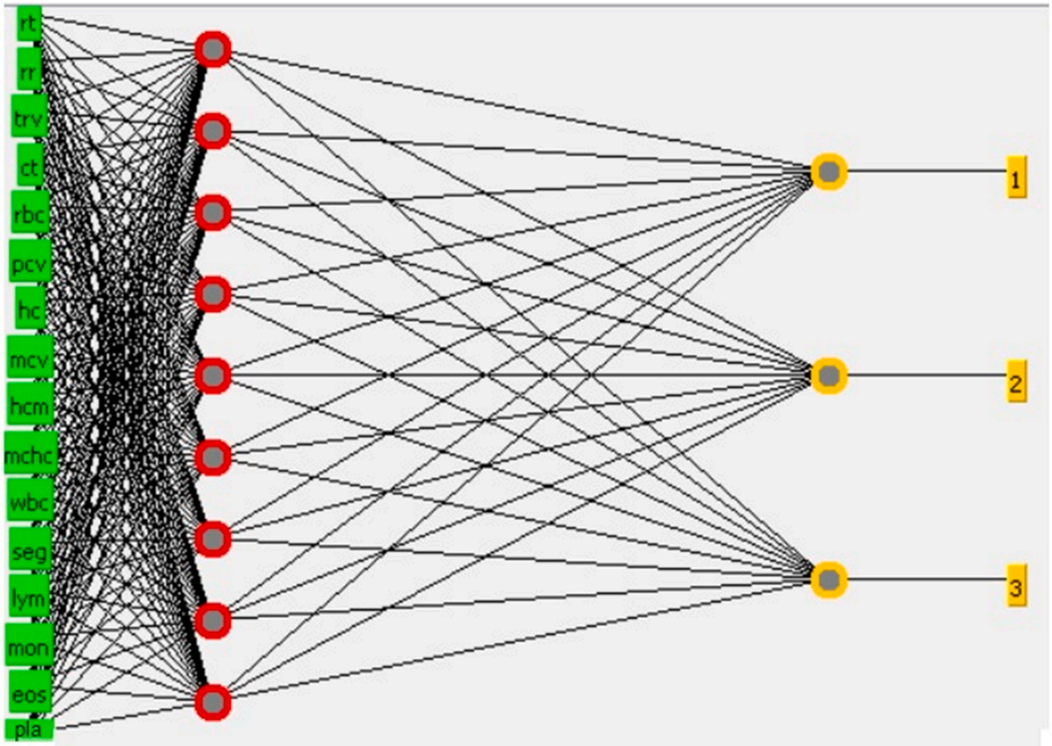

Fig. 4. Neural network using 1 layer of neurons for thermoregulatory, morphological, hematological and immunological responses according to the adaptive profile, RR – Respiratory rate (breaths/min^{-1}); TRV – Tidal respiratory volume; RT – Rectal temperature (°C); CT – Coat thickness (cm); RBC – Red blood cell (×10$^{6/}$mL); PCV – Packed cell volume (%); HC – haemoglobin concentration (g/dL^{-1}); MCV – Mean corpuscular volume (fl); MCH – Mean corpuscular haemoglobin (pg/hm); MCHC – Mean corpuscular haemoglobin concentration (g/dL^{-1}). WBC – White blood cell (cells/mL^{-1}); SEG – Segmented cells (cells/mL^{-1}); LYM – Lymphocytes (cells/mL^{-1}); MON - Monocytes (cells/mL^{-1}); EOS – Eosinophils (cells/mL^{-1}); PAL – Platelets (10^{3}/mm^{3}), Adaptive profile: 1 Animal model with thin coats, 2 Animal model with medium thick coats, 3 Animal model with high coat thickness. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

study (Castanheira et al., 2010). An interesting question is whether these goats with a thicker coat have a consequent greater volume of sweat glands? Consequently, they sweat more? Finally, hairs were collected to assess density of the coat, hair length and thickness, but this biological material was lost on trips from laboratories from Spain to Brazil.

We encourage studies which use the responses evaluated here along with the behavioral responses, as goats are social animals and behavioral observations are necessary to ensure that stress due to isolation does not influence the results. An interesting question to be answered is: *Does the animal model profile with thicker coat thickness seeks more shade when compared to the animal model with thinner coat thickness?* Studies evaluating thermoregulation must be accompanied by a behavioral analysis (Farias et al., 2021). It is noteworthy that the systematic machine learning approach suggested and adopted here supports the use of big data.

9. Final remarks and future research

The identification of adaptive mechanisms in farm animals and the search for biomarkers requires the use of specialized methods that model these relationship patterns and identify response variables that correspond to animal adaptation mechanisms. Here, we reveal that future studies should include hematological variables to assess animal adaptation. Furthermore, coat morphology presents an important adaptive mechanism used by animals.

The systematic approach that combined three unsupervised technique with different objectives to identify biomarkers, cluster adaptive profiles and discriminate the adaptive dynamics of these profiles using thermoregulatory, morphological, hematological and immunological indicators was able to identify and validate the three profiles with low, intermediate and high coat thickness with thermoregulatory, hematological and immunological variables varying between groupings. The profile with low coat thickness corresponds to animals of the Majorera breed, a breed adapted to arid climates that presented lower respiratory rate and high concentration of erythrocytes, demonstrating phenotypic plasticity when compared to the other groupings that are related to the Tiñerna do Norte and Palmera breeds.

Factors such as age, lactation order, body weight, meteorological variables and thermal comfort indices influence thermoregulatory, hematological and immunological responses, and these factors must be considered as covariates in the statistical model when using a univariate and multivariate approach.

Most of the variables under study and other indicators such as behavioral, biochemical, metabolic and physical related to the physiology of thermoregulation in farm animals are already measured by sensors. This includes measurements of morphophysiological responses, metabolism, disease, and feed and water intake. All these variables can be used simultaneously in a machine intelligence approach for assertive decision-making by producers, since the use of sensors in animal production is an approach that seeks to maximize animal welfare and provide better alternatives in real terms for evaluating animal response to the environment.

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Table 6
Standardised coefficients from path analysis using environmental and thermal comfort indices and general characteristics (age. lactation. body condition score. thermoregulation patterns and haematology) of animal model.

From	Direction	To	Estimate	Standard Error
Age	===>	RR	0.007*	0.048
Lactation	===>	RR	−0.019*	0.047
BCS	===>	RR	0.026*	0.048
RH	===>	RR	0.472*	0.364
BGT	===>	RR	7.595*	6.097
RHL	===>	RR	−1.265*	0.933
BGTI	===>	RR	−5.999*	5.039
WS	===>	RR	0.126**	0.047
CT	===>	RR	0.175***	0.051
ERY	<==>	RR	0.100**	0.038
PCV	<==>	RR	0.061*	0.031
RR	<==>	TRV	−0.701***	0.043
HC	<==>	TRV	−0.023	0.045
MCV	<==>	TRV	0.065	0.045
MCH	<==>	TRV	0.069	0.045
MCHC	<==>	TRV	−0.032	0.044
WBC	<==>	TRV	0.005	0.030
SEG	<==>	TRV	0.019	0.044
LYM	<==>	TRV	−0.024	0.044
MON	<==>	TRV	0.091*	0.044
EOS	<==>	TRV	−0.025	0.044
PLA	<==>	TRV	−0.092***	0.028
HC	<==>	RR	0.100**	0.037
MCV	<==>	RR	0.018	0.026
MCH	<==>	RR	0.033	0.032
MCHC	<==>	RR	−0.005	0.031
WBC	<==>	RR	−0.054***	0.002
SEG	<==>	RR	−0.037	0.040
LYM	<==>	RR	0.044	0.041
MON	<==>	RR	−0.047	0.037
EOS	<==>	RR	−0.015	0.041
PLA	<==>	RR	0.018***	0.001
HC	<==>	RT	0.027	0.044
MCV	<==>	RT	−0.004	0.044
MCH	<==>	RT	−0.017	0.044
MCHC	<==>	RT	−0.033	0.045
WBC	<==>	RT	0.014	0.043
SEG	<==>	RT	−0.070	0.044
LYM	<==>	RT	0.070	0.045
MON	<==>	RT	0.002	0.044
EOS	<==>	RT	0.010	0.045
PLA	<==>	RT	0.021***	0.001
TV	<==>	RT	−0.248***	0.044
RR	<==>	RT	0.271***	0.039

BCS – Body condition score; CT – Coat thickness; ERY – Erythrocytes; HC – Hemoglobin concentration; MCV – Mean corpuscular volume; MCH – Mean corpuscular hemoglobin; MCHC – Mean corpuscular hemoglobin concentration; WBC – White blood cell; SEG – Segmented cell; LYM – Lymphocytes; MON – Monocytes; EOS – Eosinophils; PLA – Platelets; RH – Relative humidity; BGT – Black globe temperature; RHL – Radiant heat load; BGTI – Black globe and temperature index; WS – Wind speed; RR – Respiratory rate; TRV – Respiratory tidal volume; RT – Rectal temperature; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$;
==> – Unidirectional;
<==> – Bidirectional.

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11. Ethics approval

This experiment was approved by the Ethics Committee of the UFERSA, Mossoró, Brazil (process identification: 23091003895/2014-71).

CRediT authorship contribution statement

Robson Mateus Freitas Silveira: Conceptualization, Methodology, Data curation, Data analysis, Writing – review & editing, Final review. **Débora Andréa Evangelista Façanha:** Data collect, Final review, Resources, Validation, Visualization. **Concepta McManus:** Data curation, Data analysis, Final review. **Luis Alberto Bermejo Asensio:** Final review, Validation, Visualization. **Iran José Oliveira da Silva:** Conceptualization, Writing – review & editing, Final review, Validation, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compag.2023.108502.

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Corrigendum

Corrigendum to “Intelligent methodologies: An integrated multi-modeling approach to predict adaptive mechanisms in farm animals” [Comput. Electron. Agric. 216 (2024) 108502]

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The authors regret that the name Sergio Álvarez Ríos [Animal Production Unit, Pastures and Forages in Arid and Subtropical Zones, Canarian Institute of Agricultural Research (ICIA), PBOX n° 60, 38200 La Laguna, Santa Cruz de Tenerife, Spain] was missing from the original article and correct the affiliation of the author Luis Alberto Bermejo Asensio to Departamento de Ingeniería Agraria y del Medio Natural, Carretera general de Geneto, 2, Apartado 456, Código postal 38200, San Cristóbal de La Laguna, S/C de Tenerife, Spain, which has been corrected in this corrigendum updated as above. In addition, Sergio Álvarez’s contribution:

Sergio Álvarez: Data collection, Final review, Resources; Validation.

The authors would like to apologise for any inconvenience caused.

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