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1 Non-invasive measure of heat stress in sheep using machine learning techniques and 2 infrared thermography

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9 Abstract

10 Heat stress (HS) leads to altered sheep behavior, physiological, and biochemical processes
11 which negatively affects their welfare and performance. While suitable strategies are needed to
12 ameliorate the impacts of HS in sheep, it is equally important to accurately and non-invasively
13 measure HS. Traditionally, rectal temperature (RT) is considered an indicator of thermal balance
14 and is used to assess the impacts of hot conditions on sheep. However, measuring RT itself can be
15 a stressor as it often requires restraining of the animals. The main objective of this study was to
16 establish whether a combination of infrared thermography (IRT) and machine learning techniques
17 can be applied to predict sheep RT when subjected to HS. Thermal images and RT were taken
18 twice weekly from Dorper, and 2nd Cross (Poll Dorset X (Border Leicester X Merino)) lambs
19 (n=24/breed, 4-5 months old), for two weeks. Sheep were randomly allocated to either (i)
20 thermoneutral (TN; 18–21 °C, 30–50% relative humidity (RH), n = 12/group) or (ii) cyclic HS
21 treatments (28–40 °C, 40-60% RH, the cycle comprised of high temperatures 38-40 °C between
22 0800 and 1700 h daily and 28 °C, 30-40% RH maintained overnight). The head was selected as
23 the region of interest because of less wool cover; specifically, the IRT of forehead, eye, ear, nostril,
24 and face locations were measured. Artificial neural network (ANN) models were developed using
25 three different backpropagation algorithms with temperature-humidity index (THI), and IRT
26 temperatures as inputs and RT measured manually as targets. Results showed that the forehead
27 and eye IRT temperatures had the highest correlation (P<0.01) with THI and RT. Further, Bayesian
28 Regularization, with one hidden layer containing 10 neurons with a tangent sigmoid transfer
29 function, showed the best correlation (R=0.92) and highest performance (MSE=0.02). The model
30 developed may be a rapid and cost-effective technique to monitor real-time body temperatures in
31 sheep and also to detect HS with minimal restraint.

32 **Keywords:** heat stress, infrared thermography, machine learning, rectal temperature

33 **Introduction**

34 Excessive heat load or heat stress (HS) describes a situation where the thermoregulatory
35 mechanisms of an animal fail to regulate the body temperature effectively within the normal range.
36 HS is a major constraint to the wellbeing and productivity of farm animals, such as sheep reared
37 under subtropical and tropical conditions (Marai et al., 2008; Joy et al., 2020a; Baida et al., 2021).
38 The primary physiological response to HS in ruminants involves increased body temperature and
39 respiration rate (normal body temperature and respiration rate range in sheep is 38.1-39.9 °C and
40 12-30 beats/min; but the values may vary under different conditions such as level of activity, diet,
41 breed and age) (Chauhan et al., 2014b; Joy et al., 2020b). Prolonged heat stress often leads to low
42 productivity (Koluman and Daskiran, 2011; Maurya et al., 2016), compromised immune function
43 (Chauhan et al., 2014a; Shi et al., 2020; Chauhan et al., 2021), high morbidity, and mortality
44 (Phillips, 2016). Early identification of animals under moderate /extreme HS is vital to enable
45 suitable interventions such as providing access to shade, cool drinking water and in severe cases
46 artificial cooling of stressed animals using pedestal fans to mitigate HS, improve animal welfare,
47 and reduce the risk of sheep mortality. However, systematic screening to identify signs of HS is
48 particularly difficult under farm conditions, especially in grazing systems, where animals are
49 present in large numbers. Although weather indices such as temperature-humidity index (THI) and
50 heat load index (HLI) could act as a guide for estimating HS severity in livestock, they carry a set
51 of limitations. The primary constraint for using bioclimatic indices is their poor relationship to the
52 thermoregulatory dynamics of the animals under excessive HS. Typically, the level of HS in
53 animal depends upon the inherent genetic potential of the individual animal to the stressful
54 conditions, which may vary for species, breed, age, physiological stage etc. (Osei-Amponsah et
55 al., 2019; Joy et al., 2020a). Therefore, irrespective of the available information, methods to
56 measure and monitor the physiological responses such as body temperature in real-time may give
57 more information on the early detection of HS in sheep.

58 The animal's core body temperature estimates the temperature of vital internal organs such
59 as the heart, liver, and brain. There are several indicators established in ruminants as an indirect
60 measurement to represent core body temperature comprising rectal (Goodwin, 1998), vaginal
61 (Hillman et al., 2009), tympanic (Brown-Brandl et al., 1999) and rumen temperature (Ipema et al.,
62 2008; Lees et al., 2019). Among those, rectal temperature (RT) is used as a conventional "gold
63 standard" indicator of core body temperature in sheep. Nevertheless, measurements of RT are

64 time-consuming, labor-intensive, and often require manual handling, which can affect animal
65 welfare. Infrared thermography (IRT) provides an alternative approach for quantifying animal
66 body temperature. This approach measures the surface temperature based on proportional
67 emissions of heat radiation from the body (Salles et al., 2016; Macmillan et al., 2019). Infrared
68 images also indicate the difference in the blood flow resulting from high body temperature under
69 stressful environmental conditions (McManus et al., 2015). Hence, the temperature of different
70 body locations such as the eye (Hoffmann et al., 2013; Daltro et al., 2017), fore-head (Peng et al.,
71 2019), muzzle (Fuentes et al., 2020b), rump (Baida et al., 2021), flank (McManus et al., 2015),
72 feet (Montanholi et al., 2008) and udder temperatures (Metzner et al., 2014; Osei-Amponsah et al.,
73 2020), measured using IRT, have been used to quantify physiological parameters and stress in
74 various livestock species. Thermal imaging is fast, reliable and has the advantage that it could
75 screen many animals with little or no restraint (Idris et al., 2021). Also, this method is more
76 advanced, non-invasive and has greater potential for automation than conventional methods (Salles
77 et al., 2016; Fuentes et al., 2020b). However, there are some limitations and factors that must be
78 taken into consideration while using IRT. Accurate measurement of IRT often requires a consistent
79 image angle and distance to the subject, along with constant ambient temperature, wind speed, and
80 direct sunlight (Idris et al., 2021). Also, it is not possible to predict RT from IRT imaging.
81 However, a model could be developed to predict it from the surface temperatures of the body and
82 because of non-linearity of the relation between the inputs and the target, machine learning could
83 provide a more accurate prediction.

84 Machine learning and computer vision algorithms provide new opportunities to non-
85 invasively examine farm animals in terms of behavior (Stewart et al., 2017; Fuentes et al., 2020a),
86 physiology (Jorquera-Chavez et al., 2019; Fuentes et al., 2020b), and production changes (Fuentes
87 et al., 2020c). Artificial neural networks (ANN) are widely applied in multiple agricultural fields,
88 designed to learn, and find patterns among the input data to predict specific outputs (Gonzalez
89 Viejo et al., 2019; Taheri et al., 2021). Model development is achieved by a process of training
90 where these algorithms process the data by modifying weights and biases to obtain the best
91 correlation (Taheri et al., 2021). Applications of IRT and ANN have been recently implemented
92 to analyze environmental-related stress responses in farm animals based on changes in body
93 temperature (Jorquera-Chavez et al., 2019; Fuentes et al., 2020b). However, using the ANN
94 technique usually requires selecting the best neural network structure with optimum model factors

95 such as the number of hidden layers, neurons, training function, and the activation function for
96 hidden layers and the output function (Taheri et al., 2021). A hidden layer is located between the
97 input and output of the algorithm, in which the function applies weights to the inputs and directs
98 them through an activation function as the output. The number of hidden layers and neurons is
99 mostly determined by trial-and-error to obtain the best model with a minimum error and high
100 performance in predicting the target values (Gonzalez Viejo et al., 2019). Compared to other
101 ruminant species (cattle and goats), sheep have thick wool that acts as a significant resistance layer
102 to the skin (Fuentes et al., 2020b). We propose that the facial area of the head would be suitable
103 for measuring skin temperature using IRT as this location contains minimal wool in sheep.
104 Therefore, the main objective of this research was to explore the correlations between the
105 temperatures obtained from IRT images of a sheep's head and develop an ANN model to predict
106 the RT using these images.

107 **Materials and Methods**

108 **Animals and Experimental Design**

109 The live animal study was approved by the Faculty of Veterinary and Agricultural Sciences, The
110 University of Melbourne Animal Ethics Committee (Ethics ID: 1714357.1) and was conducted at
111 The University of Melbourne, Dookie Campus, Victoria, Australia (36°23'01.9"S 145°42'52.1"E)
112 over two weeks. The details on animals and experimental design have been previously reported by
113 Joy et al. (2020b) and Zhang et al. (2021). Briefly, 48 lambs of two different breeds, Dorper and
114 2nd Cross [SC; Poll Dorset X (Border Leicester X Merino)] lambs (24 lambs from each breed; 4-5
115 months old with live weight = 40.9 ± 0.91 kg, (Mean \pm SD) were used in the study. The lambs
116 were acclimatized to indoor facilities for two weeks before starting the measurements. They were
117 fed a mixed ration (50% pellets, 25% oaten hay, and 25% lucerne chaff) *ad libitum*, complimented
118 with freshwater *ad libitum*. After acclimatization, lambs were randomly allocated to two
119 treatments (i) thermoneutral (TN; 18–21 °C, 30–50% relative humidity (RH), n = 12/group) and
120 (ii) cyclic heat stress (HS; 28–40 °C, 40-60% RH, the cycle comprised of high temperatures 38-
121 40 °C between 800 and 1700 h daily and 28 °C, 30-40% RH maintained overnight).

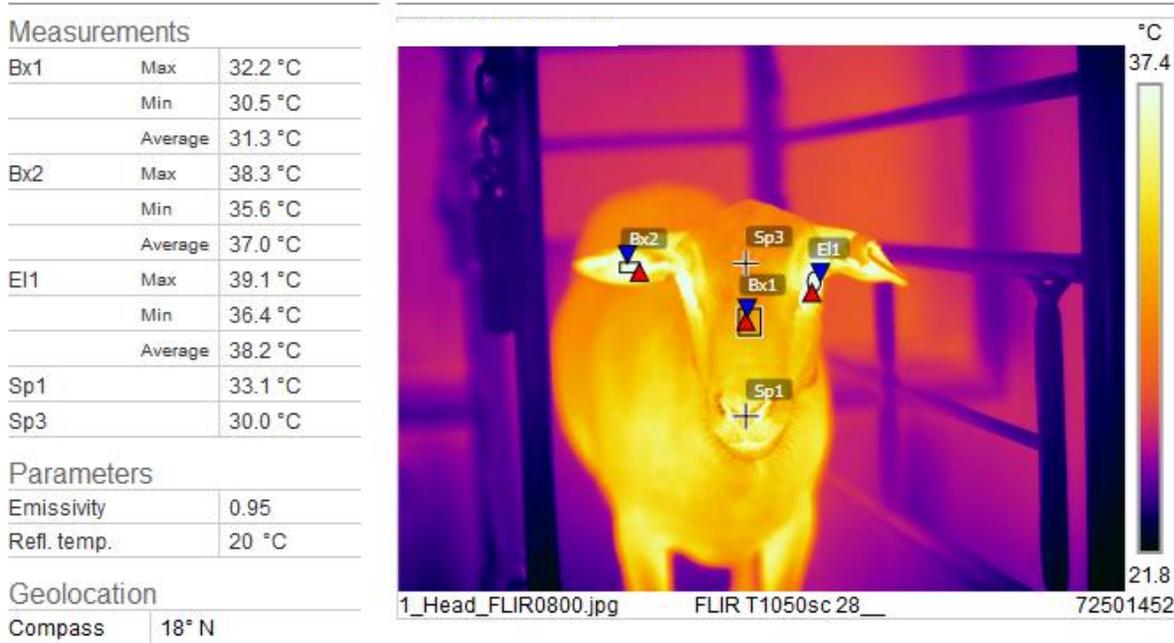
122 **Data Acquisition**

123 During the current study, the temperature and RH of the treatment rooms (TN and HS) were
124 recorded at 30-minute intervals using a universal serial bus (USB) temperature and humidity data

125 logger (TechBrands; Electus Distribution, Rydalmere, NSW, Australia). Based on the weather
126 variables, the THI was calculated according to the formula described by Marai et al. (2007), given
127 T and RH as dry-bulb temperature (°C) and relative humidity (%) respectively.

$$128 \text{ THI} = T - \{(0.31 - 0.0031 * \text{RH}) * (T - 14.4)\}. \quad \dots (1)$$

129 Thermal images were obtained twice weekly at 1700 h using a handheld portable infrared
130 thermal camera FLIR T1050sc (FLIR Systems Inc.; Wilsonville, OR, USA) with thermal
131 sensitivity of <20 mK and a wide temperature range (-40 °C to +2000 °C). The camera has an
132 accuracy of ±2 °C or ±2% of reading at 25 °C for temperatures up to 1200 °C, with an emissivity
133 of 0.985 (FLIRSystems, 2015; Osei-Amponsah et al., 2020). Sheep were restrained in a standing
134 position and thermal imaging was performed at approximately 0.5 m distance from the animal at
135 an angle of between 30° to 40° with emissivity set to 0.95, as indicated for animal skin (Stelletta
136 et al., 2012). Thermal images were analyzed using the FLIR's ResearchIR Max software
137 (FLIRSystems, 2015) to record the skin temperature of the lambs in various body locations.
138 Specifically, the head was selected as a region of interest (ROI) for temperature estimation as this
139 area contains less wool and other regions of the body may create a bias for data extraction between
140 hair and wool sheep breeds (Fuentes et al., 2020b). Henceforth, nostril (nostril T), forehead (FH
141 T), ear (ear T), eye (eye T), and face (face T) were selected as ROIs for this current study (Fig 1).
142 One of the main constraints in extracting FH T from SC lambs was that they had less wool in the
143 forehead region which may create biases in the data obtained. Therefore, FH T on both breeds
144 were obtained from a slighter lower location, level with the eye to avoid any interruptions from
145 wool (Fig 1). The RT of the animals was also simultaneously measured using a digital thermometer
146 (Model: DT-K11A; Honsun, Shanghai, China).



147

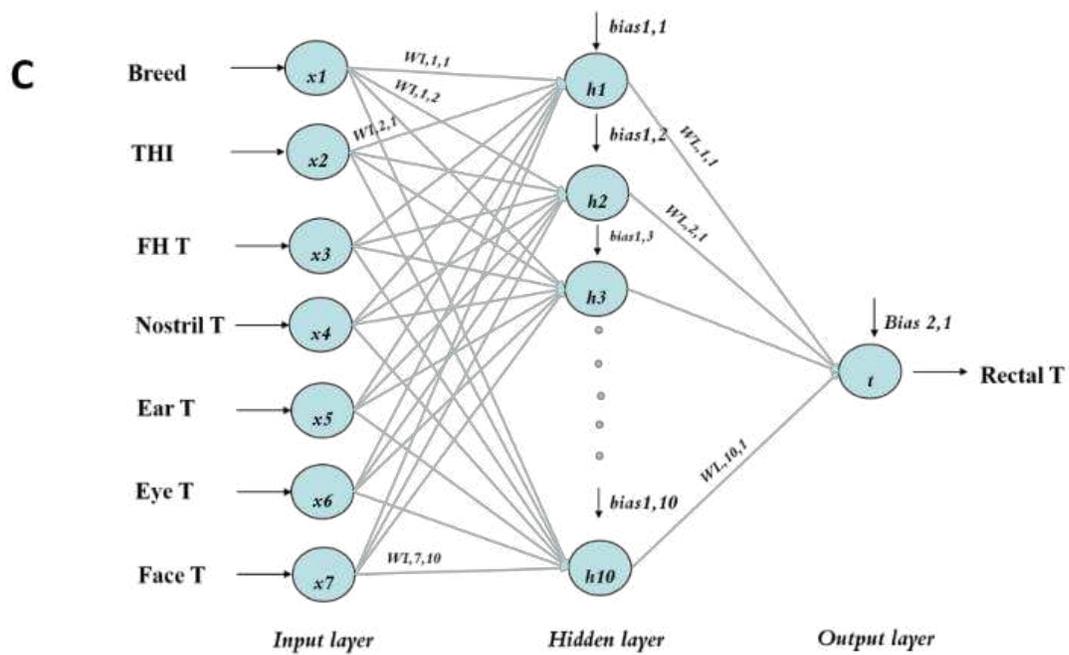
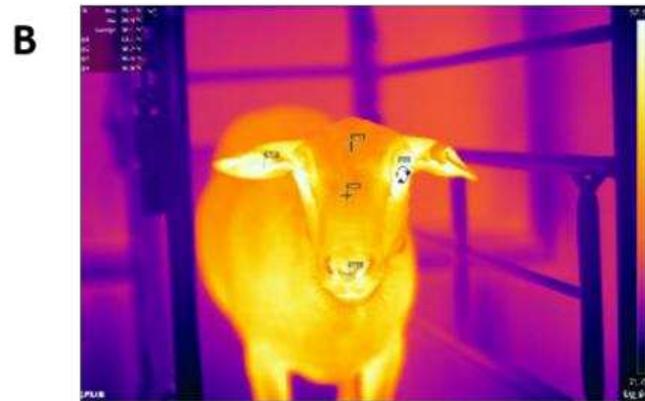
148 **Fig 1.** Example of a thermal image of a Dorper sheep’s head showing the region of interests
 149 (ROIs) selected; Bx1- Face, Bx2- Ear, EI1- Eye, Sp1- Nostril and Sp3- Fore-head

150 **Artificial Neural Network (ANN) modeling**

151 **ANN model structure**

152 The basic structure of an ANN model consists of an input layer, one or more hidden layers,
 153 and an output layer. In every layer, there are several nodes, or neurons, with each layer using the
 154 output of the preceding layer as its input, so neurons interconnect with distinct layers. Each neuron
 155 specifically has weights that are modified during the learning process, and as the weight decreases
 156 or increases, it adjusts the strength of the signal of that neuron (Taheri et al., 2021). In this current
 157 study, the model was constructed based on a two-layer feedforward network with a tan-sigmoid
 158 activation function in the hidden layer and a linear transfer function in the output layer. Fig 2
 159 illustrates the graphical representation of the entire study design. The input variables selected for
 160 the prediction of RT were forehead, eye, ear, nostril, and overall face temperature obtained from
 161 infrared thermography, THI and breed type (represented as binary numbers; Dorper and SC as 0,1
 162 respectively). The neural fitting app of MATLAB 2020a (MathWorks Inc., Natick, MA, USA)
 163 allows for selecting data, creating, and training a network and validating its performance using
 164 correlation coefficient (R) and mean square error (MSE) as the goodness of fit criteria. Three back-
 165 propagation training algorithms (Levenberg-Marquardt, Bayesian Regularization (BR), and Scaled

166 Conjugate Gradient) were used to train the ANN model to predict RT in sheep. A neuron trimming
167 exercise (5, 7, 10 and 15 neurons) was performed to obtain the best-hidden layer structure based on
168 the highest accuracy (highest R and lowest MSE) for each algorithm (data not shown). Fifteen was
169 the highest number of neurons considered for all the models because the use of a large number of
170 neurons would most likely result in overfitting (Gonzalez Viejo et al., 2019). The original dataset,
171 corresponding to 192 observations, was randomly divided into 70% for training (N=134), 15% for
172 testing (N=29) and 15% for validation (N=29) for each model. In the BR algorithm, 85% of the
173 data was applied for training and 15% for testing. This algorithm has an implemented cross-
174 validation, which is performed on the training data (85%).



176 **Fig 2.** Graphical representation of the study design: (A) the experimental layout, (B) the thermal
177 image capturing and processing with the selected region of interests in the head and extraction of
178 temperature (°C) values from the infrared thermal image (IRTI) analysis, (C) the schematic
179 illustration of artificial neural network model to predict rectal temperature. Model diagram
180 abbreviations: THI: temperature-humidity index; T: temperature; w: weights.

181 **Statistical analysis**

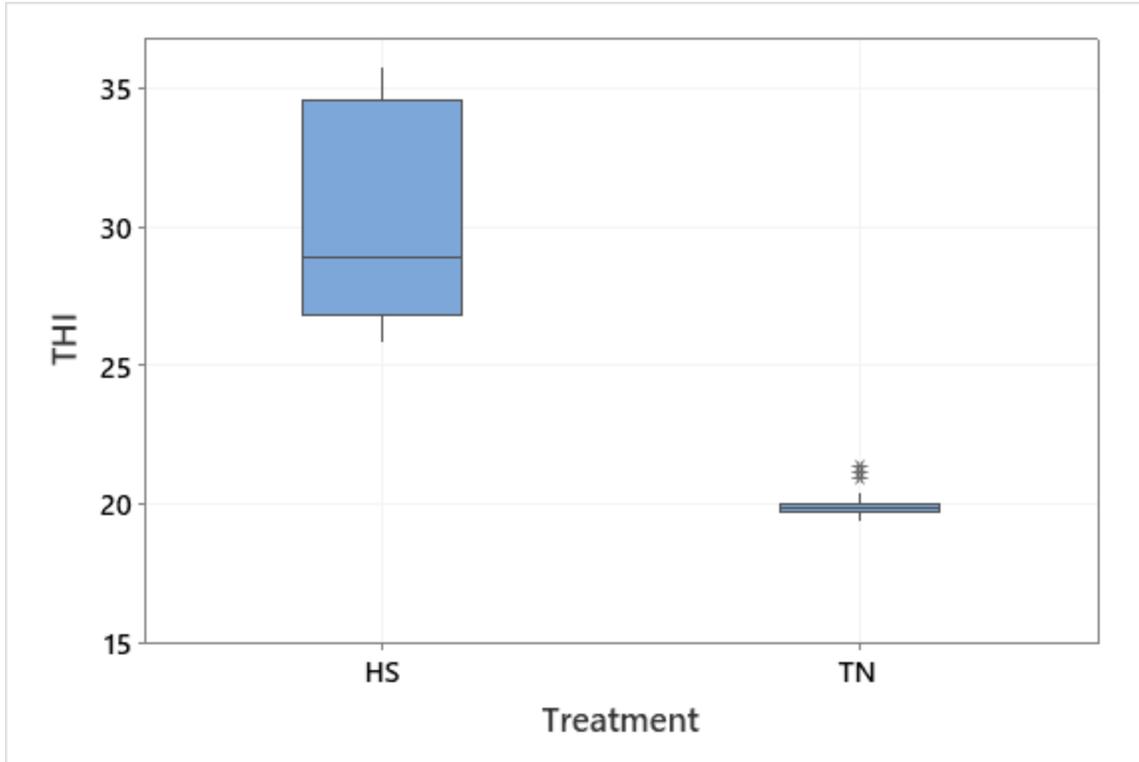
182 The correlation among THI, RT and skin temperatures (obtained from IRTs) were estimated
183 using correlation analysis in Genstat (GenStat 19th Edition; VSN International Ltd., Hemel
184 Hempstead, UK) with a significance level set at $P \leq 0.05$. Further, the statistical analysis to evaluate
185 and compare the accuracy of the developed models consisted of R, MSE to assess performance
186 and slope for each of the (i) training, (ii) validation, (iii) testing, and (iv) overall model stages. For
187 the best model, the percentage of outliers using 95% confidence boundary was calculated. Linear
188 regression analysis for temperature data with the intercept passing through the origin and $P \leq 0.05$
189 as the criteria were used to compare the RT measurements using the manual methods against the
190 predicted RT using the ANN model with Minitab® 19 (Penn State University, PA, USA).

191 Dimension reduction using principal component analysis (PCA) was performed in Minitab to
192 find relationships and patterns among the data between THI, manual RT measurements, skin
193 temperatures obtained from IRTs and estimated RT using the ANN model proposed. Both breed
194 type and THI were also included in PCA analysis.

195 **Results**

196 The THI values calculated for the study period (2 weeks) ranged from 19.2-21.8 for TN
197 conditions to 26.5-35.3 for HS treatment (Fig 3A) (Joy et al., 2020b).

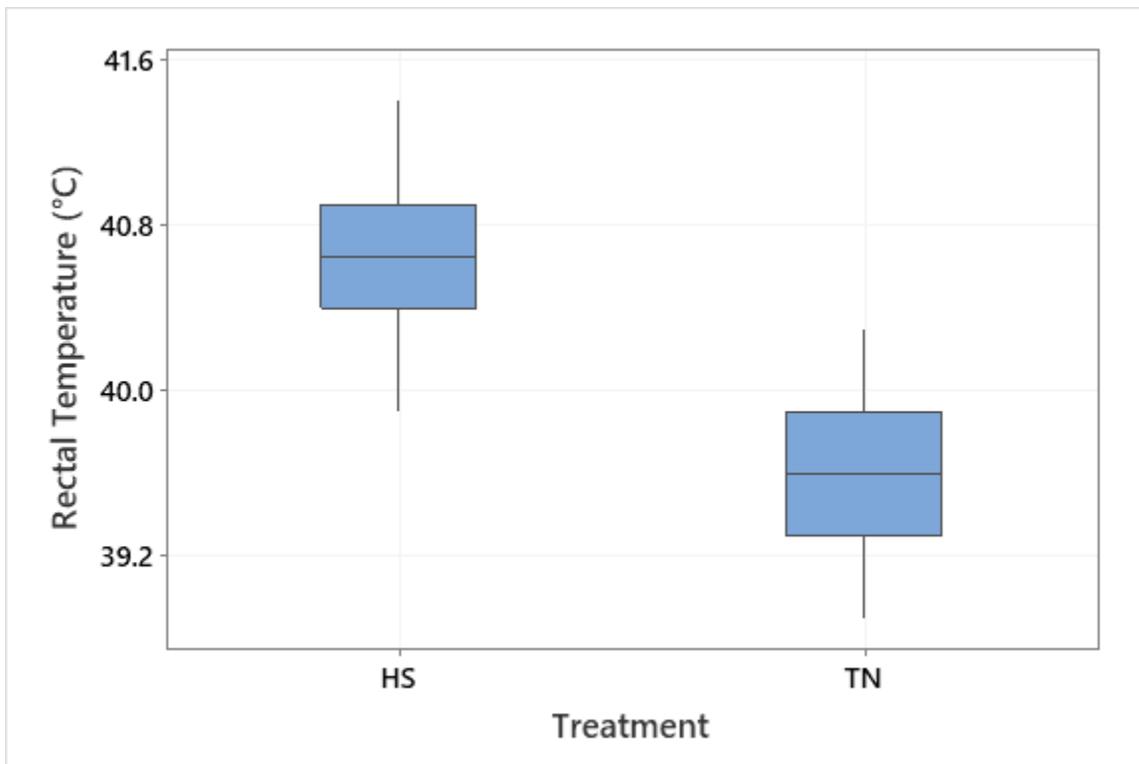
(A)



198

199

(B)



200

201 **Fig 3.** Boxplots showing (A) THI and (B) RT values for both TN and HS treatments over two
 202 weeks

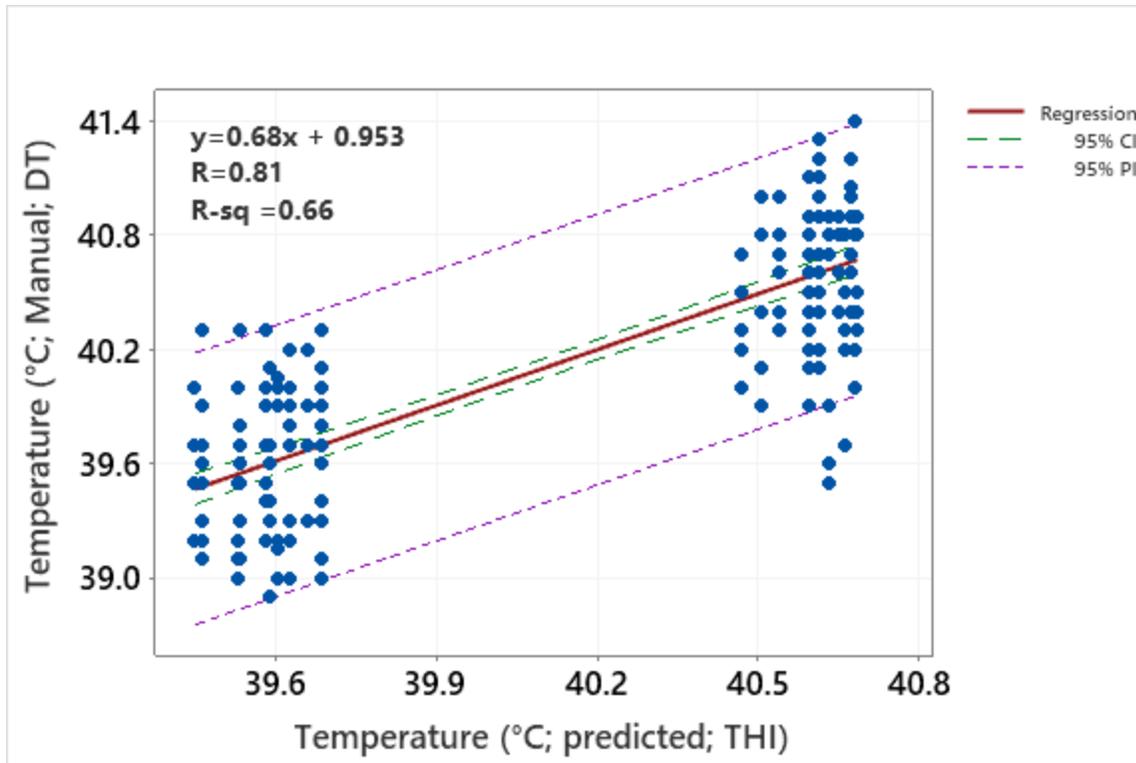
203 Table 1 shows the correlations between THI, RT, and skin temperatures (forehead, eye, ear,
 204 nostril, and face region) obtained from IRT. Overall, skin temperatures obtained from IRT showed
 205 a positive correlation ($P<0.01$) with THI and RT in the afternoon. Among various ROIs selected,
 206 FH T ($R=0.84$; $P<0.01$) and eye T ($R=0.68$; $P<0.01$) had the highest correlation with THI.
 207 Significant positive correlations ($P<0.01$) were also obtained between RT and FH T, nostril T and
 208 eye T ($R=0.68, 0.58, 0.52$ respectively; Table 1). Since, sheep head surface temperatures, measured
 209 using IRT, had a positive correlation ($P<0.01$) with RT, they were all considered as inputs for model
 210 development.

211 **Table 1.** Pearson correlation coefficients between THI, RT and skin temperatures (Forehead,
 212 eye, ear, nostril and face) in sheep (n=192)

	THI	RT	FH T	Eye T	Ear T	Nostril T	Face T
THI	1						
RT	0.81**	1					
FH T	0.85**	0.68**	1				
Eye T	0.60**	0.67**	0.66**	1			
Ear T	0.56**	0.65**	0.65**	0.78**	1		
Nostril T	0.62**	0.55**	0.75**	0.72**	0.74**	1	
Face T	0.68**	0.50**	0.46**	0.72**	0.73**	0.48**	1

213 THI: Temperature-humidity index; RT: rectal temperature; FH T: fore-head temperature; Eye T:
 214 Eye temperature; Ear T: Ear temperature; Nostril T: Nostril temperature; Face T: Face temperature.
 215 **correlation differs ($P<0.01$) from zero.

216 Figure 4 shows the linear regression between RT measured manually and predicted using
 217 Bayesian training algorithm with THI as the input variable. As depicted, the correlation and
 218 determination coefficients were relatively low ($R=0.81, R^2= 0.66$; $P<0.001$) with $MSE=0.16$ and
 219 slope= 0.68 .



220

221 **Fig 4.** Comparing results from rectal temperatures measured manually using a digital thermometer
 222 vs. temperature predicted using linear regression fitted to THI-RT data. Abbreviations: R:
 223 Correlation, R^2 : Coefficient of determination; CI: confidence interval; PI (prediction interval).
 224

224

225 Table 2 shows the statistical data of the best models developed using various training
 226 algorithms. Overall, correlations from all models were highly significant with $P < 0.001$. The scaled
 227 conjugate gradient algorithm with 7 neurons had the lowest R (0.87) and highest MSE (0.09; Table
 228 2). However, models developed using Levenberg-Marquardt and BR algorithms with 10 neurons
 229 showed higher overall performance with $R > 0.90$. Comparatively, BR showed the best performance
 230 with the least MSE (< 0.05) with R values being consistently over 0.90 for all stages. Also, the BR
 231 model had the highest slope close to unity ($b = 0.83$) when compared to others. Hence, according
 232 to these results, BR was selected as the best training algorithm for this network.
 233

233

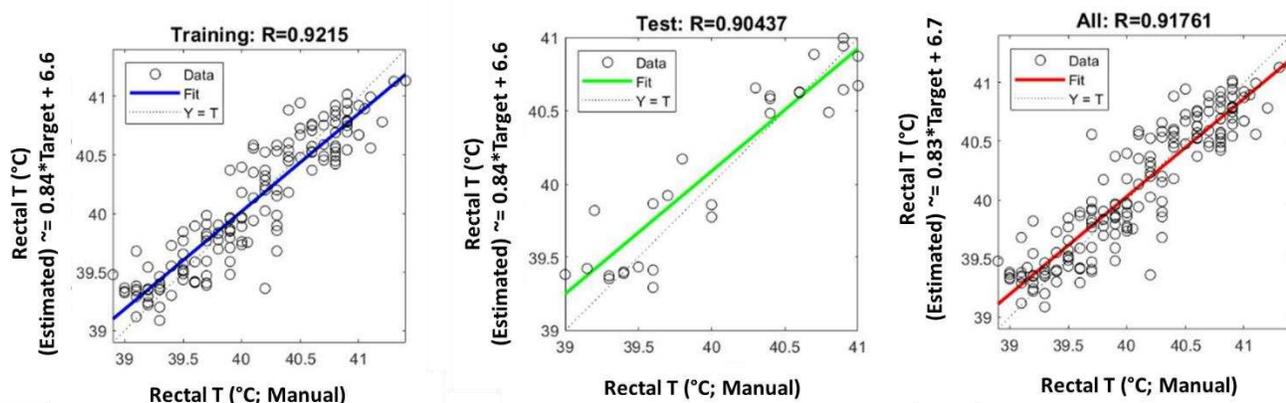
234 **Table 2.** Statistical results of the models developed using different algorithms.

Algorithm	Neurons	Stage	R	Slope (b)	MSE
Levenberg-Marquardt	10	Training	0.90	0.78	0.06
		Validation	0.93	0.77	0.09
		Testing	0.91	0.88	0.07

		Overall	0.90	0.79	0.05
Bayesian	10	Training	0.92	0.84	0.03
Regularization		Validation	-	-	-
		Testing	0.90	0.84	0.05
		Overall	0.92	0.83	0.02
Scaled Conjugate	7	Training	0.87	0.77	0.09
Gradient		Validation	0.87	0.76	0.09
		Testing	0.88	0.82	0.08
		Overall	0.87	0.78	0.07

235 R- correlation coefficient; MSE- mean square error.

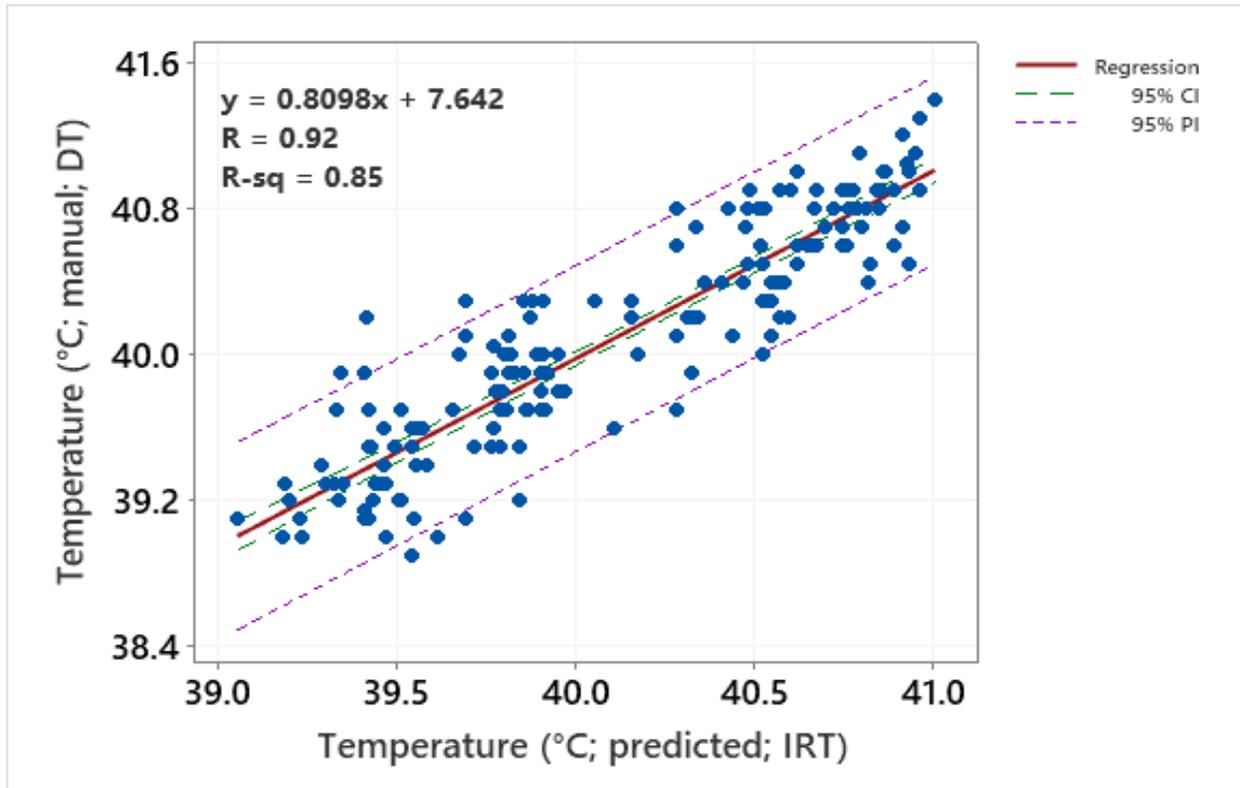
236 Figure 5 shows the model's performance, with 10 hidden neurons, on the training, testing and
 237 overall data, which was trained with the BR algorithm. Better performance was found for the
 238 training stage (R=0.92; slope=0.84) while for the testing R= 0.90 and slope=0.84 and overall model
 239 had an R= 0.92, ($R^2=0.85$) and slope=0.83. The weights and biases in this model's hidden and output
 240 layers are available as supplementary material.



241
 242 **Fig 5.** Comparison of the estimated and measured RT in training, test, and overall datasets, the
 243 neural network model was trained with the Bayesian Regularization algorithm to estimate rectal
 244 temperature from infrared thermal images (IRTIs) displaying the correlation coefficient (R) and
 245 95% confidence bounds.

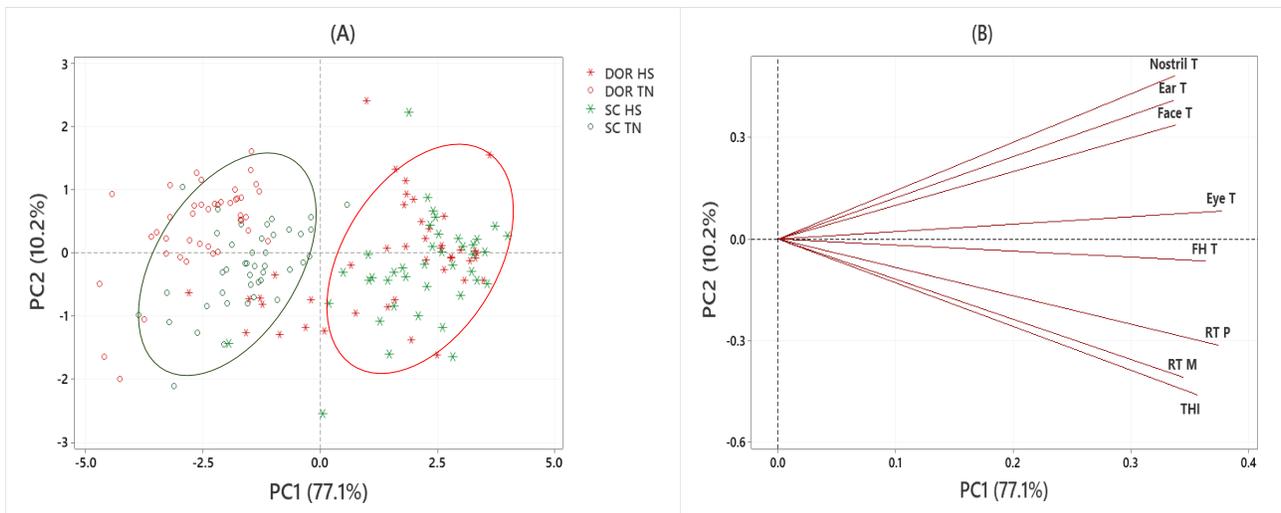
246

247 Figure 6 shows the linear regression model between RT measured manually and predicted
248 temperature from the ANN model using IRT analysis. The linear regression model showed a high
249 correlation ($R=0.92$) and coefficient of determination ($R^2= 0.85$) and was statistically significant
250 ($P<0.01$) with slope=0.81. The model also showed 5.2% outliers (10 out of 192) based on the 95%
251 confidence intervals.



252
253 **Fig 6.** Linear regression model comparing results from rectal temperatures measured manually
254 using a digital thermometer vs. temperature from the infrared thermal image analysis (IRT).
255 Abbreviations: R^2 : Coefficient of determination; CI: confidence interval; PI (prediction interval).

256 Figure 7 shows the PCA comparing RT measured manually (RT M), RT predicted using model
257 (RT P), THI and skin temperatures of various ROIs obtained from IRT analysis for two sheep breeds
258 (Dorper and SC) under both TN and HS conditions in different days of measurements. The PCA
259 described 87.3% of the total data variability with 77.1% and 10.2% for PC1 and PC2, respectively.
260 The results showed that RT M and RT P were closely related (Fig 7B). There was a clear difference
261 in temperatures between HS and TN treatments such that the HS group showed higher THI and
262 temperature values in both the breeds (Fig 7A).



263 **Fig 7.** Principal components analysis: (A) Score plot and (B) Loading plot of data measured with
 264 (1) manual techniques (i) RT M: rectal temperature manual, (2) infrared thermal images (i) Nostril
 265 T: nostril temperature, (ii) Face T: face temperature, (iii) FH T: forehead temperature, (iv) Ear T:
 266 ear temperature (v) Eye T: eye temperature and those using the machine learning model (i) RT P:
 267 rectal temperature predicted from each day of the control and heat stress treatments for both Dorper
 268 and second cross sheep breeds.
 269

270 Discussion

271 Infrared thermography has been used as a non-invasive remote sensing tool to assess
 272 changes in heat transfer and blood flow in ruminants via detecting slight variations in body
 273 temperature (Paim et al., 2012). Our study proposed selecting IRT of specific body locations such
 274 as the forehead, eye, muzzle, ear, and face, for estimating RT in sheep. The results demonstrated
 275 that THI and IRT, along with machine learning models, could help in the automated measurement
 276 of RT/body temperature in sheep to assess HS.

277 In recent years, with the growing awareness and interest of consumers in animal welfare,
 278 there is an urgent need to develop non-invasive measures of stress in animals to promote animal
 279 welfare. Producers and consumers are paying more attention to farm management conditions
 280 (Bittner et al., 2021) particularly on the procedures that prevent pain and discomfort. Thus, non-
 281 invasive techniques of measuring HS in ruminants such as IRT (Paim et al., 2012; McManus et
 282 al., 2015) and estimation of fecal cortisol metabolites (Rees et al., 2016) are gaining importance,
 283 but it demands further research for the optimization of the methods. In this current study, we used
 284 two different sheep breeds: 1) Dorpers with loose white hairy fleece with the head being free of
 285 wool and 2) SC breeds that had a chalky white dense fleece with a less wool in the forehead region
 286 (Joy et al., 2020b). Special care was taken while measuring FH T in SC to avoid wool interruptions.

287 Positive correlations among the IRTs, THI, and RT indicated that these variables were altered with
288 a similar trend such that elevating THI corresponded to increasing RT and thermographic
289 measurements (FH T, ear T, eye T, nostril T, and face T). The FH T and eye T showed the highest
290 correlation with THI, suggesting that real-time monitoring of these regions may help to signify
291 potential impacts of the increased environmental temperature on the thermoregulatory responses
292 of sheep. This is in accordance with findings of previous studies (Daltro et al., 2017; Peng et al.,
293 2019) that also indicated THI was highly correlated with FH T, and eye T in cattle. Also, RT
294 showed a high correlation with FH T, eye T and nostril T. Measuring IRT of the eye region has
295 been established as the best proxy of core body temperature in cattle (Daltro et al., 2017). There
296 was also a moderate correlation between IRT of eyes and RT has been established in cattle (Gloster
297 et al., 2011). Interestingly, nostril T showed a moderate correlation with the RT in sheep. This
298 could be because the nose region in our study has more hairless skin exposed in sheep along with
299 a large number of blood vessels (Dawes and Prichard, 1953), which allows measuring changes in
300 blood flow and heat transfer more accurately.

301 Generally, the best ANN model is indicated by high correlation coefficient values (R) and
302 training performance (MSE). To ensure that there is no overfitting, R and MSE values of the
303 training and testing steps should be close to each other (Steyerberg et al., 2010). Considering a
304 part of the data for validation before testing helps to reach this goal (Gonzalez Viejo et al., 2019).
305 Although BR does not have a validation stage in particular, it has an implemented cross-validation
306 which randomly divides the training data into training and validation and automatically trains the
307 data several times until reaching the optimal combination of errors and weights using different sets
308 of training data. This means the BR is more robust than the other algorithms (Taheri et al., 2021).
309 Although THI showed a high correlation with RT, the observed correlations and determination
310 coefficients of the model developed using THI as an input for predicting RT were relatively low.
311 On the other hand, inclusion of IRT measurements as inputs improved the model performance and
312 accuracy. Based on the performance of the three training algorithms applied for the model
313 development, it can be concluded that BR with a NN structure of one hidden layer, containing 10
314 neurons with a tan-sigmoid transfer function was the most accurate for estimating RT using the
315 IRT technique. This is based on the highest correlation coefficient ($R=0.92$), best performance
316 ($MSE= 0.02$) for overall data, good fit within confidence bounds with a low number of outliers
317 (5.2%), overall slope close to 1 ($b=0.81$), and fewer signs of overfitting. Similarly, Gonzalez Viejo

318 et al. (2019) and Taheri et al. (2021) proved that BR was the most effective algorithm for training
319 the neural network. The BR is a back-propagation algorithm based on Levenberg-Marquardt
320 optimization, which works based on calculating the second derivatives of a cost function with an
321 additional term for updating weights and biases (Tiwari et al., 2013) and minimizes a combination
322 of squared errors and weights. Several studies stated some of the important advantages of BR over
323 other training algorithms such as good generalization for small datasets (Kayri, 2016), avoids
324 overfitting (Bruneau and McElroy, 2006), and does not require a separate validation stage
325 (Gonzalez Viejo et al., 2019). However, this algorithm is slower and requires more memory than
326 the Lavenberg-Marquardt training function (Tiwari et al., 2013; Taheri et al., 2021).

327 As expected, HS increased RT and IRTs in both sheep breeds, implying compromised
328 thermoregulatory mechanisms in sheep exposed to high THI (Chauhan et al., 2016; Joy et al.,
329 2020b). Further, there was a close association between THI, RT M and RT P obtained from PCA
330 analysis which further indicates the acceptable precision of the proposed model in predicting RT
331 of sheep under different THI conditions. Also, this model, if implemented, would be very useful
332 in the remote monitoring of a large number of animals (i.e., at flock level), where the image/video
333 is taken of the flock, but the data is analyzed for each animal individually (using image recognition
334 software tools). As indicated before, the positioning of cameras, environmental conditions and
335 excessive motion of animals could have an impact on applying these techniques under large scale
336 conditions. Thus, further research is required to investigate the feasibility of implementing these
337 techniques on at a flock scale and to reduce the impact of environmental factors on the accuracy.
338 Additionally, the established model could be implemented in an intelligent interface to monitor
339 the real-time sheep temperature on farms, which would allow a reduction in time and handling
340 cost for producers while screening the stressed animals. Moreover, the implementation of artificial
341 intelligence (AI) for automated data gathering using IRT images and video analysis will extend a
342 reliable and completely automated system to identify stressed sheep during summer.

343 **Conclusions**

344 Infrared thermography measurements in sheep offer a reliable, precise, and non-invasive
345 technique to measure HS. Among the ROIs studied using IRT, FH T and eye T showed the highest
346 correlation with THI, and FHT, eye T, and nostril T were strongly correlated with RT. Further, a
347 combination of IRT and machine learning techniques, namely ANN, was applied to model the RT

348 in sheep. The best algorithm for the specific model developed in this current study was the BR
349 with one hidden layer, containing 10 neurons with tangent sigmoid transfer function. The model
350 showed the highest correlation ($R=0.92$) and least error ($MSE=0.02$). Therefore, it is concluded
351 that IRT and machine learning could be used as a rapid and cost-effective technique to monitor
352 real-time body temperatures in sheep for early detection of HS with minimal restraint to improve
353 sheep welfare.

354 **Conflict of interest**

355 The authors declare no conflict of interest.

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