Uso da espectroscopia de infravermelho próximo com Transformada de Fourier (FT-NIR) para acompanhar o processo de Tristeza Parasitária Bovina

Use of near-infrared spectroscopy with Fourier Transform (FT-NIR) to accompany the Bovine Parasitic Sadness process

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ABSTRACT
Cattle are animals that stay in the herd for a large period of the day in the open air pasture. This daily routine leaves them exposed and susceptible to infectious diseases. One of them is the Bovine Parasitic Sadness which is considered one of the greatest cattle health problems due its high rate of mortality and morbidity. In this work preliminary results are presented from calf inoculated with anaplasmosis. Blood samples were collected and analyzed through Fourier Transform Near Infrared spectroscopy (FT-NIR). The animal was monitored for 60 days until its recovery. This exploratory work shows the potentiality of the FT-NIR in the detection, follow-up and recovery of the anaplasmosis. The possibility of prevention of this disease in the herd via a fast optical analysis may bring a great breakthrough in disease control in dairy and beef cattle.

Keywords: infrared, cattle, Bovine Parasitic Sadness, babesiosis, anaplasmosis, FT-NIR.

RESUMO
O bovino é um animal que permanece grande parte do dia no rebanho em pastagem a céu aberto. Essa rotina diária o deixa expostos e suscetíveis a doenças infecciosas. Uma delas é a Tristeza Parasitária Bovina que é considerada um dos maiores problemas de saúde do gado devido ao seu alto índice de mortalidade e morbidade. Neste trabalho são apresentados resultados preliminares de bezerros inoculados com anaplasmose. Amostras de sangue foram coletadas e analisadas por espectroscopia de infravermelho próximo com transformada de Fourier (FT-NIR). O animal foi monitorado por 60 dias até sua recuperação. Este trabalho exploratório mostra a potencialidade do FT-NIR na detecção, acompanhamento e recuperação da anaplasmose. A possibilidade de prevenção desta doença no rebanho por meio de uma análise ótica rápida pode trazer um grande avanço no controle da doença em bovinos leiteiros e de corte.

Palavras-chave: infravermelho, gado bovino, Tristeza Parasitária Bovina, babesiose, anaplasmose, FT-NIR.

1 INTRODUCTION

According to the Municipal Livestock Survey (MLS), released in 2020 by the Brazilian Institute of Geography and Statistics (BIGS), Brazil is the holder of the second largest herd of cattle in the world. Brazil is still the largest exporter and second largest producer of beef, according to the

United States Department of Agriculture (United States Department of Agriculture - USDA). In 2019, in the Municipal Livestock Survey, the registered herd was about 214.7 million head of cattle [1]. Even in the face of the impact on the world economy, which according to experts, was already underway, but was accelerated due to the Covid-19 pandemic [2], agribusiness exports in Brazil were not negatively affected, in which beef can be highlighted beef with foreign sales of 637.81 million dollars in March 2020 [3]. Generally, cattle live in herds and spend most of the day together in the pasture, being exposed to risk factors that can trigger various diseases: infectious bovine rhinotracheitis (IBR), foot-and-mouth disease, mastitis, leptospirosis, neosporosis and bovine parasitic sadness (BPS), among others [4] [5] [6]. The knowledge of the several factors that can cause diseases is extremely important for the prevention of diseases in the herd, and consequent economic losses [5].

Ticks are one of the transmitters of diseases to cattle, causing considerable damage. The tick *Rhipicephalus* (*Boophilus*) *microplus* (*R.microplus*) can transmit two protozoa to cattle: *Babesia bovis* and *Babesia bigemina*, and a rickettsia *Anaplasma marginale*. So, babesiosis is transmitted by Babesia and anaplasmosis by Rickettsia [7][8].

BPS consists of a complex of diseases that include babesiosis and anaplasmosis. It is considered one of the health problems of greatest economic damage in cattle raising, which translates into high rates of mortality and morbidity, with a significant reduction in the production of milk and/or meat, causing high cost with special handling and treatments [9][8][10]. The basic clinical symptoms are: fever, anemia, lack of appetite, among others [9][8]. Tropical and subtropical climates facilitate the vector's direct action on the host, which greatly affects cattle ranching in these climates [10]. This vector is endemic in India, Asia, northeastern Australia, Madagascar, southeastern Africa, the Caribbean, countries in South and Central America, and Mexico [11]. Due to the great economic impact caused by *R. microplus*, the United States started a national campaign to eradicate *Boophilus* in 1906, and in 1943 this program was declared complete [11]. However, *R. microplus* reinfestations can occur in the states of Texas and California [12]. Young animals show non-specific immunity to BPS agents until approximately 7 to 10 months of age. In places where climatic conditions allow the tick to be present practically all year round, BPS agents are continuously inoculated in animals from birth, when they are more resistant, allowing them not to get sick and to develop strong specific immunity, which it will make them tough adults. These sites or regions are known to be endemic, stable or of enzootic stability, that is, there are normally no clinical cases of BPS in native animals. On the contrary, where climatic conditions or tick management and control issues do not allow the tick to be constantly present, there is no continuous transmission of BPS agents to cattle, which can pass the young phase without being inoculated, not

developing adequate specific immunity and becoming sensitive adults. These places or regions are known as epidemic, unstable or of enzootic instability, that is, where an outbreak of clinical disease can occur, with a large number of deaths [9].

Optical spectroscopy studies the interaction of electromagnetic radiation with matter, in order to determine excitations of energy from the atoms or molecules that constitute which can be electronic, vibrational or rotational [13][14].

Spectroscopic techniques combined with multivariate statistical methods have been shown to be efficient in many areas of scientific research because they allow to analyze, interpret and extract information quickly and accurately, with a minimum of sample preparation [15]. Infrared spectroscopy is one of the most relevant technologies in the analysis of raw materials, process control and specifications of final products in the dairy industry [16]. Techniques such as near infrared (NIR), medium infrared (MIR) and Energy Dispersion Spectroscopy (EDS) have already been successfully applied in the food industry, for quality control [17] and in the evaluation of the quality of milk and dairy products, including powdered milk, whey and cheese [15][18][19][20], as well as in the analysis of veterinary drug residues in liquid milk [4][21][22] and powder [23].

In view of the aforementioned scenario, this work aims to use FT-NIR spectroscopy to carry out a preliminary study of the analysis of bovine blood from a calf to monitor the development of BPS in it.

2 METHODOLOGY

2.1 ANIMAL AND ANAPLASMOSIS INOCULATION

Because it was a preliminary experiment, blood samples were collected only from a single animal, a calf with a genetic makeup above 85% Dutch. This animal was accompanied and monitored from its birth, February 2019, until the end of the experiment, in December of the same year, remaining for this period in the Experimental Field from Embrapa Dairy Cattle, located in the city of Coronel Pacheco, Minas Gerais, Brazil. The calf was housed in a closed, screened shed, with a double door, free of contact from anaplasmosis dissipating agents, and in an individual shavings stall until weaning. Between 90 and 120 days of age, the calf was regrouped in a concrete floor corral, free from contact with vectors that dissipate anaplasmosis, for socialization with other calves and adaptation. To inoculate anaplasmosis in the calf, the UFMG1 sample was used, obtained from naturally infected calf blood [24][25]. This has an appendix and has low virulence [26], as characterized by sequencing the gene responsible for the production of the MSP1a protein and registered on GenBank under the number: EU676176 (UFMG1). When the animal tested for anti-A antibodies. negative marginale by the immunofluorescence reaction test (IFR) demonstrating the

end of passive antibodies, he was subjected to inoculation (2x 107 erythrocytes infected by the isolated UFMG1) [27]. After inoculation of *A. marginale* on day zero (D-0), they were collected every 7 days to evaluate the evolution of rickettsemia. From the identification of the first erythrocyte parasitized by *A. marginale* in a blood smear, vacuum venipuncture of the jugular vein was performed to monitor the globular volume (GV) and smear every 48 hours and, after the beginning of the fall, every 24 hours. hours. When the GV reached 50% of the average value of the 100 individual healthy period, the animal was treated with enrofloxacin at a dose of 7.5 mg/kg of LW, intramuscularly in a single dose [28].

When necessary, supportive treatment of animals was performed by enteral fluid therapy, aiming to correct hydroelectrolytic and acid - base imbalances. The activities involving experimental animals are approved by the Animal Use Ethics Committee (AUEC) Embrapa Dairy Cattle, under protocol number 4498240316.

2.2 BOVINE BLOOD SAMPLES

Bovine blood samples were collected at the Experimental Field of the Embrapa Dairy Cattle. Soon after collection, a fraction of the blood was reserved and the EDTA coagulant was used. Then they were taken to the Materials Spectroscopy Laboratory (MSL), located in the Department of Physics UFJF, Juiz de Fora, Minas Gerais, Brazil, being maintained at a temperature storage of about 4 ºC [29]. In this, the samples were analyzed, for 2 months, following all biosafety protocols during the achievements of the measures. After the analyzes, the samples were immediately collected and sent to Embrapa Dairy Cattle for proper disposal.

2.3 ANALYSIS USING THE FT-NIR METHOD

The samples were analyzed with the Bruker MPA FT-NIR Multi Purpose Analyzer operating in the mode reflectance with number of waves in the 13500 cm^{-1} to 3700 cm^{-1} and detected by 4 cm^{-1} Te-InGaAs sensor resolution. 300 µL of bovine blood were pipetted into a cuvette of borosilicate 8 mm thick. Each analysis was performed in triplicate with 32 scans. After the measurements, the cuvettes were properly sterilized. For data acquisition, OPUS® software version 5.5 was used.

3 RESULTS AND DISCUSSIONS

The figures below show the reflectance spectra obtained by FT-NIR spectroscopy. It is important to note that in this preliminary phase the measurements were carried out over 2 months, and that the days mentioned in the spectra, on the right, are the days of analysis in the MSL/UFJF,

that is, they are not consecutive days. The days at the top of the spectrum refer to the days after inoculation.

3.1 VIBRATIONAL SPECTRA

Figure 1 shows the vibrational spectrum obtained by FT-NIR for the samples of blood with EDTA coagulant from a calf since anaplasmosis inoculation until his recovery. The spectra with the thickest lines in the green, purple and blue colors correspond to the healthy, sick animal in the most critical and recovered phase, respectively. In a first analysis it can be seen that there is a reduction in the intensity of the spectrum, between the numbers of wave 8000 cm^{-1} and 9000 cm^{-1} during the phase when the animal starts to get sick until its recovery. But this cannot be considered a predominant factor to conclude, across the spectrum, that the animal is sick. In this same region, around 8500 cm^{-1}, it was observed that the signal in the range between 8400 cm^{-1} and 8700 cm^{-1} is inversely proportional to the wave number when the animal is healthy and recovered. The reverse occurs when the animal is sick in the most critical phase, that is, the signal in the aforementioned region is directly proportional to the wave number.

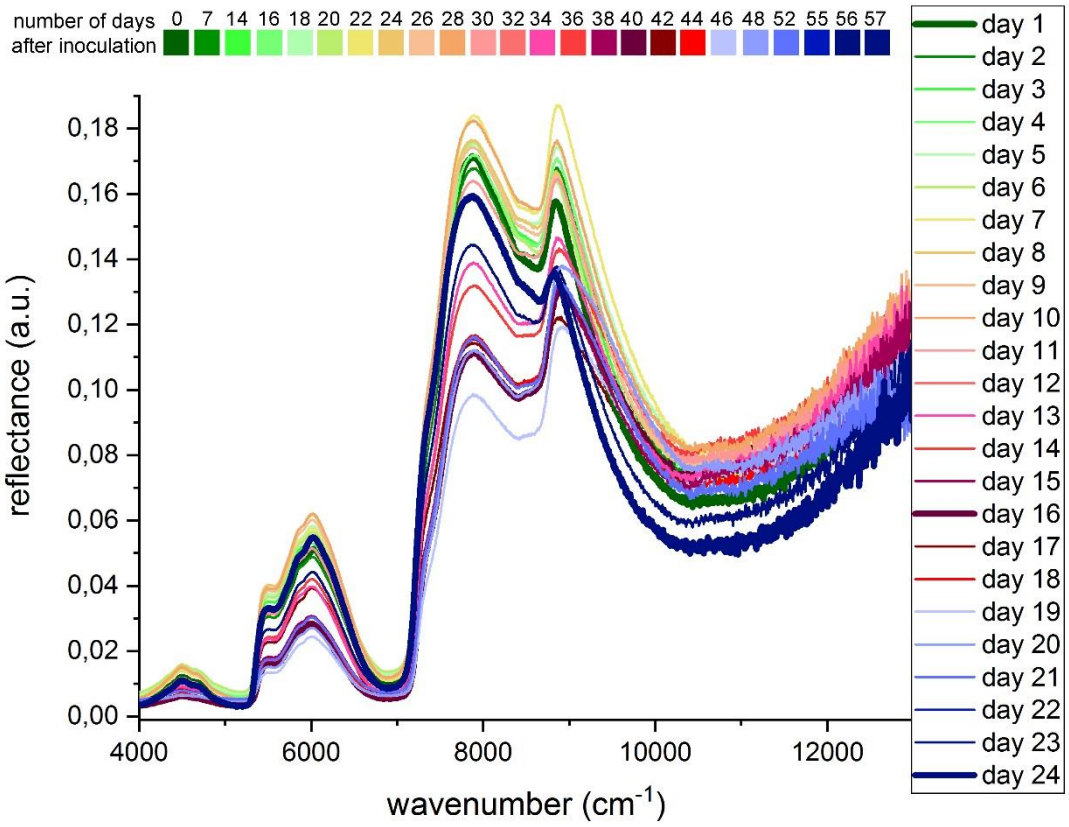

Figure 1: FT-NIR Vibrational Spectra, in reflectance mode, for monitoring blood samples, with EDTA coagulant, from a calf inoculated with anaplasmosis.

Figure 2 shows the region around the wave numbers 7300 cm^{-1} and 9500 cm^{-1} of figure 1, however enlarged. It can be observed that in the experimental days 12 and 13 (respectively, 32 and 34 days after inoculation), the slope of the spectra in the 8500 cm^{-1} region is altered, becoming slightly positive, showing that the animal started to get sick. This assumption was confirmed through microbiological monitoring measures carried out by Embrapa researchers. Subsequently, on days 14 and 15, the angle of inclination of the derivative of the curve increases until reaching a maximum, on day 16 (38 days after inoculation). Then, in the following days, the straight lines, in the same region, change the slope again (negative slope), being similar to those of healthy animals, which indicated the animal's recovery. That is, between the last experimental day, 57 days after inoculation and the first day, the FT-NIR spectra in the 8400 cm^{-1} and 8700 cm^{-1} regions are similar, indicative of a healthy animal.

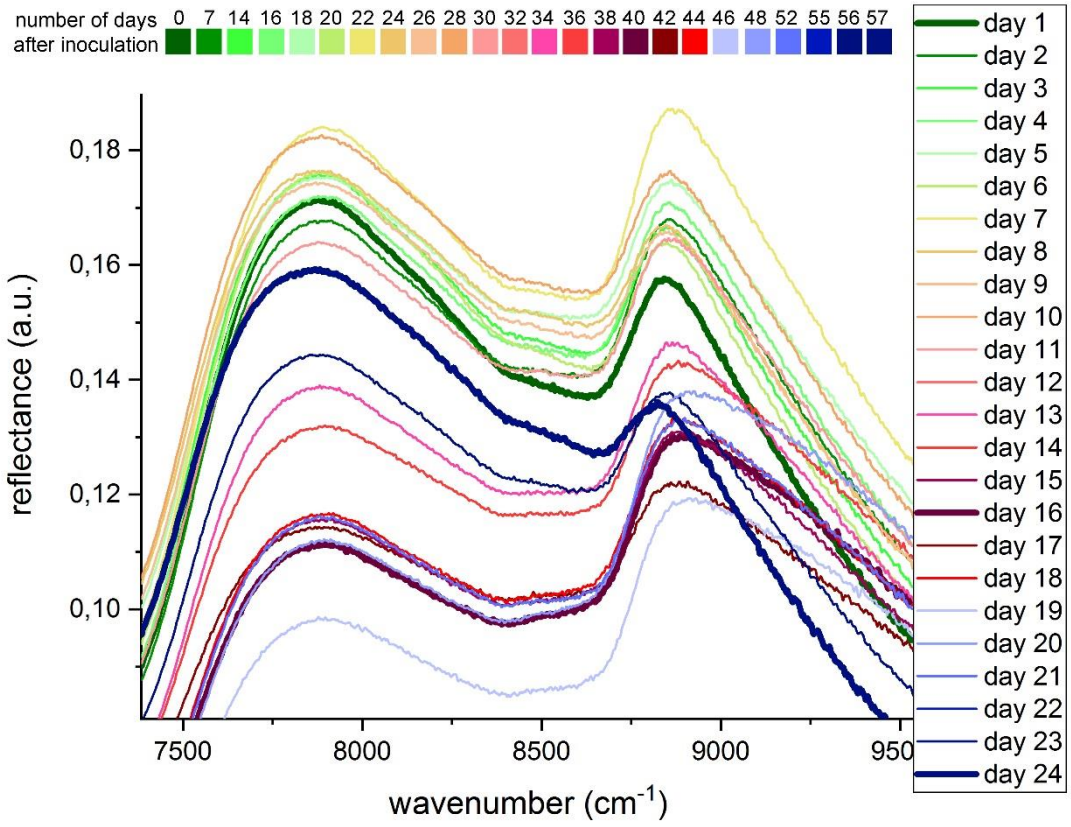

Figure 2: FT-NIR Vibrational Spectra, in reflectance mode, for monitoring blood samples, with EDTA coagulant, from a calf inoculated with anaplasmosis, enlarged between the wave numbers 7300 cm^{-1} and 9500 cm^{-1}.

Figure 3 shows the initial region of the spectrum in Figure 1 enlarged. In Figure 3, it was also observed a significant reduction in intensity in the animal's spectrum when it was sickest, 38 days after inoculation, in the regions close to 6000 cm^{-1} and 4500 cm^{-1}. However, it is observed that,

even in the recovery phase, 48 and 52 days after inoculation, the spectra show lower intensity compared to day 38 after inoculation, in the region of 6000 cm^{-1}. Therefore, it was decided to rule out that the intensity reduction may be an indicative factor that the animal is getting sick until measures with a group of animals are included.

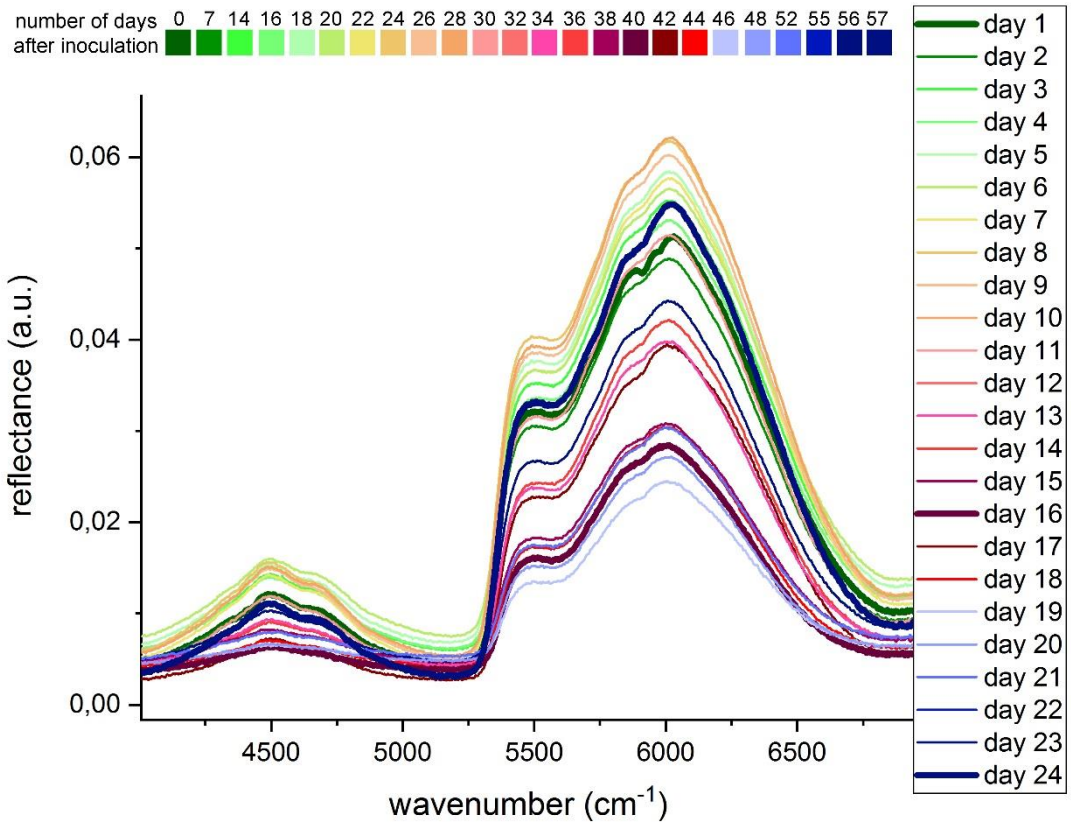

Figure 3: FT-NIR Vibrational Spectra, in reflectance mode, of blood sample monitoring, with EDTA coagulant, from a calf inoculated with anaplasmosis, enlarged between the wave numbers 4000 cm^{-1} and 7000 cm^{-1}.

Figure 4 represents the first derivative of the spectral data on the first day of measurement, on the most critical day of the disease in the animal and on the last day of measurement. In Figure 4, it was observed that the curves between the healthy animal (not inoculated) and the recovered animal (57 days after inoculation) are similar. While for the sick animal, the curve differed, not only in intensity, but in the regions that influenced the spectra of the previous figures (8000 cm^{-1}, 6500 cm^{-1}, 5000 cm^{-1} and 4500 cm^{-1}). In these regions the derivatives either have opposite signs or have a decrease in the module of the same.

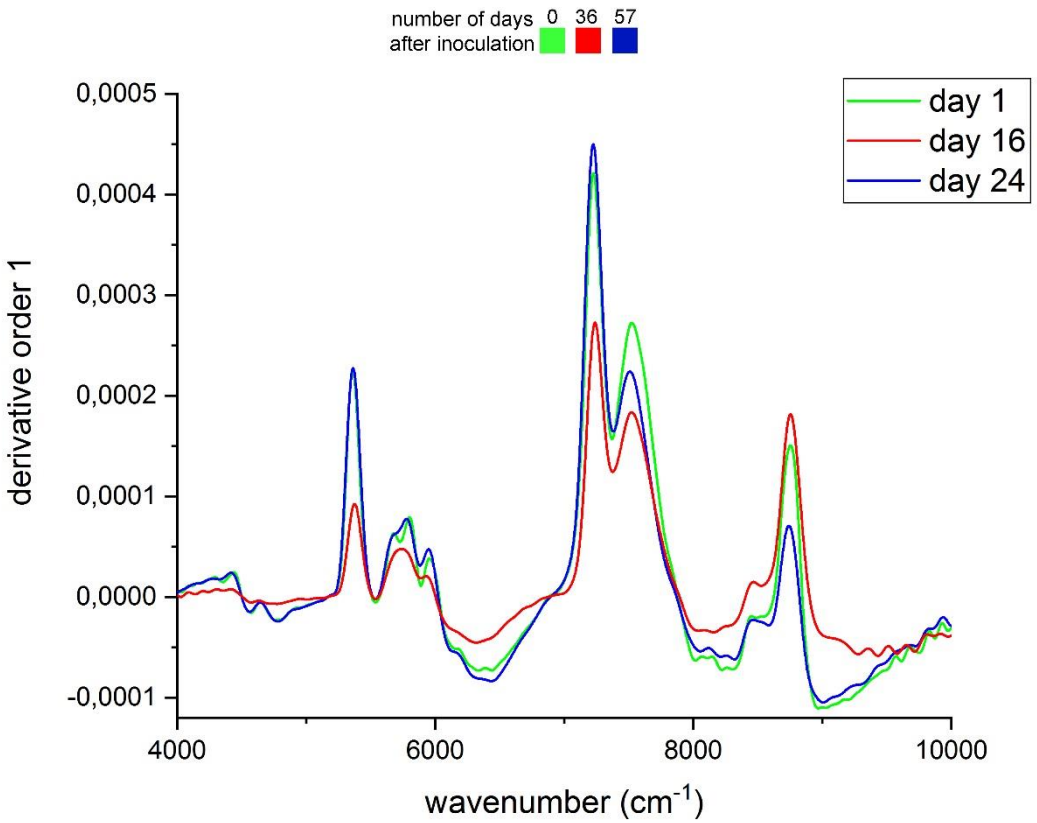

Figure 4: First derivative of spectral data from analyzes carried out on blood samples with EDTA coagulant, from a calf inoculated with anaplasmosis.

4 CONCLUSIONS

The present work showed that FT-NIR spectroscopy through the reflectance spectra of blood samples from a calf inoculated with anaplasmosis was able to monitor the development of the disease in the animal, as well as its recovery. Checked out that the numbers of waves coming 8000 cm^{-1}, 6500 cm^{-1}, 5000 cm^{-1} and 4500 cm^{-1} contributed to predict and identify the day when the animal became ill. The FT-NIR analysis proved to be fast, about 1 minute for each sample, and needs to monitor the disease in the animal. With a larger number of animals it will be possible to find other identification regions and confirm or discard the intensity hypothesis of the spectrum. It will also be possible to perform a static analysis to discriminate between sick animals, healthy animals (control) and those that even if inoculated did not become sick.

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