INEFICACY OF PORTABLE DEVICE ACCUTREND® PLUS IN EQUINE INTERNAL MEDICINE

INEFICÁCIA DO APARELHO PORTÁTIL ACCUTREND® PLUS NA CLÍNICA MÉDICA DE EQUINOS

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SUMARY

Four blood components (glucose, lactate, triglycerides and cholesterol) were determined and compared using a portable device (Accutrend® Plus, ROCHE) and laboratory methods. Blood samples from 20 horses were analyzed (11 geldings and 9 mares, from various breeds with age and weight varying between 8 ± 5 years and 327.60 ± 64.02 Kg, respectively. After complete clinical examination, blood was withdrawn through venipunction of the external jugular and these samples were analyzed using a portable device and compared to laboratory results. Statistical analyses were used to compare both methodologies. The results showed that glucose data were not different with a weak Pearson's correlation and no agreement according to Bland–Altman method. The results obtained for lactate according to both methods were also not in agreement. Triglycerides and cholesterol data could not be compared due to the fact that reference values for resting horses lie below the reading range of the portable device. The present study showed that the use of Accutrend®Plus to determine glucose, lactate, triglycerides, and cholesterol levels, is not adequate in equine internal medicine.

KEY-WORDS: Biochemical. Dry chemistry. Horses. Portable device.

RESUMO

Comparou-se nesse estudo a determinação de quatro constituintes sanguíneos (glicose, lactato, triglicérides e colesterol), entre um aparelho portátil e métodos laboratoriais convencionais. Foram analisados o sangue de 20 equinos (11 machos e 9 fêmeas), de diversas raças com idades variando entre 8 ± 5 anos e peso corpóreo de 327,60 ± 64,02 Kg. Após exame clínico completo de cada animal realizou-se punção da veia jugular externa para coleta de amostra sanguínea, de maneira que a mesma amostra, foi utilizada para determinação no aparelho portátil e no laboratório. Na comparação entre as metodologias, em relação a glicose, não foi notada diferença entre as médias (teste T de Student), a correlação de Pearson revelou-se fraca e não houve concordância segundo o método de Bland-Altman. Para o lactato também não se observou concordância entre os dois métodos. Quanto aos triglicérides e colesterol foi possível apenas a análise descritiva dos resultados, devido as concentrações dos padrões fisiológicos de equinos em repouso, se apresentarem inferiores ao intervalo de leitura do aparelho portátil. Este trabalho demonstrou que o uso do referido aparelho para determinação de glicose, lactato, triglícerides e colesterol não é adequado na clínica médica equina.

PALAVRAS-CHAVE: Bioquimicos. Equinos. Equipamento portátil. Química seca.

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INTRODUCTION

Glucose is the organism main source of energy, therefore knowing glucose levels in the body is very important when trying to assess the metabolic situation of a patient (SWENSON & REECE, 1996a). There are various types of equipment to determine blood glucose levels, portable glucose meters are quite effective; however, laboratory bench determinations are more accurate since they are done in a controlled environment.

Another important substrate of energy metabolism is lactate. During exercise, lactate is released into the bloodstream to be used to form glucose from gluconeogenesis. At rest, lactate concentration in the plasma seems to be related to the physical condition of anaerobically trained individuals (REHUNEN et al., 1982).

Lipids act on several organic functions, and interpreting the results of this blood component should be done carefully, since even some normal physiological state may change its profile. For example, the third trimester of pregnancy causes a negative energy balance and consequently, an increase of triglycerides concentration in the bloodstream (SWENSON & REECE, 1996). The monitoring of lipid concentration in horses is increasingly more important, as the rate of animals with metabolic syndrome is increasing, caused by ingestion of unbalanced diets or supplements, in a bid for a better diet.

Some of the portable devices used for veterinary purposes were developed for humans, and therefore do not display the sensitivity required when measuring the blood components of animals and should be tested. The feasibility of using the portable device Accutrend®Plus as an auxiliary diagnoses method for resting horses is determined by comparing the results of the following blood components: glucose, lactate, cholesterol and triglycerides to results from conventional laboratory methods.

MATERIAL AND METHODS

Twenty horses (11 male and 9 female) within the age interval of 8 ± 5, and body weight 327.60 ± 64.02Kg (mean ± standard deviation) were used. Blood samples were drawn through venipunction of the external jugular while the animals were resting. Before the blood samples were drawn, a complete clinical examination was performed in each animal.

From the 5 mL-blood samples collected, one drop was used immediately in the portable device. The remaining sample was stored in negative pressure tubes (Vacuette®), one without anti-coagulant for biochemical tests, another containing EDTA to be used for the hemogram and the third containing sodium fluoride to measure glucose and lactate in the plasma. Sodium fluoride is a hypotonic solution, with respect to erythrocytes, that causes hemolysis and inhibits glycolysis, thus capable of preventing blood coagulation and lactate production by erythrocytes (SIMÕES et al. 1998). All samples were stored in Styrofoam boxes containing ice, for about 4 hours and sent to the Clinical Pathology Laboratory of the Veterinary Hospital, at UnB. In the lab, the samples were centrifuged during 5 minutes at 3000 rpm and separated into serum and plasma aliquots of 1.5 mL. The serum was used to determine triglycerides and cholesterol levels using enzymatic kits (Liquiform/Labtest®) in the semi-automatic biochemical analyzer Bio2000. Blood counts were performed by the device PocH-100 *iv Diff* and differential counts were obtained from blood smears stained with Panoptic. Plasma samples were frozen, stored in a Styrofoam box containing ice and sent to Laboratório de Farmacologia e Fisiologia do Esforço Equino at UNESP-Jaboticabal, where lactate and glucose were measured by a YSI 2300 STAT PLUS device, in which electrodes determine the electric current generated by the reaction catalyzed by glucose-oxidase or lactate-oxidase (PLANCHE et al. 2001).

The comparison of glucose and lactate results obtained by the portable device Accutrend®Plus and in the laboratory, was performed by the statistical methods Student's t test with paired samples and significance $P<0.05$, Pearson correlation test in order to determine if there was a pattern of distribution that would allow to correlate the results and the agreement investigation method by Bland&Altman (1986). The comparison of triglycerides and cholesterol levels measured by the device and the laboratory is given only as a descriptive analysis, since the portable device indicated that the values were below the operating range of the instrument, by displaying the word "LOW".

RESULTS AND DISCUSSION

The horses used in the study were patients in the Hospital Veterinário de Grandes Animais, UnB, during the final phase of treatment, and needed only daily dressings. To ensure that they were all healthy, both clinical examination and complete blood count were performed for each animal. Table 1 shows the results for physiological (heart rate, breathing, body temperature) and hematological variables (erythrocyte, hematocrit and hemoglobin counts) for the 20 horses used in the study and reference values as well. When the horses were removed from their stalls and during containment in the squeeze chute for the clinical examination, they became restless, which triggered an adrenergic stimulation and thus increased heart and breathing rates (SWENSON & REECE, 1996b). The other variables were within the reference values for the species. Table 2 shows glucose, lactate, triglycerides and cholesterol levels in the bloodstream measured by the portable device and the laboratory reference methods.

Sample storage time that is the time elapsed between sample collection and components determination is very important. In the case of glucose at room temperature, the erythrocytes promote glucose depletion at the rate of 10% (of the total) per hour (KANEKO, 1997). In the present study, the samples with sodium fluoride were stored in

Table 1 - Physiological and hematological variables (mean ± SD) determined during equine clinical examination (n=20) and reference values for the species (Brasília, 2010).

	Physiological variables	**Reference values [1]**
Heart rate (bpm)	46 ± 7.7	24 – 48[2]
Breathing rate (mpm)	25 ± 8.7	18 – 20[3]
Body temperature (°C)	37.5 ± 0,62	37.2 – 38.2[4]
Hematocrit	36 ± 5.32	32 – 52[1]
Hemoglobin (g/dL)	11.88 ± 2.27	11 – 19[1]
Total erythrocyte (x10^3/uL)	10.51 ± 2.71	5.5 – 12.5[1]

1. KANEKO et al., 1997, 2. MACORIS, 1994, 3. SPEIRS, 1999, 4. CUNNINGHAM, 1999.

Table 2 - Bloodstream levels of glucose, lactate, triglycerides and cholesterol (mean ± SD) in equines (n=20) determined by a portable device (Accutrend® Plus) and by laboratory reference method (Brasília, 2010).

	Glucose (mg/dL)	**Lactate (mmol/L)**	**Triglycerides (mg/dL)**	**Cholesterol (mg/dL)**
Accutrend®Plus	4,67 ± 0,69	1,57 ± 0,39	low	low
YSI2300 STAT PLUS	4,44 ± 0,43	0,66 ± 0,27*	-	-
Bio2000 – BioPLUS®	-	-	19 ± 7,39	62,65 ±14,17
Reference Values	4,27 - 7,32[1]	0,55 - 1,11[2] 1,11 - 1,78[3]	4 – 44[4]	75 – 150[5]

* shows the statistical difference between the two methods (p<0,05).
1. HYYPPA et al., 1997; 2. RODIEK & STULL, 1997; 3. KANEKO, 1989; 4. BRUSS, 1980; 5. KANEKO et al., 1997

Styrofoam boxes containing ice during 5 hours, and after that frozen. Mean glucose values (and standard deviation) were 4.689 ± 0.69 and 4.44 ± 0.43 mmol/L for Accutrend®Plus and YSI 2300 STAT PLUS, respectively. The values determined by both devices were within the standard values required for horses at rest according to Hyyppa et al. (1997) and Ferraz et al. (2008), who reported glucose physiological values between 4.5 and 6.0 mmol/L. However, there was no difference (P=0.18) between the two methods according to Student's t test. Also, Pearson's correlation was weak (r=0.08) for the two values. The agreement investigation method by Bland&Altman (1986) did not show good agreement between the results given by the portable device and the laboratory. A mean difference of 0.25 mmol/L was observed between Accutrend®Plus and YSI2300 and a concordance limit of -1.3 and 1.79 mmol/L, since these values are significant, there was no good agreement between the two methods (Figure 1).

Lactate results were given by measurements carried out in total blood samples using Accutrend®Plus (that determines the values from total blood samples and converts them to plasma values by calculations given by the manufacturer) and plasma measured by YSI 2300 STAT PLUS. Both methods determined lactate in mmol/L. In the portable device, the mean value (and standard deviation) was 1.57 ± 0.39 mmol/L, and in the laboratory 0.66 ± 0.27 mmol/L. These results corroborate Soares (2008), who stated that lactate measurements conducted from the plasma of horses displayed lower values compared to results obtained from total blood and also Muñoz et al. (1996), who mentioned that this difference between the results may be due to higher concentration of lactate in blood cells compared to plasma. In this study, the values obtained by Accutrend®Plus are in agreement with the ones by Franchini et al. (2004), who reported that when values fall below 5 mmol/L, another portable device (Accusport®), that uses the same technology, also gave higher readings. However, blood lactate levels were within physiological standards for horses at rest (1.11 a 1.78 mmol/L), according to Kaneko (1989). When using portable devices, special attention should be given to hematocrit values, since when they fall below 30%, the results may be falsely high, while when hematocrit values are above 50%, they might have been underestimated (WESS & REUSCH, 2000). In the present study, hematocrit values were within the reference values for the species, with means and standard deviation of 36.27±5.55.

The results given by the portable device and laboratory method were different (P<0.001) by Student's t test, and displayed no good agreement since the mean was 0.91 mmol/L and concordance limits (95%) of 0.17 and 1.65 mmol/L (Figure 2). Pearson's correlation (r=0.407) was considered moderate, and differed from the results reported by some authors (FRANCHINI et al., 2004, KOBAYASHI, 2007), who compared another portable device (Accusport®), with

the same reference equipment used in this work, and strong correlations were observed r=0.9632 and r=0.95, respectively.

Average serum concentrations (±SD) of triglycerides and cholesterol determined by the laboratory reference method were 19 ± 7.39 mg/dL and 62.65 ± 14.16 mg/dL, respectively. In the Accutrend®Plus the reading interval for triglycerides lies between 70 and 600 mg/dL, while for cholesterol from 150 to 300 mg/dL. The results found in this study were in agreement with the reference values established for horses at rest; however, outside the reading interval of the portable device, which was indicated by the word "LOW" on the monitor.

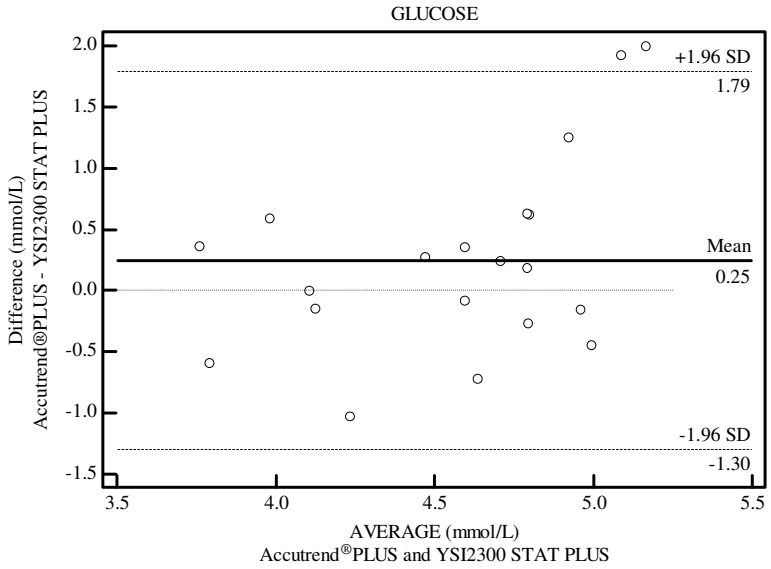

Figure 1- Representation of the agreement between the devices Accutrend®Plus and YSI2300 STAT PLUS according to Bland and Altman (1986). The horizontal axis shows the means for glucose levels obtained by the two devices and the vertical axis shows the differences between the two methods.

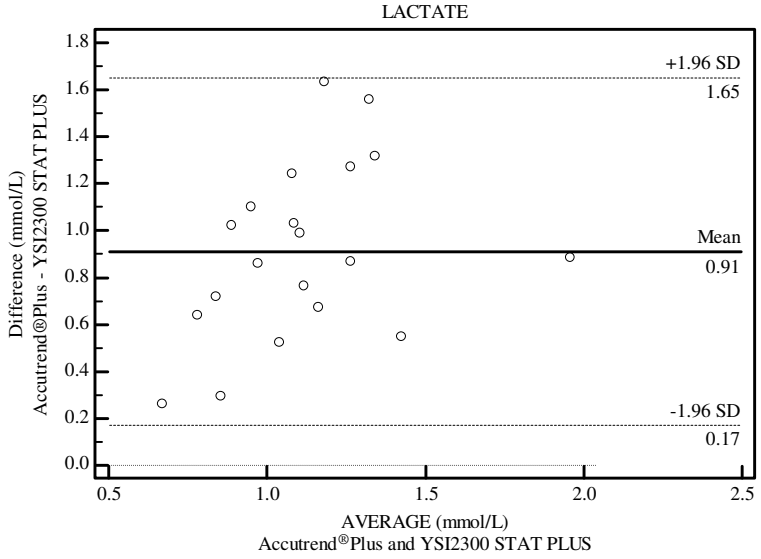

Figure 2 - Representation of the agreement between the devices Accutrend®Plus and YSI2300 STAT PLUS according to Bland and Altman (1986). The horizontal axis shows the means for lactate levels obtained by the two devices and the vertical axis shows the differences between the two methods.

CONCLUSIONS

From the analysis of the results given by the laboratory reference method, it can be concluded that the portable device Accutrend® Plus, used according to the manufacturer's recommendation to determine glucose, lactate, triglycerides and cholesterol in the bloodstream is not a reliable diagnosis method to be used in equine internal medicine.

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