

A device to improve the Schleger and Turner method for sweating rate measurements

Alfredo Manuel Franco Pereira · Alexandre Alves ·
Paulo Infante · Evaldo A. L. Titto · Flávio Baccari Jr. ·
J. A. Afonso Almeida

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Abstract The objective of this study was to test a device developed to improve the functionality, accuracy and precision of the original technique for sweating rate measurements proposed by Schleger and Turner [Schleger AV, Turner HG (1965) Aust J Agric Res 16:92–106]. A device was built for this purpose and tested against the

original Schleger and Turner technique. Testing was performed by measuring sweating rates in an experiment involving six Mertolenga heifers subjected to four different thermal levels in a climatic chamber. The device exhibited no functional problems and the results obtained with its use were more consistent than with the Schleger and Turner technique. There was no difference in the reproducibility of the two techniques (same accuracy), but measurements performed with the new device had lower repeatability, corresponding to lower variability and, consequently, to higher precision. When utilizing this device, there is no need for physical contact between the operator and the animal to maintain the filter paper discs in position. This has important advantages: the animals stay quieter, and several animals can be evaluated simultaneously. This is a major advantage because it allows more measurements to be taken in a given period of time, increasing the precision of the observations and diminishing the error associated with temporal hiatus (e.g., the solar angle during field studies). The new device has higher functional versatility when taking measurements in large-scale studies (many animals) under field conditions. The results obtained in this study suggest that the technique using the device presented here could represent an advantageous alternative to the original technique described by Schleger and Turner.

A. M. F. Pereira (✉)
Instituto de Ciências Agrárias Mediterrânicas,
Universidade de Évora,
Apartado 94,
7000-554 Évora, Portugal
e-mail: apereira@uevora.pt

A. Alves
Universidade de Évora,
Apartado 94,
7000-554 Évora, Portugal
e-mail: aalves@uevora.pt

P. Infante
Departamento de Matemática, Universidade de Évora,
Apartado 94,
7000-554 Évora, Portugal
e-mail: pinfante@uevora.pt

E. A. L. Titto
FZEA-Universidade de São Paulo,
São Paulo, Brazil
e-mail: titto@usp.br

F. Baccari Jr.
UNESP,
Botucatu, Brazil
e-mail: bacjr@uol.com.br

J. A. A. Almeida
Instituto de Ciências Agrárias Mediterrânicas,
Departamento de Zootecnia, Universidade de Évora,
Apartado 94,
7000-554 Évora, Portugal
e-mail: afonso@uevora.pt

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Introduction

The importance of evaporative thermolysis, mainly sweating rate, for heat tolerance has been stressed by several researchers (McLean 1963a, b; Finch et al. 1982; Gatenby 1986). Most knowledge concerning sweating in cattle has

been derived mostly from studies in climatic chambers, under well-controlled environments. However, such studies cannot accurately simulate the animal's reactions under field conditions (Murray 1966; Pereira et al. 2008) because solar radiation increases the temperature of the epidermis, which has a direct effect on sweating rate (Murray 1966).

The different methods available for sweating rate measurements under field conditions (McLean 1963a; Murray 1966; Amakiri and Mordi 1975; Gatenby 1986) have several limitations, the major one being related to short-term fluctuations in environmental conditions, which make it difficult to obtain complete and precise information (McDowell et al. 1953). The variability of sweating rate data obtained by different methods limits their widespread utilization in the area of physioclimatology (Schleger and Turner 1965). The more accurate and precise methodologies for sweating rate measurements are used mostly under laboratory conditions, but their utilization under field conditions and in large scale studies is difficult and expensive (Murray 1966; Finch et al. 1982). Practical inference regarding differences in sweating rates between breeds can be gained only from studies under field conditions on large numbers of animals (Amakiri and Mordi 1975; Schleger and Turner 1965).

The great advantage of the Schleger and Turner (1965) cobalt chloride colorimetric method in field studies is its simplicity and speed, allowing a significant number of animals to be tested in a short time interval, while environmental conditions remain relatively constant. The accuracy of this method is quite acceptable but the repeatability of sweating rates so measured, while highly significant and adequate to reveal various sources of variance, is lower than desirable.

The major source of variation with the Schleger and Turner method is due to the inevitable sliding of the filter paper discs on the skin, mainly when dealing with more reactive animals and when measurements take longer due to low sweat production.

The objective of this study was to test a device developed to improve the functionality, accuracy and precision of the original technique proposed by Schleger and Turner (1965).

Material and methods

The Schleger and Turner method (1965) is based on the utilization of filter paper discs impregnated with cobalt chloride. The time taken for the cobalt chloride color change from blue to pink allows measurement of the sweating rate. The original method utilises adhesive tape to fix the filter paper discs onto the animal's skin. In this study all the original procedures were followed, except that the

filter paper fixation (which represents the main source of variation) was substituted by the utilization of a device.

Description of the device

Figure 1 presents a plan (I), elevation (II) and perspective (III) views of the device. The device was built from two transparent acrylic plastic plates of 20.0 cm² (10.0×2.0 cm; plate 1) and 12.0 cm² (6.0×2.0 cm; plate 2). Plate 2 was glued in a central position on plate 1 to increase thickness, which was needed to provide better adhesion of the paper discs to the skin and to minimize lateral slide movements. Two 2.0×2.0 cm strips of Velcro were glued on the free ends of plate 1 to attach the device to the surface of the animal (wide arrows represent the direction of movement to attach the device to the animal's hide). Three paper discs with cobalt chloride, prepared as described by Schleger and Turner (1965), were mounted on a double adhesive face of a 4.0×2.0 cm cellulose strip. The adhesive strip was fixed on plate 2 (small black arrows) immediately prior to attachment of the device to the animal's hide.

Experimental procedure

To compare results obtained using the original technique described by Schleger and Turner (1965) to those obtained with the new technique using the described device, six heifers of the Mertolenga breed (*Bos taurus* portuguese native cattle breed), with initial average body weights of 235.0±28.8 kg, were subjected to four thermal levels (TL; Table 1) in a 45 m² climatic chamber.

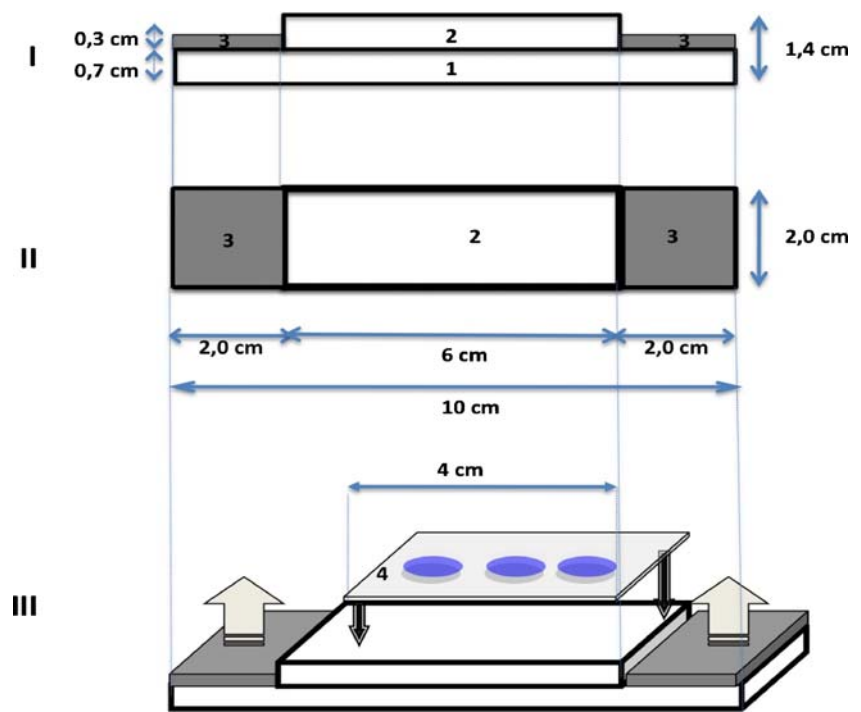
The characteristics of the climatic chamber are as described by Pereira et al. (2008).

Two months before the experiment, all animals were handled, head halted and trained, in order to decrease reactivity and to obtain a better adjustment to the new management and environment. The heifers were kept in individual stalls (3.0×1.1 m) and restrained by a head halter. Feed and water were provided ad libitum. Fresh food was provided in excess twice per day at 0830 hours and 1630 hours through an automatic waterer.

The experiment lasted 17 days: 5 for adaptation [with air temperature (T_a) and relative humidity (RH) kept at 20°C and 56%, respectively] and 12 days for data collection (3 days for each TL). For each technique, a total of 432 sweating rate (SR) measurement was performed, with 108 SR measurements for each TL.

The mid-trunk region was chosen for SR measurements because the consistency of results is higher in the forequarters, and the mid-trunk region is known to better represent the average SR of the whole animal (McLean 1963b; Pan et al. 1969).

Fig. 1 Scheme of the device: *I* Plan, *II* elevation, *III* perspective view. Components: *1* Plate 1, *2* plate 2, *3* Velcro strips, *4* cellulose strip with paper discs. *Wide arrows* Direction of movement to attach the device to the animal' hide. *Black arrows* indicate fixation of the cellulose strip to plate 2



At the middle of the trunk, between the 10th and 11th ribs, two vertical contiguous hide patches, 25.0 cm² each (one for the original technique and the other for the device), were close-clipped and brushed free of dust. The hair coat was trimmed (5.0 cm²) at the two edges of the patch for the device (D) before gluing (2-ethyl cyanoacrylate) two 4.0 cm² pieces of Velcro (Fig. 2) to the hide to assure a good adhesion to the Velcro strips of the device.

Sweating rates were measured simultaneously either by the original technique of Schleger and Turner (1965) or by the technique using the newly built device. Sweating rates were calculated and reported as proposed by Schleger and Turner (1965).

All measurements were performed simultaneously by the same operator (A.M.F.P.) everyday at 1100 hours, 1230 hours and 1400 hours. Whenever results seemed erroneous, all findings were discarded and new measurements were made. Data were discarded only when the color did not change uniformly on the same filter paper disc, or if the final pink color coexisted with some blue spots.

Statistical analysis

The following statistical procedures were adopted. First, normality (Kolmogorov-Smirnov test) and homocedasticity (Levene test) were tested; all data exhibited normal distribution and homocedasticity.

Paired comparisons were used between techniques. After running this test, the lowest *P*-value obtained was 0.218, which confirms the randomness of the samples.

Pitman's *t* statistic was used to test the null hypothesis in order to ensure that the two sets of data arise from populations with identical variances:

$$T = \frac{(F - 1)\sqrt{n - 2}}{2\sqrt{F(1 - \rho^2)}} \cap t_{n-2}$$

where *F* is the ratio of the larger variance to the smaller variance, *n* is the number of pairs of scores and ρ is the correlation between the scores in the traditional and the device techniques.

Table 1 Thermal levels (TL) in the four periods of the experiment

Thermal level	Temperature (°C)	Relative humidity (%)	Temperature cycle
TL 1	28	46	No cycle
TL 2	30	44	No cycle
TL3	35	41	0800–1600 hours
	28.5	46	1600–0800 hours
TL 4	39	37	0800–1600 hours
	28.5	46	1600–0800 hours

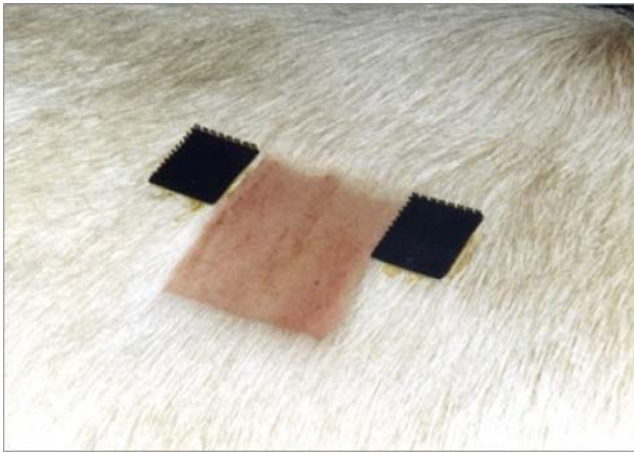


Fig. 2 Two pieces of Velcro stuck to trimmed hair of the animal at the edges of the patch

Dispersion of the values was studied by descriptive statistics and outlier analysis. Pearson correlation coefficients were also calculated.

Based on the assumption that the source of variation could be present within each day and between days within each TL, the ANOVA used was a repeated measures model, with two fixed factors—the technique of sweat measure and the TL—and the day and the hour within the day as repeated measures.

The traditional and device techniques were also compared by repeatability and reproducibility analysis. Repeatability—the inherent precision of the equipment measurement—is the variability of the measurements obtained by an instrument on the same item and under the same conditions. Reproducibility is the variability of the measurement system caused by differences in operator behavior or measurement conditions, and represents the degree of agreement between the results of measurements of the same item under different conditions. As there is no way to avoid the source of variation represented by the operator, subjectivity was minimized by using the same operator, and the TL was considered the factor to determine the accuracy of the device through the several levels of this factor. The gauged repeatability, reproducibility and animal-to-animal variation for the original technique and for the technique using the device, was then calculated by ANOVA (cross study method), using two-way random factors with replicates, where factor 1 is the animal and factor 2 is the TL. Calculations were performed as follows:

$$\sigma_{\text{Repeatability}}^2 = \text{MSE}; \sigma_{\text{Reproducibility}}^2 = \frac{MSB - MSAB}{nb} + \frac{MSAB - MSE}{n}$$

$$\sigma_{\text{Gauge}}^2 = \sigma_{\text{Repeatability}}^2 + \sigma_{\text{Reproducibility}}^2$$

The repeatability and reproducibility gauge (R&R) was performed as follows:

$$\begin{aligned} \text{Repeatability} &= 6 * \sqrt{\text{MSE}}; \text{Reproducibility} \\ &= 6 * \sqrt{\frac{MSB - MSAB}{na} + \frac{MSAB - MSE}{n}} \end{aligned}$$

Where MSE is the mean square of the error; MSA the mean square of factor 1; MSB the mean square of factor 2; MSAB the mean square of the interaction; b the number of thermal levels and n the number of measurements in each animal within each level.

All statistical analysis procedures were performed using the SPSS 15.0 software package

Results

Utilization of the device revealed no problems. The glue utilized guaranteed tight fixation of the Velcro to the hide without any observable allergic reaction. The two acrylic plates of the device had no adverse influence on the visibility of the color change of the filter paper. The Velcro fixed on the device and hair assured maintenance of the position of the device on the surface of the animal during the entire experimental period. There was no visible lack of adherence of the filter paper to the epidermis, despite the sweating and movements of the animal. In contrast, with the original technique, due to the sweat production, there was a lack of adherence of the adhesive tape during most of the experimental period, making it necessary to press the tape in place with fingers until the conclusion of each measurement. Contact of the person's hand against the animal surface almost always triggered animal movement resulting in lateral sliding of the filter paper discs and an overestimation of the evaluated epidermis surface. With the original technique, there were a higher number of doubtful results and 42 additional measurements (22.4%) had to be made, while with the utilization of the device results were more consistent and only 7 observations had to be repeated.

There were no significant differences between the results obtained by each technique for the different TLs (Table 2).

For all TLs, the amplitude of the SR measured with the original technique was higher (Fig. 3). The correlation

Table 2 Means and standard deviations of the sweating rates (SR) measured by both techniques in all animals for each TL

	TL1	TL3	TL3	TL4
Original	62.4±11.5	83.4±19.5	115.1±29.8	142.4±32.7
Device	59.4±8.5	79.1±16.8	111.4±27.6	140.6±31.7

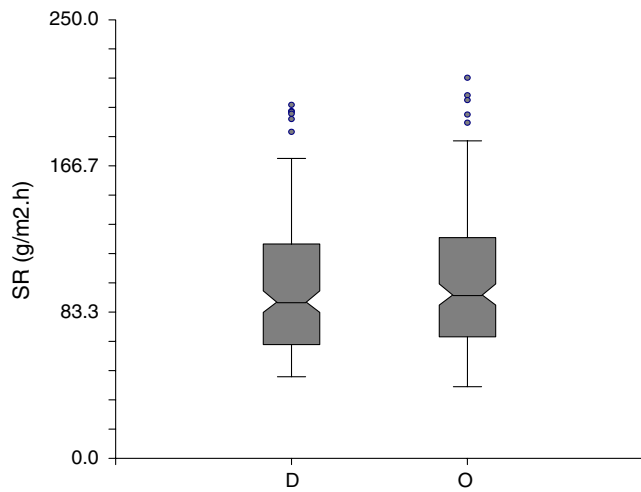


Fig. 3 Median and total outliers of sweating rate (SR) data obtained with the device developed here (D) and the original technique (O) of Schleger and Turner (1965)

coefficients of data obtained with both techniques were highly correlated (0.823 for TL 1 to 0.994 to TL 4).

Considering all SR for all 4 TL, there was a higher value dispersion relative to the median with the original method (Fig. 3). With both techniques the SR variability was higher when the thermal conditions increased (Figs. 4, 5); nevertheless the higher dispersion was always associated to the original technique.

The number of outliers was low for both techniques, probably due to the repeated measurements done whenever the first ones seemed doubtful.

The SR variability between animals was high for all TLs, being higher when measured with the original technique (Figs. 6, 7, 8, and 9). The differences were higher at the lower TLs, because the contact times of the filter paper

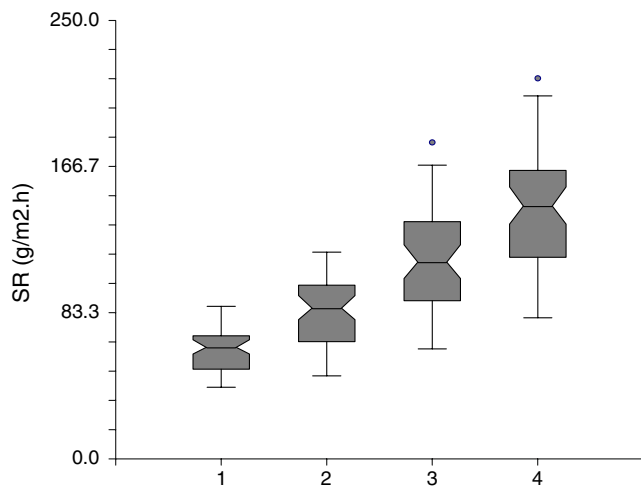


Fig. 4 Median and outliers of SR measured by the original technique at each TL

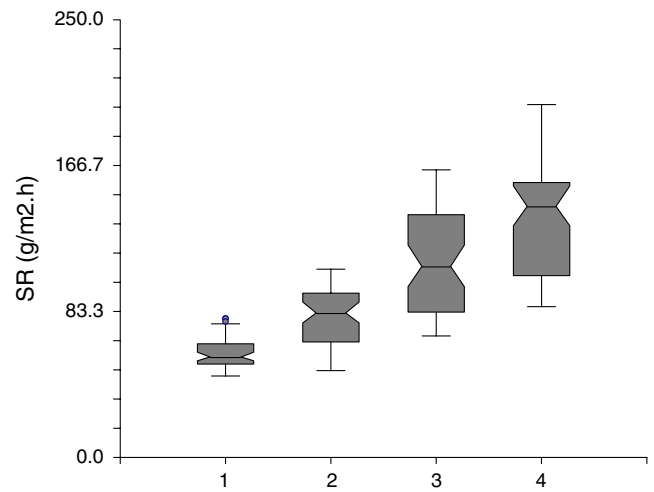


Fig. 5 Median and outliers of SR measured by the device technique for each TL

discs with the epidermis were higher. The higher variability of the results obtained with the original technique can be explained by the interaction between animal reactivity, which results in the sliding of the filter paper discs, and duration of the measurements.

The accuracy of each method was not affected by the different TLs, as there were no significant differences in the reproducibility of the two techniques; however, the repeatability of the data obtained with the device was significantly lower ($P < 0.01$; Table 3), corresponding to a lower variability and consequently to a higher level of precision.

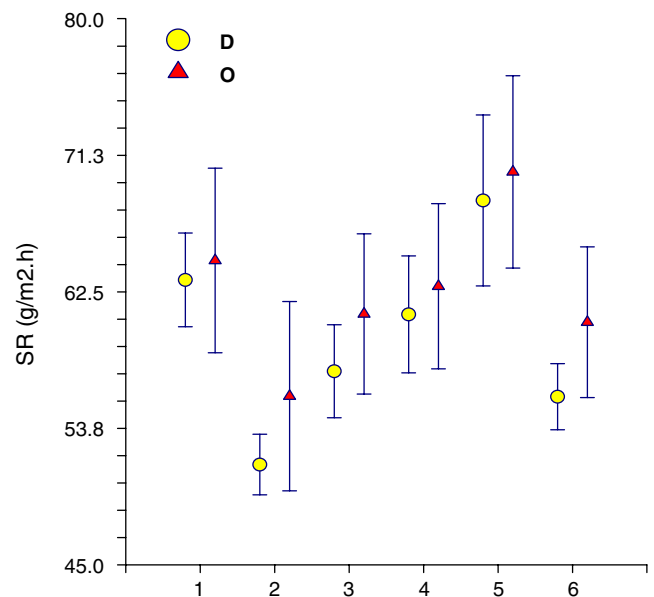


Fig. 6 Means and standard deviations of the SRs measured by both techniques in all animals at TL 1

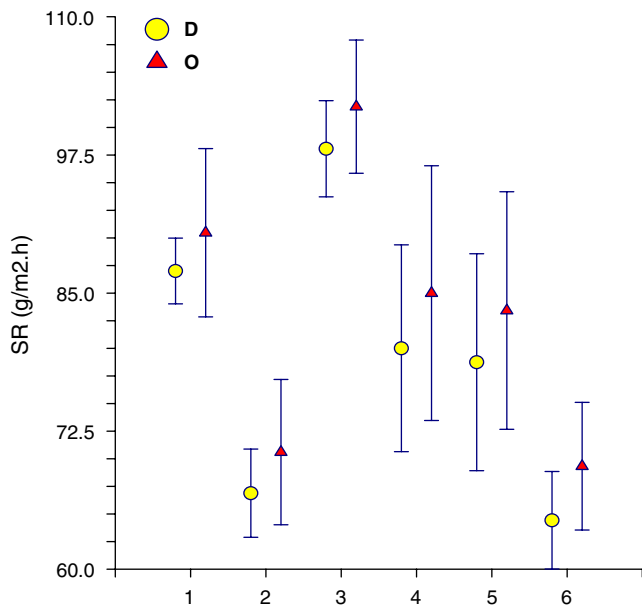


Fig. 7 Means and standard deviations of the SRs measured by both techniques in all animals at TL 2

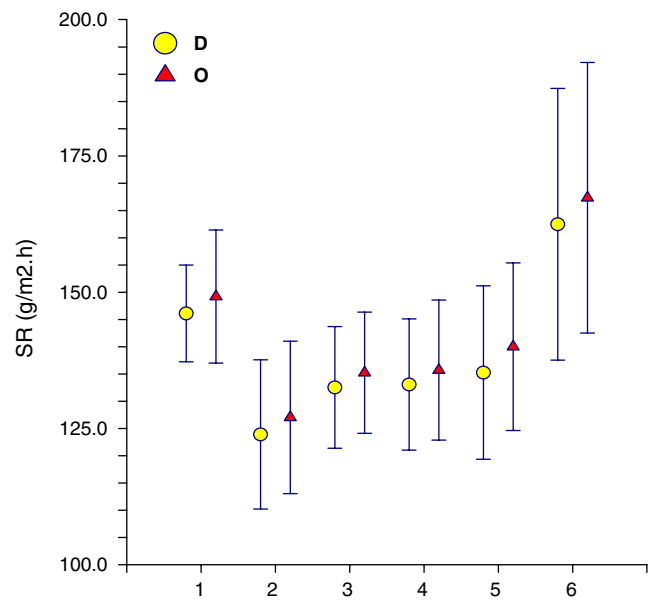


Fig. 9 Means and standard deviations of the SRs measured by both techniques in all animals at TL 4

Discussion

One of the main sources of variation in SR measurements with the original Schleger and Turner (1965) technique is the sliding of the filter paper discs on the epidermal surface, mainly under conditions of heavy sweat production in the more reactive animals. When the cobalt chloride-impregnated filter paper discs slide on the epidermis, they are influenced by a higher moistened surface area, leading to an over-estimation of SR, as demonstrated in this study.

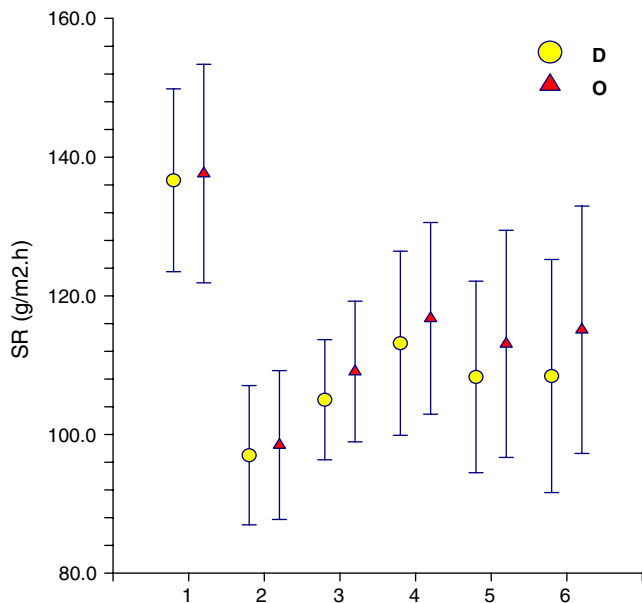


Fig. 8 Means and standard deviations of the SRs measured by both techniques in all animals at TL 3

By utilizing the device, filter paper adherence to the epidermis is not influenced by animal movements, depending only on the Velcro’s adherence capacity. Thus, there is no need for physical contact between the operator and the animal to maintain the filter paper discs in position.

There are two direct advantages of using the device: (1) the pressure on the filter paper and epidermis is constant throughout the measurement period, and (2) the animals stay quieter, in particular the more reactive ones. These advantages could not be evaluated directly in this study because the two techniques were assessed simultaneously in all animals and there was lack of adherence of the adhesive tape with the original technique. However, later measurements (unpublished data) confirmed that the animals stayed much quieter during measurements only with the device.

The excellent adherence of the device allows simultaneous measurements in several animals. During complementary studies (unpublished data) it was possible to make simultaneous measurements with six animals without interfering with the precision of the time recording. The possibility of having more measurements in a given period

Table 3 Repeatability and reproducibility (R&R) for the original (O) and device (D) techniques. In each column, mean values with different superscript letters are significantly different ($P < 0.01$)

Method	Total R&R guage	Repeatability	Reproducibility
O	40.59	12.44 ^a	38.63 ^a
D	39.19	3.80 ^b	39.00 ^a

of time, and the increased precision of the observations are the main advantages of the device; in addition, the error associated with temporal hiatus (e.g., the solar angle during field studies) is diminished (Schleger and Turner 1965; Finch et al. 1982). Using the device, the time for filter paper disc color change was much more homogeneous, thus reducing significantly the need for repetitions. With the original technique, many more repetitions were needed, mainly either during periods of lower temperature or when the animals were more reactive.

However, some limitations remain with both techniques. There is a certain degree of subjectivity in the evaluation of the time for color change of the cobalt chloride-impregnated filter paper discs, mainly during profuse sweating. However, the device behaved more homogeneously, exhibiting higher precision and the same degree of accuracy throughout several TLs, as evidenced by the repeatability and reproducibility gauge.

Conclusions

Higher precision and a lower repeatability of SR measurements were associated with utilization of the described device, as compared to the measurements obtained with the original Schleger and Turner technique. The accuracy of SR measurements with the device was also higher.

Utilization of the device described here could have several advantages: easy construction and low cost; ease of utilization; no requirement for permanent physical contact between the operator with the animal; simultaneous measurements in several animals is possible. Finally, the device has higher functional versatility when measurements are made in large-scale studies (many animals) under field conditions.

The results obtained in this study suggest that the technique utilizing this device represents an advantageous alternative to the original technique described by Schleger and Turner (1965).

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