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RESEARCH PAPER
https://doi.org/10.1071/AN24165

ANIMAL PRODUCTION SCIENCE

Use of two different methods for glucose determination in sheep under normoglycemic, hypoglycemic, and hyperglycemic conditions: an evaluation of practical diagnostic methods in ovines

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Handling Editor:
Sathya Velmurugan

Received: 28 April 2024
Accepted: 21 June 2024
Published: 16 July 2024

Cite this: Chenard MG *et al.* (2024) Use of two different methods for glucose determination in sheep under normoglycemic, hypoglycemic, and hyperglycemic conditions: an evaluation of practical diagnostic methods in ovines. *Animal Production Science* **64**, AN24165. doi:10.1071/AN24165

ABSTRACT

Context. Animals can present abnormal blood glucose concentrations because of various diseases or pathological conditions, stress, or hunger. Early diagnosis prevents complications, economic losses, and death. The use of a portable glucometer (PGM) has been shown to be a good, simple, and practical alternative method with good precision and accuracy for assessing blood glucose in humans and companion animals. **Aims.** The objective of this work was to evaluate the accuracy and reliability of a portable glucometer (PGM) for assessing glycemia in normoglycemic, hypoglycemic, and hyperglycemic sheep. **Methods.** Blood glucose was evaluated in 60 normoglycemic, 15 hypoglycemic, and 15 hyperglycemic sheep. Blood samples were collected and analysed within 2 h by using PGM and the enzymatic method (EM). Each test was evaluated for sensitivity, specificity, and the area under the receiver operating characteristic (ROC) curve for two cutoff points, namely, one for hypoglycemia and the other for hyperglycemia. **Key results.** The results of the Kolmogorov–Smirnov test ($P < 0.05$) for all groups evaluated did not show a normal distribution for the values evaluated by PGM and EM. Despite the significant difference found between the medians of the methods and the low homogeneity according to the coefficient of variation (CV), there was a homogeneous and linear dispersion of the results. The Bland–Altman test showed that the mean difference between the two methods was close to zero, denoting good agreement, precision, and accuracy of PGM when compared to EM. **Conclusions.** PGM presents high accuracy and precision for assessing glycemia in sheep, providing satisfactory and reliable results when compared with EM. **Implications.** The use of PGM facilitates the veterinarian's routine, promoting early diagnosis, field examinations, and monitoring of metabolic diseases.

Keywords: blood, enzymatic method, glucose, hyperglycemic, hypoglycemic, metabolism, normoglycemic, portable glucometer, sheep.

Introduction

Blood glucose concentrations in animals may vary depending on their general health status, diseases or pathological conditions, stress, and periods of starvation (Stämpfli *et al.* 2015). Early diagnosis might help prevent complications, economic losses, and death in farm animals (Radostite *et al.* 2007). The use of a portable glucometer (PGM) has proven to be a good, simple, and practical alternative method for glucose measurements with good precision and accuracy in different species (Helayel *et al.* 2020; Chenard *et al.* 2022).

In studies involving diverse farm-animal species and health conditions, blood glucose measurements have shown high accuracy and precision compared with enzymatic laboratory methods (Pichler *et al.* 2014; Carvalho *et al.* 2020; Helayel *et al.* 2020). Previous findings, published by the same research team as part of their research interest in this work, demonstrated the effectiveness of PGM in evaluating glucose concentrations in goats (Chenard *et al.* 2022). However, the efficacy of PGM in sheep remains unconfirmed.

Therefore, this study aims to present data from the same project, focusing on sheep species, to investigate the applicability of the portable glucose monitor (Accu-Chek® Advantage; Roche Diagnosis Brasil) for assessing blood glucose in sheep (*Ovis aries*) under normoglycemic, hypoglycemic, and hyperglycemic conditions.

Materials and methods

This study was authorised by the Ethics Committee on the Use of Animals of the Universidade Federal Fluminense (CEUA/UFF) under number 4350220519. Sixty sheep of both sexes, aged between 6 and 36 months, and weighing between 15 and 40 kg, were utilised in this research, all of which were deemed clinically healthy based on clinical examinations (Feitosa 2008) and haematological analyses (Thrall 2015).

The methodology employed in this study strictly adhered to the experimental design previously outlined by Chenard *et al.* (2022). The animals were categorised into three groups. Group G1 comprised 60 sheep from which blood samples were collected. Group G2 consisted of 15 randomly selected animals from G1, subjected to hypoglycemia induction via subcutaneous administration of 0.7 IU per kg of ultrarapid insulin (recombinant DNA lispro insulin; Humalog, Eli Lilly do Brasil, São Paulo, Brazil), as described by Reynolds (1989), with blood samples collected after 1 h. For G3, 15 randomly selected animals from G1 were subjected to hyperglycemia induction by intravenous bolus infusion of 150 mg/kg of sterile 50% glucose solution, according to the technique of Regnault *et al.* (2004), with blood samples collected after 10 min. Samples from all three groups were analysed using PGM and EM (Fig. 1).

After trichotomy and antisepsis, a volume of 6 mL of blood was collected from each animal via puncture in the jugular vein by using a 25 × 8 mm hypodermic needle and a 10 mL disposable plastic syringe. Immediately, a drop of blood was utilised in an Accu-Chek® PGM (Roche Diabetes Care Brasil Ltda.), following the manufacturer's instructions for assessing blood glucose. A volume of 3 mL of the total blood was placed in tubes containing EDTA for blood count analysis, according to Thrall (2015), and the remaining 3 mL was placed in tubes containing sodium fluoride. Blood glucose was then assessed by EM according to Chenard *et al.* (2022), using glucose kits (labtest) on a Labmax 240 Premium device. All samples were refrigerated and processed within 2 h of collection.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA), ver. 8.0® software; MedCalc Statistical Software ver. 19.2.6 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024). Descriptive tests, including Pearson correlation, significance level for Levene's test (PLT), and significance level for Pearson correlation (PCP), were conducted to describe the results of the variables across the different groups (G1, G2, and G3). The Kolmogorov–Smirnov test was used to assess the normality of distribution, the Sign test for median, the Levene test for homogeneity of variances, and Pearson correlation for the degree of correlation. EM served as the standard for comparison purposes.

The efficiency of the PGM and EM methods was compared by defining two cutoff points for the evaluations of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Kappa index, area under the receiver opearting characteristic (ROC) curve, and confidence interval of these analyses. These cutoff points were defined based on the first and third quartile of the distribution of results assessed using EM, with values below the first quartile indicating hypoglycemia and values above the third quartile indicating hyperglycemia.

Results

In all analysed groups, neither the portable glucometer (PGM) nor the enzymatic method (EM) demonstrated a normal distribution following the Kolmogorov–Smirnov test ($P < 0.05$).

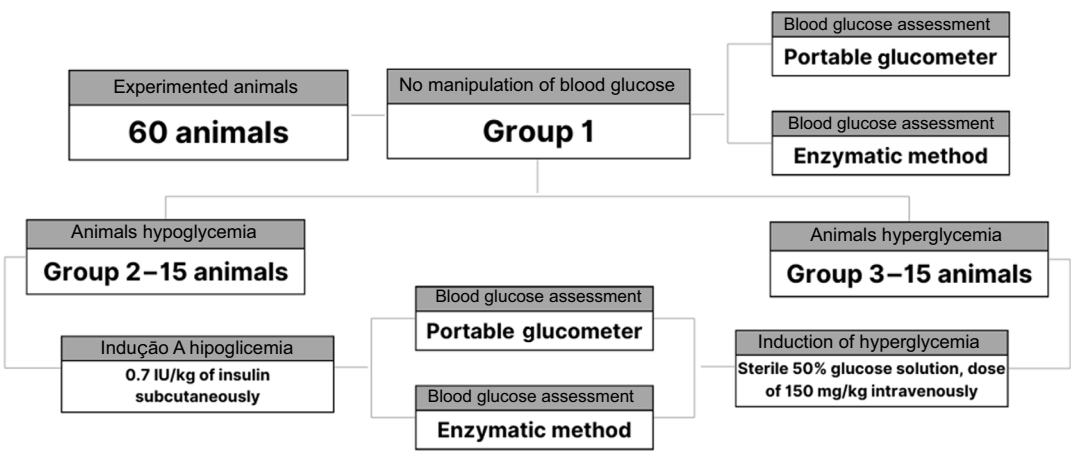

Fig. 1. Steps of the experimental procedure.

The distribution of blood glucose concentration among the samples showed distinct quartiles; the first quartile encompassed animals with blood glucose concentrations below 48.75 mg/dL, the third quartile included values exceeding 70.50 mg/dL, and the second quartile comprised values falling between 48.75 and 70.50 mg/dL. Across the 90 samples assessed, blood glucose concentrations ranged from 13 to 271 mg/dL for the portable glucometer (PGM) and from 6 to 268 mg/dL for the enzymatic method (EM), with mean values of 63.20 mg/dL (PGM) and 65.58 mg/dL (EM) (refer to Table 1).

Despite the significant difference found between the medians of the methods, and the low homogeneity according to the coefficient of variation (CV) (Table 2), there was a homogeneous and linear dispersion of the results, confirmed by a PLT of 87% (>0.05) and a positive and significant correlation demonstrated by CP of 99% (Table 2). A significant difference was found in the median for the 60 sheep evaluated (G1); however, there was a linear and homogeneous dispersion of PGM and EM results, confirmed by CV (low dispersion), PLT of 54% (>0.05), and PC of 85% (Table 2).

The medians did not show a significant difference for G2, and the CV indicated a linear and homogeneous dispersion of the results between the methods, corroborated by a PLT of 47.5% ($P > 0.05$) and PC of 74% (Table 2). In G3, the medians showed a significant difference; however, a linear and homogeneous dispersion of results was also observed, as evidenced by the CV, PLT of 85% ($P > 0.05$), and PC of 99.4% (Table 2).

The medians did not show a significant difference for the first quartile; despite the high CV, the PLT was 91%, and the PC was 93%, denoting excellent homogeneity. Despite

Table 1. Results of glycemia in sheep assessed through a portable glucometer (PGM) and enzymatic method (EM), according to stages of evaluation (G1, with no changes in glycemia; G2, hypoglycemia, G3, hyperglycemia).

Group	N	Age	Sex	Weight (kg)	PGM (mg/dL)	EM (mg/dL)
G1	14	12	Female	35.77 ± 3.4[A]	54.78 ± 11.7[A]	55.55 ± 10.7[A]
	2	12	Male	39.3 ± 0[A]	54.00 ± 4.2[A]	52.50 ± 0.7[A]
	40	24	Female	37.35 ± 2.2[A]	56.13 ± 8.9[A]	59.67 ± 9.6[A]
	4	24	Male	39.33 ± 0.8[A]	52.50 ± 4.7[A]	53.75 ± 2.5[A]
G2: hypoglycemia	5	12	Female	34.18 ± 3.8[A]	20.4 ± 5.8[A]	21.20 ± 5.8[A]
	2	12	Male	38.95 ± 1.1[A]	25.00 ± 7.1[A]	22.00 ± 8.5[A]
	8	24	Female	37.09 ± 2.8[A]	22.25 ± 5.0[A]	22.88 ± 8.1[A]
G3: hyperglycemia	14	24	Female	37.39 ± 2.5[A]	136.00 ± 48.8[A]	139.86 ± 46.1[A]
	1	24	Male	38.2 ± 0[A]	124 ± 0[A]	125 ± 0[A]
Total	90[B]	NA	NA	37.08 ± 2.7[A]	63.2 ± 40.12[A]	65.58 ± 40.52[A]

[A]Averages plus or minus standard deviations.
[B]Total sum of animals.
NA, not applicable.

Table 2. Descriptive statistics of results of glycemia in sheep assessed through a portable glucometer (PGM) and enzymatic-colorimetric method (EM), according to stages of evaluation.

Group	Method	N	M	X ± s.d.	CV%	PLT	PC (R^2)	PCP
Total	PGM	90	54a	63.2 ± 40.11	63.47	0.873	0.992*	<0.001
	EM	90	55b	65.58 ± 40.53	61.80			
G1	PGM	60	54a	55.5 ± 9.21	16.59	0.541	0.850*	<0.001
	EM	60	55b	58.1 ± 9.63	16.57			
G2	PGM	15	21a	22.0 ± 5.28	24.00	0.475	0.742*	0.002
	EM	15	22a	22.2 ± 6.98	31.44			
G3	PGM	15	122a	135.2 ± 47.1	34.84	0.857	0.994*	<0.001
	EM	15	124b	138.87 ± 44.62	32.13			

G1, no changes in glycemia; G2, hypoglycemia; G3, hyperglycemia; *M*, comparison of results by the Sign test (median test); PC, Pearson's correlation; R^2, coefficient of determination; *X* ± s.d., mean plus or minus standard deviation; CV, coefficient of variation; PLT, significance level for Levene's test ($P > 0.05$ means equal variances between groups); PCP, significance level for Pearson's correlation.
Values within a group in a column followed by different letters are significantly different from each other. *$P < 0.05$.

presenting a significant difference, the CV was low in the second quartile, with PLT of 96%, denoting high homogeneity between methods, and PC of 75%, denoting good correlation between results. In the third quartile, despite the Sign test showing statistical differences and the CV showing average homogeneity, a PLT of 79% and PC of 99% showed that the values presented by the methods showed a high correlation (Table 3). These results showed that the PGM has excellent accuracy in the different interquartile ranges.

The Bland–Altman test indicated that the mean difference between the two methods was close to zero, suggesting good agreement, precision, and accuracy of the portable glucometer (PGM) compared with the enzymatic method (EM). Despite the greater dispersion of the means, because of blood glucose being further from 50 mg/dL, most results are within the 95% confidence interval, indicating similarity between the methods and high reliability of the PGM compared with the EM. These results validate the use of PGM as a diagnostic method for assessing glycemia in sheep, providing satisfactory and reliable results in comparison to EM (Fig. 2).

Two specific cutoff points were defined to evaluate the test validation parameters and their agreement indices (sensitivity, specificity, PPV, NPV, kappa index, and area under the receiver operating characteristic curve (ROC curve)). At the cutoff point below 48.75 mg/dL, sensitivity was 94.1%, with specificity of 95.5%, PPV of 98.5%, and NPV of 84.0%; the kappa index was 94.4%, and the area under the ROC curve was 98.0% (Fig. 3*a*). At the cutoff point above 70.5 mg/dL, sensitivity was 86.4%, with specificity of 100.0%, PPV of 100.0%, NPV of 95.8%, kappa index of 96.7%, and area under the ROC curve of 97.4% (Fig. 3*b*). All results above 80.0% denote excellent reliability and accuracy of the PGM. The results showed that the PGM presents high reliability, accuracy, and precision in the different glycemic indices considered in this study, with high sensitivity and specificity, presenting values greater than 80.0% when compared with the EM, with greater accuracy in conditions of hypoglycemia. These findings validated PGM as an efficient and reliable alternative diagnostic tool to assess glycemia in sheep.

Comparing the costs of the methods, EM includes a needle (R$0.10 each), a syringe (R$0.20 each), a tube with sodium fluoride for collection (R$1.50 each), reagent for blood glucose assessment (R$60.00/100 analyses = R$0.60 each analysis), Bioplus L2000 device for analysis (R$18,000.00), distilled water for washing the device (R$2.00 each analysis), an isothermal box (R$25.00), and ice for transportation from the property to the laboratory (R$10.00 for each analysis). The cost of using the PGM includes the portable device (R$80.00), a needle (R$0.10 each), and a disposable reagent strip (R$1.50 per test). The cost recorded for performing 1000 analyses by the EM is R$31,940.00 (R$31.94 for each analysis), whereas the PGM requires an investment of R$1680.00 (R$1.68 for each analysis).

Table 3. Descriptive statistics of results of glycemia in sheep assessed using a portable glucometer (PGM) and enzymatic-colorimetric method (EM), according to cutting points (first, median, and third quartile).

Quartile and method (*N*)	*M*	$X \pm$ s.d.	CV%	PLT	PCP
First quartile					
PGM (22)	27a	29.36 ± 12.30	41.89	0.910	0.933*
EM (22)	28a	29.68 ± 12.6	42.45		<0.001
Second quartile					
PGM (46)	107a	54.85 ± 6.6	12.03	0.968	0.758*
EM (46)	115b	57.13 ± 6.28	10.99		<0.001
Third quartile					
PGM (22)	67a	114.5 ± 49.72	43.42	0.796	0.993*
EM (22)	78b	119.14 ± 46.97	39.42		<0.001

N, numbers of animals in the group; M, comparison of results by the Sign test (median test); PC, Pearson's correlation; R^2, coefficient of determination; $X \pm$ s.d., mean plus or minus standard deviation; CV, coefficient of variation; PLT, significance level for Levene's test ($P > 0.05$ means equal variances between groups); PCP, significance level for Pearson's correlation.
Values between the two methods within a quartile in a column followed by different letters are significantly different from each other. *$P < 0.05$.

Discussion

The extreme blood glucose concentrations observed after the induction protocols used exceeded those typically found in natural conditions, even in diseased states (Kaneko *et al.* 2008). The efficiency of these protocols has been previously demonstrated (Reynolds 1989; Regnault *et al.* 2004; Chenard *et al.* 2022). Additionally, measuring blood glucose concentrations at extreme hypoglycemic and hyperglycemic concentrations posed a challenge for the PGM tested in this study. The experimental design was shown to be effective, with the quartiles obtained resembling physiological parameters (Chenard *et al.* 2022).

Although imbalances in haematocrit concentrations in animals can potentially affect results, all animals in this study exhibited haemogram indices within normal ranges. Analytical quality may also be influenced by factors such as blood volume, environmental conditions such as altitude, humidity, and air temperature, as well as variations in reagent-strip batches (Rebel *et al.* 2012).

Values exceeding those reported in the literature were observed in hyperglycemic animals. Santos *et al.* (2011) reported values between 69.8 and 130.5 mg/dL, and Silva *et al.* (2008) found hyperglycemic values of >90 mg/dL. The glucometer investigated is designed for human use and can evaluate blood glucose concentrations of up to 600 mg/dL, thus not affecting the results.

Bland–Altman plots demonstrated satisfactory agreement between the two methods in terms of analytical and clinical accuracy, being consistent with findings by Hirakata and

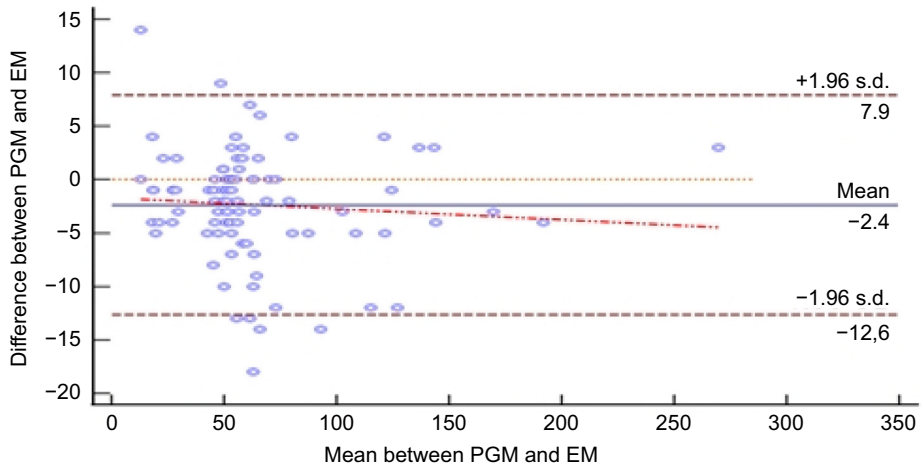

Fig. 2. Distribution of individual results of glycemia in 90 sheep assessed by using a portable glucometer method (PGMM) and enzymatic method (EM) by the Bland–Altman method.

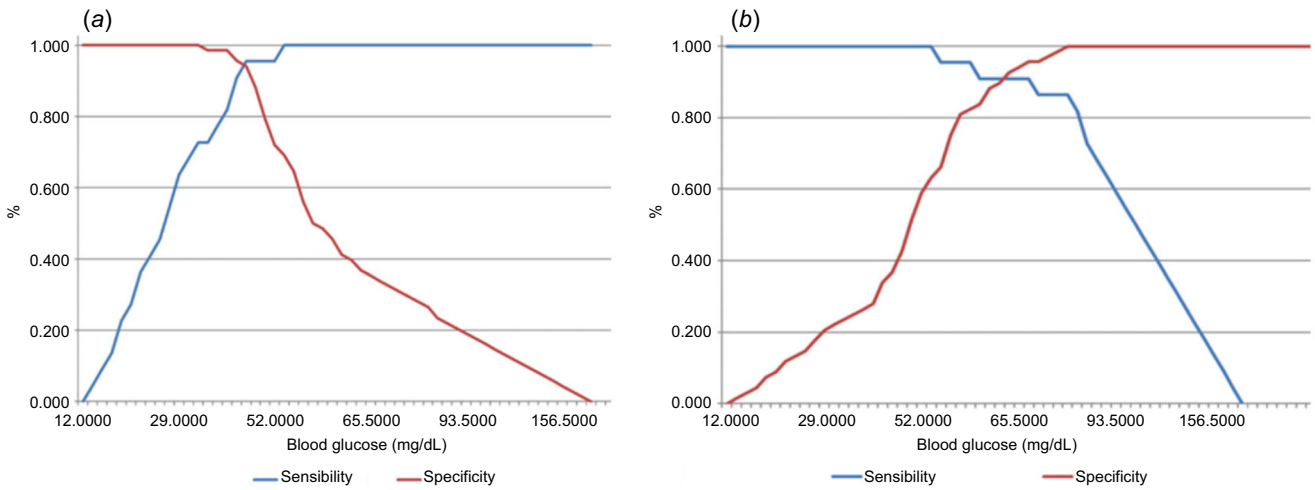

Fig. 3. Variation in sensitivity and specificity of a portable glucometer (PGM) in relation to enzymatic method (EM) for assessing glucose in sheep, considering the cutoff point of (*a*) 48.75 mg/dL (hypoglycemia), and (*b*) 70.50 mg/dL (hyperglycemia).

Camey (2009) and Chenard *et al.* (2022). The differences between the methods fall within the calculated limit of agreement, as described by Monteiro *et al.* (2015).

Therefore, the PGM exhibits high sensitivity and specificity, as also reported by Panousis *et al.* (2012), who tested the Precision Xceed® device in sheep and reported sensitivity of 98.6% and specificity of 98.2%. Katsoulos *et al.* (2011) utilised a One Touch Vita® device and confirmed the suitability of PGM in the field for cattle and sheep, describing linear results and accuracies of 95% for cattle and 88% for sheep using the Bland–Altman method.

The quality of the device was confirmed on the basis of sensitivity and specificity values, as well as other parameters, including an area under the ROC curve exceeding 80%, indicating acceptable performance (Chenard *et al.* 2022), effectively classifying hypoglycemic and hyperglycemic animals. It is important to note that, in practice, tests with high sensitivity and specificity do not reach 100% (Kramer 1988).

Regarding costs, this is the first report to evaluate and compare the costs of assessing blood glucose in sheep by using a portable glucometer and the enzymatic method. Comparing the costs of both methods shows that the use of PGM reduces process costs. Additionally, unlike the enzymatic method, PGM provides immediate results, with blood glucose concentrations available seconds after collection.

On the basis of the results, it can be concluded that the evaluated PGM (Accu-Chek®) is a reliable and safe alternative for assessing blood glucose in sheep, as previously reported by Chenard *et al.* (2022) in goats. This differs from other PGM brands, such as the FreeStyle® Optium Neo glucometer, which has been shown to underestimate plasma glucose

concentrations (Katsoulos *et al.* 2011; Pichler *et al.* 2014; Carvalho *et al.* 2020).

The results indicated that the PGM evaluated offers a viable option for measuring blood glucose concentration in sheep (*Ovis aries*) under the conditions studied. It allows for field tests for early diagnosis and monitoring of metabolic diseases. The results obtained do not differ from those obtained using the enzymatic method, and the statistical tests and parameters validate the precision and accuracy of the measurements taken with the PGM.

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Data availability. The datasets generated and/or analysed during the present study are available in the Google Drive repository at [https://drive.google.com/drive/folders/11K57jN0krqP3udEKxncV5g4x ×Tyco7x ×SP?usp=sharing].

Conflicts of interest. The authors declare no conflicts of interest.

Declaration of funding. This work received support from the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) through scholarships awarded to the author Marina G. Chenard.

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