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Behavioural and physiological measures: correlation and relevance in evaluating the welfare of beef cattle

Medidas comportamentais e fisiológicas: correlação e relevância para avaliação do bem-estar de bovinos de corte

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Highlights

Cortisol is a reliable indicator of temperament in beef cattle.
Cortisol in beef cattle can be estimated using the glycaemic index.
The portable glucometer is accurate in determining blood glucose levels in cattle.

Abstract

This study investigated the correlation between the scale score and flight distance with physiological stress indicators and its relevance to welfare assessments in beef cattle. Plasma glucose testing in the laboratory was compared to capillary blood testing using a portable glucometer to test/validate the methodology in cattle. Thirty-six cattle were studied, divided into two treatments, where each animal was considered one experimental unit. Each group comprised 18 randomly chosen cattle, which were evaluated over 490 days. A descriptive analysis (mean, standard deviation and coefficient of variation), an analysis of repeated measurements over time, and the Pearson correlation were carried out, followed by a randomisation test. The effect of the treatments was measured using the following indicators: flight distance, composite scale score, plasma glucose, capillary glucose and blood cortisol, evaluated in repetitions over time. The results showed a moderate association (0.57414) between the composite scale score and flight distance, while the composite scale score, capillary glucose (0.63870), plasma glucose (0.63386) and cortisol (0.62329) were highly correlated. The composite scale score and the

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levels of glucose and cortisol are reliable indicators for determining the degree of animal well-being. The portable glucometer was accurate in determining glucose levels in cattle.
Key words: Animal welfare. Flight distance. Composite scale score. Glucose. Cortisol.

Resumo

Este trabalho investigou a correlação entre o escore de balança e distância de fuga com os indicadores fisiológicos de estresse e sua relevância para avaliações de bem-estar de bovinos de corte. Comparou-se pesquisa de glicemia plasmática por exame laboratorial com pesquisa em sangue capilar medida por glicosímetro portátil para testar/validar metodologia para bovinos. Foram estudados 36 bovinos distribuídos em dois tratamentos, onde cada animal foi considerado uma unidade experimental. Os grupos foram formados aleatoriamente com 18 bovinos cada, sendo esses avaliados por 490 dias. Foi realizada análise descritiva (média, desvio padrão e coeficiente de variação), análise de medidas repetidas no tempo e estudo de correlação de Pearson seguido de teste de aleatorização. O efeito dos tratamentos foi mensurado pelos indicadores distância de fuga, escore composto de balança, glicemia plasmática, glicemia capilar e cortisol sanguíneo, avaliados em repetições no tempo. Os resultados demonstraram que escore composto de balança e a distância de fuga são medidas moderadamente (0.57414) associadas enquanto escore composto de balança, glicose capilar (0.63870), glicose plasmática (0.63386) e cortisol (0.62329) estão altamente correlacionadas. Escore composto de balança, glicose e cortisol são indicadores confiáveis para determinação do grau de bem-estar dos animais. O glicosímetro portátil demonstrou precisão na determinação dos níveis glicêmicos em bovinos.
Palavras-chave: Bem-estar animal. Distância de fuga. Escore composto de balança. Glicose. Cortisol.

Introduction

Behavioural assessments can indicate the level of temperament of an animal in the face of stressful situations during the various stages of animal production. Stressful situations for cattle include the influence of the environment, the facilities, handling from birth to slaughter, health, the supply of food and water, and transportation, all of which overload their control systems and reduce their adaptation. Stress and its lack of control can therefore lead to delayed growth, reproductive damage, or disease (Broom & Fraser, 2010). The need to meet new trends in the market, together with more demands from consumers, highlights the need for good production practices aimed at animal welfare to reduce economic losses in the industry, a cost borne by both the producer and the consumer (Queiroz et al., 2014).

Different techniques such as the flight distance test (Silveira et al., 2008), the temperament score (Cooke et al., 2017), the scale behaviour score (Vetters et al., 2013; Cooke et al., 2017; Francisco et al., 2020), and exit speed (Cooke, 2014; Bruno et al., 2018) can be used to measure cattle temperament.

The lack of homeostatic balance in stressful situations has a negative impact on animal behaviour and performance, immunity, and the quality and tenderness of the meat (Kumar et al., 2023). Stressful

situations trigger a response from the hypothalamic-pituitary-adrenal axis (Brown & Vosloo, 2017), promoting the synthesis and release of glucocorticoids such as cortisol by the adrenal cortex, which together with catecholamines from the adrenal medulla, induce high concentrations of plasma glucose, the result of greater hepatic glycogenolysis and gluconeogenesis (Ralph & Tilbrook, 2016).

Cortisol is still the main physiological indicator of stress; however, the metabolism of ruminants, like other species, is characterised by a high demand for glucose, which in situations of stress or excitement, or during transport, causes transient hyperglycaemia due to the release of catecholamines and endogenous glucocorticoids that return to normal levels once the stress has passed (Cobanovic et al., 2020).

The physiological changes associated with stress in ruminants are therefore related to changes in blood concentrations of cortisol and glucose (Kim et al., 2018; García-Torres et al., 2021), with glucose possibly an important metabolic marker for stress since it undergoes significant changes in tense situations (Abbas et al., 2020; Tufarelli et al., 2023).

Assessing cortisol levels requires time and laboratory and financial resources. These can be reduced by using a substitute indicator. This is why it is important to assess glucose levels together with cortisol levels, and correlate them with behavioural indicators, thereby obtaining more reliable results. However, as with cortisol, determining plasma glucose requires time, and financial and laboratory resources. To determine plasma glucose, a portable glucometer, commonly used in humans, can be used. This is a quick and less invasive technique, which needs further testing to establish its validity and suitability for use in animals.

The aim of this study was to verify the existence of a correlation between the physiological stress indicators cortisol and glucose, with the scale score and flight distance, as well as to test/validate the use of a portable glucometer as a method of measuring blood glucose levels in cattle.

Material and Methods

The field research was carried out on the Alegrete Campus of the Farroupilha Federal Institute of Education, Science and Technology in Passo Novo, in the 2nd District of Alegrete, Rio Grande do Sul, located at -29º67'33" S and -55º50'27" W, at an altitude of 104 metres. The climate is subtropical, with an average annual rainfall of 1500 mm, an average temperature of 18.6ºC and a relative humidity of 75%. The animals and people involved in the research were treated in line with ethical principles, and the project was approved by the Ethics Committee for Animal Experimentation (CEEA) of UFPel under Process no 3843.

The study initially evaluated two methods of managing and handling the animals: those subjected to good handling practices as recommended by the 'Guidance manual for good agricultural practices: beef cattle' (Valle, 2011) or animals submitted to the traditional system of beef-cattle production in the West-Border region of Rio Grande do Sul. Thirty-six Aberdeen Angus cattle were used, with an initial average age

of 210 ± 9 days, an average body weight of 196.27 ± 7.23 kg, and an average body condition score of 2.7 ± 0.1, monitored for 490 consecutive days, until close to slaughter at approximately 23 months of age. To this end, the animals were first divided into two groups and subjected to different handling methods.

In the system employing good production practices, the use of prods, sharp objects and sticks was avoided, with flags used as the only tool for driving the animals. The animals were driven by the handlers on horseback in more-distant areas, and on foot when travelling over short distances, always calmly, without shouting or hurrying. Dogs were not used in any of the activities, a common practice in traditional management systems. An important adjustment was made to the handling pens, removing the spaces between the interspersed boards, to avoid distracting the animals, and prevent them from stopping, backing up or attempting to jump, which might delay completion of the work and potentially cause bruising (Grandin, 2000).

For animals submitted to the traditional beef-cattle production system used in the West-Border region of Rio Grande do Sul (traditional management), the traditional practices were maintained, namely: the use of dogs in the field and for handling, the use of rapid movements with the handlers always on horseback showing no regard for the animals' blind spots, and the use of aggressive instruments (whips, sticks, rods, nooses) and shouting. The facilities were the same as in the system employing good handling practices, albeit maintaining the original structure, with alleys and runs made from boards with empty spaces between them.

The two groups had no visual contact with each other throughout the experiment. The same facilities were used for handling, albeit with one group at a time, with no contact between the animals from the different treatments. All the handling practices were carried out by the same handlers, who simulated aversive or non-aversive behaviour depending on the treatments. To ensure that diet had no effect on the results, areas of pasture with similar botanical characteristics, soil and shelter were used; these had access to water and mineral salt and were rotated every 14 days. Health management was the same for all the animals in the experiment, employing the same products and procedures, and on the same dates.

Health management related to vaccines was in line with that proposed by the Secretary for Agriculture of Rio Grande do Sul. The control of endo- and ectoparasites was preventive or carried out according to infestation, respectively, using products authorised by the Ministry of Agriculture, Fisheries and Supply (MAPA). These activities used the same products and procedures, and were carried out on the same dates for both production systems in an individual containment chute. The males were castrated at the start of the experiment using non-surgical castration with Burdizzo pliers and local anaesthetic.

The composite scale score and flight distance were used as behavioural assessment measures, adapted from Piovesan (1988) and Boivin et al. (1992), respectively. All the measurements and repetitions were carried out by the same evaluator throughout the experiment. Five evaluations were made over 490 days, the first

21 days after setting up the handling systems (animal adaptation), and the others after 138, 256, 372 and 490 days. The composite scale score was assessed during the individual weighing of each animal, 10 seconds after entering the scales. These were rated: 1 = calm, no movement, no audible breathing; 2 = restless, alternating the position of the feet; 3 = twitching, shaking, occasionally moving the scales, occasional audible breathing; 4 = continuous and vigorous movements, moving the scales, audible breathing; 5 = vigorous and continuous movements, moving the scales, turning or struggling violently, audible breathing. In situations where the observed combinations did not follow the established pattern, a value of plus or minus 0.5 was assigned. The test for flight distance was carried out after weighing and assigning the scale score. The floor of the pen at the exit of the scales had been previously marked out into square metres (m2) to calculate the avoidance distance between the evaluator and the animal under evaluation, determining the distance the animal could be approached. The handler attempted to approach the animal, checking the avoidance distance against the floor markings. Animals with a greater flight distance were considered more reactive.

Glucose and cortisol were measured as physiological indicators of welfare. To validate a methodology not previously described in cattle, two methods of testing for glycaemia were compared: a laboratory blood test using the enzymatic colorimetric method, a technique commonly used in studies of this metabolic indicator, and glucose testing in fresh capillary blood, measured by portable glucometer.

Plasma glucose was measured by laboratory blood test (enzymatic colorimetric method) in four repetitions over time; while capillary glucose was measured using a portable blood glucose monitor (Accu-Chek Active® portable glucometer from Roche) in five repetitions, with the first four repetitions carried out simultaneously. The greater number of repetitions when measuring the glucose was due to the ease and cost of execution. Blood samples to determine plasma glucose were collected by jugular venipuncture using vacuum equipment, in a test tube containing anticoagulants; these were homogenised, cooled to 5ºC in a cool box containing ice, sent to the laboratory, and kept in a refrigerator for later analysis. Blood samples to measure capillary blood glucose were collected by puncturing the medial surface of the external ear using a disposable hypodermic needle, placing the drop of blood in contact with the test strip and immediately introducing this into the portable glucometer, which instantly displayed the blood glucose concentration in mg/dL.

Blood cortisol was analysed by chemiluminescence in four repetitions carried out at the same time as the plasma glucose. The blood samples were collected by jugular venipuncture using vacuum equipment, in a test tube with no anticoagulants, cooled and sent to the laboratory together with the samples intended for the plasma glucose tests. All the samples were collected after the behavioural assessments.

Earlier graphical analyses were carried out with the help of the MS Excel® software to verify the dispersion and behaviour of the data and obtain an overview of its consistency and heterogeneity. Based on these analyses,

hypotheses were formulated regarding the variables that might show a correlation. The Pearson correlation test was carried out between the behavioural variables and physiological stress indicators using the Proc Corr procedure (Statistical Analysis System Institute [SAS Institute], 2018). A dispersion analysis was then performed between the blood glucose measurements from the different methods used in the study. Polynomial regression was applied between these variables to demonstrate the relationship between the proposed methods.

Results and Discussion

The results of the different handling systems showed similar behaviour, so the correlation data were analysed together. The correlation between the behavioural variables composite scale score and flight distance presented a nominal value of 0.57414, showing these measures to be moderately associated (Table 1). The different ways of assessing temperament measure the animal's fear responses, possibly describing distinct components of reactivity (Smolinger & Škorjanc, 2021).

Table 1
Correlation matrix between behavioural variables and physiological stress indicators in growing cattle subjected to good or bad handling practices

	Scale Score	Flight Distance	Plasma Glucose	Caplillary Glucose
Scale Score	--	--	--	--
Flight Distance	0.57414	--	--	--
Plasma Glucose	0.63870*	0.42069	--	--
Capillary Glucose	0.63386*	0.41577	0.99738*	--
Cortisol	0.62329*	0.39535	0.98952*	0.98759*

* (P<0.0001)

Unlike the present study, Paranhos da Costa et al. (2002) found no significant correlations between the composite scale score and flight distance in a study with cattle, suggesting that the various temperament assessment measures may not represent the same characteristic. Our results, which are consistent with the literature, show that flight distance is a good measure of gentleness in cattle, indicating the mild or serene temperament of the individual as a quality (Sartori et al., 2019), while the composite scale score can be related to docility, which expresses the character of an individual who learns easily, is easily led and can adapt to environments and handling. Consequently, in order for the flight distance to be correlated with or influence the physiological indicators of the stress response, the animal needs to undergo greater environmental disruption.

This is because the measure does not directly involve the mechanisms regulating homeostasis.

There is a high correlation between the three physiological stress indicators: plasma glucose and capillary glucose (0.99738), plasma glucose and cortisol (0.98952), and capillary glucose and cortisol (0.98759), indicating a positive and highly significant association between glucose and cortisol levels, and confirming the hypothesis that glucose is a potential physiological indicator of stress.

Monitoring the blood glucose concentration to determine changes in metabolic parameters due to stressful situations requires obtaining a significant number of samples over a given period (Mair et al., 2016). In cattle, these samples are generally collected by venipuncture, requiring procedures to restrain the animal, which can result in hyperglycaemia induced by an increase in cortisol levels possibly resulting from improper handling (Cooke, 2014). As a response to the stressful situation, the release of cortisol stimulates glycogenolysis (Knowles et al., 2014), causing mobilisation of the stocks of liver and muscle glycogen and resulting in high blood glucose concentrations as the main regulatory mechanism for supplying immediate or 'emergency' energy to face physical or psychological threats (Dzviti et al., 2019). Such physiological mechanisms demonstrate the moderate correlation between behavioural and physiological variables. Silveira et al. (2008) and Moura et al. (2021) also found a positive correlation between reactivity scores and blood glucose levels when evaluating animals exposed to different production systems.

The high correlation between plasma glucose and capillary glucose (0.98952) can validate the use of the portable glucometer as an easy, simple, and low-cost method of researching blood glucose levels in cattle. The use of new, less invasive techniques in which small amounts of blood are collected by capillary puncture of the ear (Mair et al., 2016) to quickly measure an animal's glucose concentration are widely used in small animals (Selleri et al., 2014) and reduce any negative impacts or discomfort. This is useful, because in addition to allowing the energy metabolism to be monitored, it can now be used to determine the animal's degree of stress and try to reduce it (Mair et al., 2016). Different portable glucometers have been evaluated in dogs, with similar results to those obtained using the reference method, showing that there is no significant statistical difference between the various methodologies (Santos et al., 2022).

Comparing the use of a portable glucometer to laboratory blood testing using the enzymatic colorimetric method in cattle and sheep, Katsoulos et al. (2011) state that the portable glucometer is accurate in determining blood glucose concentrations under field conditions. Furthermore, the portable glucometer is an alternative way of measuring glucose in cattle due to its advantages in terms of ease and practicality of execution, as well as the lower cost of carrying out field tests for the early diagnosis and prevention of metabolic diseases (Helayel et al., 2020).

A moderate correlation was found between the composite scale score and the physiological variables plasma glucose, capillary glucose and cortisol, with values of 0.6387, 0.63386 and 0.62329, respectively.

This is consistent with the results of Moura et al. (2021), which show that the reactivity measure is consistent with the physiological stress responses of the animals, represented by the levels of glucose and cortisol. These findings confirm that there are at least two methods of quantifying animal stress: behavioural responses and measuring the components of fluids extracted from live animals (Chen et al., 2015; Brown-Brandl, 2018).

The correlation between the behavioural variables and physiological indicators shows that the composite scale score and levels of blood glucose and cortisol are mechanisms that can be used to determine and quantify stress. This may allow any of these measures to be chosen to classify the degree of animal welfare, both in research and in production systems. Among the methods used in this study to measure the temperament of animals in relation to handling, it was found that the composite scale score, and levels of glucose and cortisol are highly correlated and, as such, appear to evaluate the same characteristics. The correlation found in the present study corroborates the existing literature, which shows significant correlations between the different methods of assessing temperament (Vetters et al., 2013; Moura et al., 2021). Flight distance is one method that does not appear to be related to reactivity, but rather to the gentleness of the cattle. Studies of this nature are important for consolidating methods and developing new procedures for evaluating the degree of welfare of beef cattle.

An analysis of the evolution of plasma and capillary glucose in the evaluations of the present study, averaged over both handling systems (Figure 1), shows the similarity between the values obtained by the two methods of analysis. The values for plasma glucose and capillary glucose were related using simple linear regression. Based on the observed measurements, a regression equation was estimated (Figure 2) between plasma glucose (GLUC A)[1] and capillary glucose (GLUC B)[2] to predict the coefficient of variation, generating the equation y=0.8998 + 0.9638X (r2 0.9948). Although there is a need for further study, it is clear that the portable meter is reliable when measuring glucose in cattle, and is a less invasive and faster method than using plasma glucose.

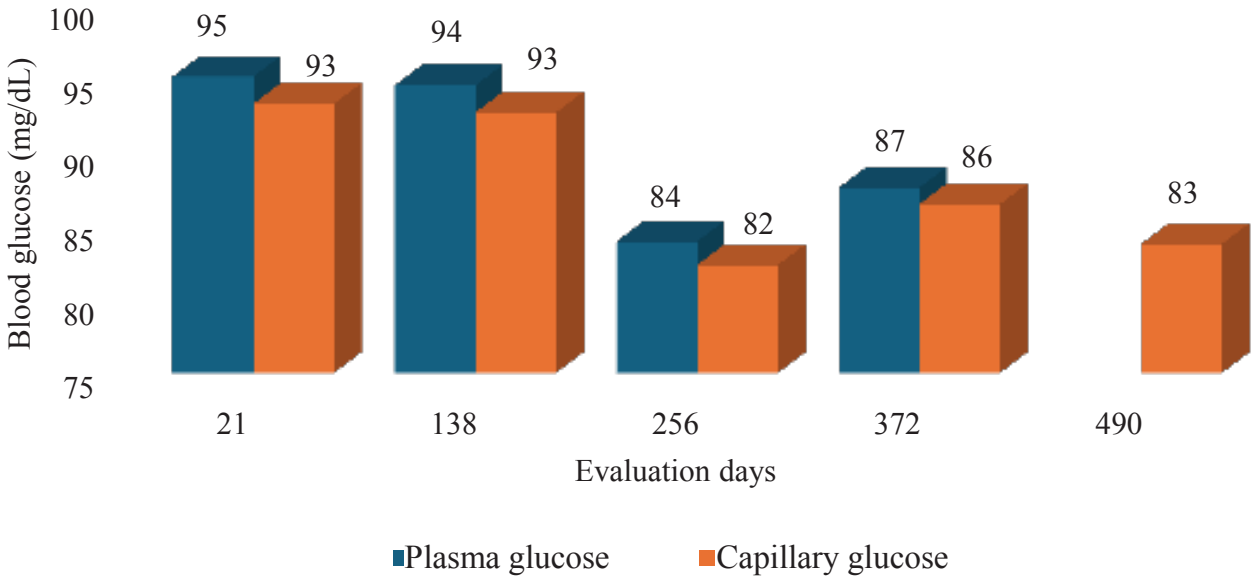

Figure 1. Plasma and capillary glucose levels (average of the Good Practice and Traditional Systems) in growing steers, regardless of the management system.

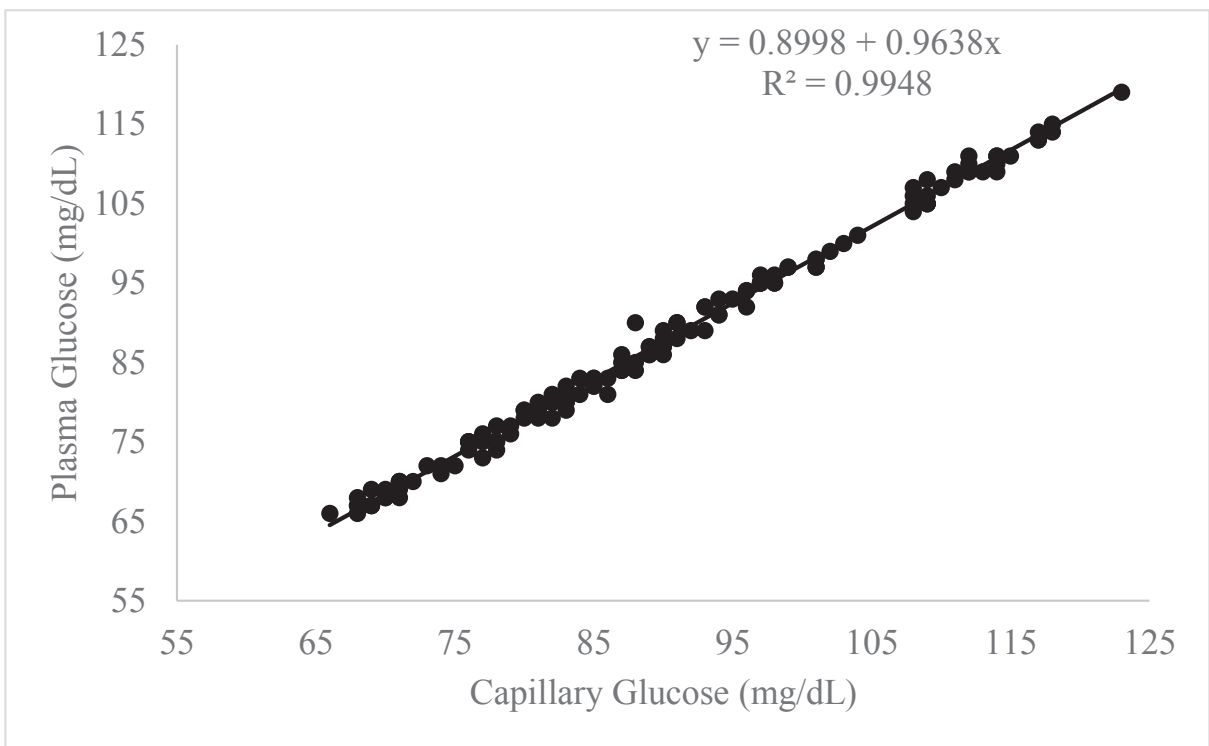

Figure 2. Regression and dispersion equation for plasma glucose (GLUC A) measured by laboratory blood test (enzymatic colorimetric method), and capillary glucose (GLUC B) measured by portable blood glucose monitor (Accu-Chek Active portable glucometer), where (n=144).

Conclusions

The composite scale score, and levels of glucose and cortisol are reliable indicators for determining stress in cattle and are correlated. The use of these measures can indicate the degree of animal welfare.

Flight distance is an important indicator and needs to be better studied so that it can be recommended as a reliable measure of animal behaviour.

The portable glucometer proved to be accurate in determining glucose levels in cattle. As the test is easy to perform, of low cost, and provides results in a few seconds, its use is recommended for determining blood glucose levels in cattle.

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