

## Erythrogram and oxidative stress in confined cattle fed with *Brachiaria sp.* hay and supplemented with antioxidants

*Eritrograma e estresse oxidativo em bovinos confinados alimentados com feno de Brachiaria sp. e suplementados com antioxidantes*

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### Abstract

*Brachiaria sp* contains sporidesmin that can be oxidized by lipoperoxidation and cause oxidative stress. In the present study we evaluated the effects of different antioxidants on lipoperoxidation of erythrocytes from Nelore cattle fed with *Brachiaria sp* hay. The experimental design was entirely randomized, in which 40 whole male cattle were divided into five treatments (G1: control - no supplementation; G2: selenium and vitamin E supplementation; G3: zinc supplementation; G4: selenium supplementation and G5: vitamin E supplementation) and allocated in feedlot pens for 105 days. The samples heparinized and with ethylenediaminetetraacetic acid (EDTA) were obtained every 28 days for hematological and oxidative stress evaluation (0, 28, 56, 84 and 105 days). In the erythrogram total erythrocyte count, hemoglobin, and hematocrit (Ht) were measured. For the evaluation of oxidative stress, in order to analyze the characteristics of the erythrocyte membrane, the thiobarbituric acid reactive substances (TBARS), total glutathione (GSH-T), glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) were determined. The results showed that regardless of the treatment there was no oxidative stress during the experimental confinement period and that the joint association of selenium and vitamin E in the bovine diet provided a lower incidence of deleterious alterations on erythrocytes.

**Key words:** antioxidant enzymes; erythrocyte; reactive oxygen species (ROS); lipoperoxidation; Nelore.

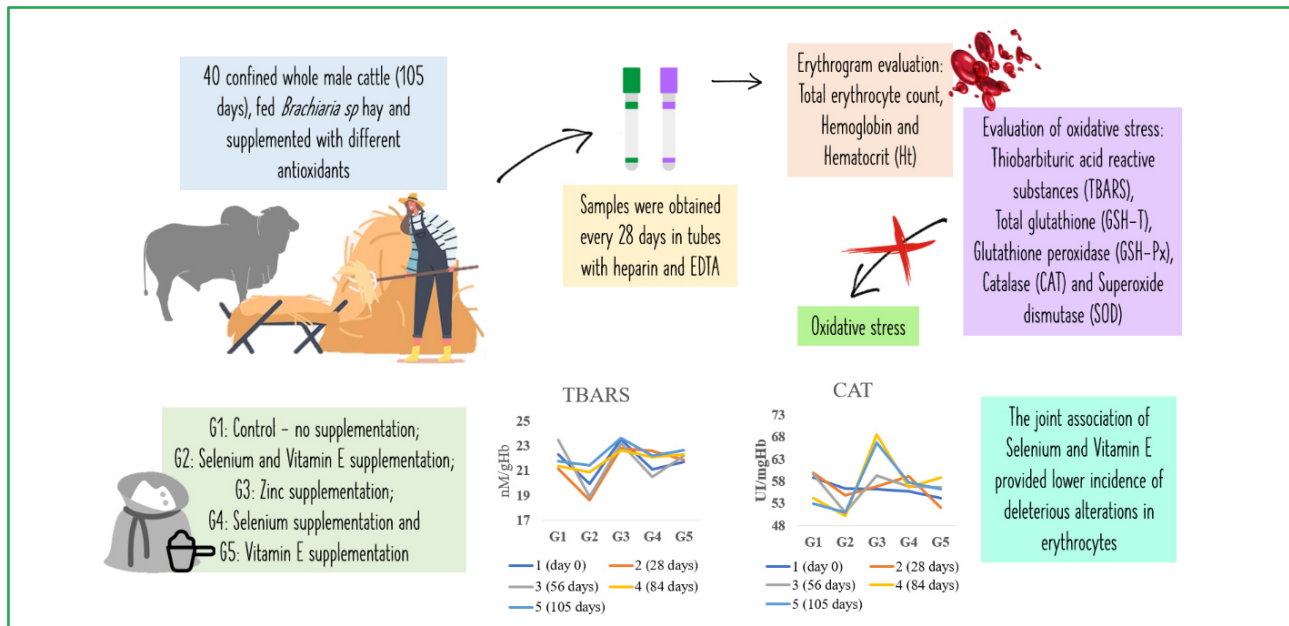
### Resumo

As *Brachiaria sp* contém esporidesminas que podem ser oxidadas por lipoperoxidação e ocasionar estresse oxidativo. No presente estudo foram avaliados os efeitos de diferentes antioxidantes na lipoperoxidação dos eritrócitos de bovinos da raça Nelore, alimentados com feno de *Brachiaria sp*. O delineamento experimental foi inteiramente casualizado, em que 40 bovinos machos, inteiros, foram divididos, em cinco tratamentos (G1: controle - sem suplementação; G2: suplementação de selênio e vitamina E; G3: suplementação de zinco; G4: suplementação de selênio e G5: suplementação de vitamina E) e alocados em baias de confinamento, por 105 dias. As amostras de plasma heparinizado ou com ácido etilenodiaminotetra-acético (EDTA) foram obtidas a cada 28 dias para avaliação hematológica e de estresse oxidativo (0, 28, 56, 84 e 105 dias). No eritrograma foi mensurado a contagem total de eritrócitos, a hemoglobina e o hematócrito (Ht). Para a avaliação do estresse oxidativo, com o objetivo de analisar as características da membrana do eritrócito foram determinadas as substâncias reativas ao ácido tiobarbitúrico (TBARS), glutatona total (GSH-T), glutatona peroxidase (GSH-Px), catalase (CAT) e superóxido dismutase (SOD). Os resultados demonstraram que independente do tratamento não houve estresse oxidativo durante o período de confinamento experimental e que a associação conjunta de selênio e vitamina E na dieta dos bovinos proporcionaram menor incidência de alterações deletérias sobre os eritrócitos.

**Palavras-chaves:** enzimas antioxidantes; eritrócito; espécies reativas de oxigênio (ROS); lipoperoxidação; Nelore.

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**Graphical abstract:** Erythrogram and oxidative stress in confined cattle fed with *Brachiaria sp.* hay and supplemented with antioxidants

## Introduction

Brazilian livestock has been developing a lot in recent years, which resulted in a contribution of 491.2 billion reais to the economy, with an expressive valuation of 24.56% of the Brazilian GDP in 2020<sup>(1)</sup>. One of the competitive advantages of the Brazilian meat production chain is the extensive system, but this system of raising cattle on pasture can lead to some inefficiencies in production<sup>(2)</sup>. To minimize these losses, the incorporation of technologies related to animal nutrition, such as the addition of some microminerals and vitamins to the diet<sup>(3)</sup>, in order to improve the antioxidant and immune response of animals and improve the efficiency of the production chain, focusing on sustainability<sup>(4,5)</sup>.

The species of the *Brachiaria* genus are important forages considered as the salvation of national livestock<sup>4</sup>. However, some species such as *B. brizantha*, *B. humidicola*, and, especially, *B. decumbens* have been described as causing hepatogenous photosensitization in ruminants<sup>(6)</sup>. The main cause of hepatogenous photosensitization in cattle is the ingestion of the toxin sporidesmin, present in the spores of the fungus *Pithomyces chartarum*<sup>(7,8)</sup>.

The main toxic effect of sporidesmin is due to the occurrence of lipid peroxidation and protein carboxylation present in the cells, resulting from the excessive amount of free radicals<sup>(9)</sup>. However, some researchers have attributed the cause of intoxication to the lithogenic steroid saponins present in *Brachiaria sp.*<sup>(10,11)</sup>. These saponins, when metabolized in the animal

organism, form insoluble salts that are deposited as crystals in the biliary system<sup>(12)</sup>. These crystals cause inflammation and obstruction of the biliary system, causing necrosis of periportal hepatocytes and resulting in jaundice, photosensitization, and hepatitis<sup>(13)</sup>.

Oxidative stress can be defined as an imbalance between oxidants and antioxidant in favor of oxidants<sup>(14)</sup>, causing changes in redox balance and control or molecular damage, with consequent functional alteration and impairment of vital functions in several organs and tissues<sup>(15)</sup>. From a functional point of view, oxidative damage promotes alterations in fluidity, permeability, and metabolic function, which results in an increase in the fragility of the erythrocyte membrane<sup>(9)</sup>. Among mammals, bovines have a particularity, in which they have a lower susceptibility to the action of free radicals<sup>(16)</sup>, due to the composition and organization of the erythrocyte membrane that contains low amounts of phosphatidylcholine<sup>(17,18)</sup>, a highly peroxidable phospholipid<sup>(19)</sup>.

Due to the oxygen transport carried out by hemoglobin, erythrocytes are constantly exposed to reactive oxygen species (ROS)<sup>(19)</sup>. The erythrocyte presents in its membrane many sulfhydryl groups, which, when oxidized, results in denaturation of membrane proteins. In this process, intracellular damage can occur, with oxidation of hemoglobin to methemoglobin, which precipitates and forms Heinz bodies<sup>(18,20,21)</sup>.

To prevent the damage caused by peroxidation, cells have an antioxidant defense system consisting of the

enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) <sup>(22)</sup>. The non-enzymatic antioxidant agents consist of ascorbic acid, vitamin E, glutathione reductase (GSH-Rd), reduced glutathione (GSH), carotenes, uric acid, transition metal-chelating proteins, among others<sup>(23)</sup>.

One of the methods for evaluating lipid peroxidation in the biomembranes of erythrocytes is the measurement of thiobarbituric acid reactive substances (TBARS) that quantifies the content of malondialdehyde (MDA) generated in lipid peroxidation of polyunsaturated fatty acids<sup>(24,25,26)</sup>.

The use of antioxidants added to the feed offered to animals has been an efficient option for improving the production system. Its use has provided the animals with improvements in health, reproduction, and production (feed conversion and weight gain) <sup>(27,28)</sup>.

In this context, this present study aimed to evaluate whether the supplementation of different sources of antioxidants in the diet of confined cattle fed *Brachiaria sp.* hay could reduce the occurrence of oxidative stress on erythrocytes.

## Material and methods

The project was approved by the Ethics Committee on Use of Animals (CEUA) of the Federal University of Goiás (UFG) and was registered under number 360/2010. The study was developed at Fazenda Tomé Pinto, owned by the School of Veterinary and Animal Science at UFG, located in the municipality of São Francisco de Goiás, State of Goiás, 110 km from the capital.

Forty uncastrated male cattle were used, with an average initial weight of 360 kg and age between 24 and 36 months, raised on *Brachiaria sp.* pastures. The animals were divided into five groups, with eight animals each, which received the following treatments: Group 1 (G1 = CG) – control (no supplementation); Group 2 (G2 = Se + Vit. E) – supplementation with 2g  $\alpha$ -tocopherol acetate (1000 UI Vitamin E/animal/day) and 10g methionine selenium (10 mg selenium/animal/day); Group 3 (G3 = Zn) – supplementation with 6g in the form of zinc methionine (600mg zinc/animal/day); Group 4 (G4 = Se) – supplementation with 10g methionine selenium (10mg selenium/animal/day) and Group 5 (G5 = Vit. E) – supplementation with 2g  $\alpha$ -tocopherol acetate (1000 UI Vitamin E/animal/day).

After the groups were formed, the animals were taken to their respective enclosures, with an area of 15m<sup>2</sup>/animal, with shaded area, troughs and drinking fountains, and diet twice a day, at 8:00am and 3:00pm. Feed (*Brachiaria sp.* hay) and water were supplied *ad libitum*. The concentrate (feed) was balanced according to the bromotological analysis of the hay offered (Chart 1),

based on an estimated daily gain of 0.880 kg/day and to meet the nutritional requirements of the animals<sup>(29)</sup>. The formulated concentrate (Integral Nutrição Animal, Goiânia, GO, Brazil) had a concentration of 34% protein and 71% of total digestible nutrients (NDT), being the main constituent's soybean meal, corn meal, and a mineral mixture (Chart 2). The cattle remained confined for 119 days, with 14 days of adaptation and 105 days of experiment.

**Chart 1** – Means values of the bromotological composition of *Brachiaria sp.* hay used in the experiment

Parameters	%	SD
Dry matter	94.43	2.34
Total digestible nutrients	55.13	14.29
Crude protein	1.43	0.27
Acid detergent fiber	37.30	8.51
Neutral detergent fiber	72.36	5.06

**Chart 2** – Composition of the total ration formulated based on the dry matter used to feed the animals in the different experimental groups<sup>\*</sup>.

Ingredients	% in dry matter
<i>Brachiaria sp.</i> hay	70.0
Ground corn	20.5
Soybean meal 45%	7.5
Minerals <sup>†</sup>	2.0

<sup>\*</sup>The formulation was performed using RLM 3.2 software (2009)

<sup>†</sup>Minerals without urea and ionophore

During the experimental period, antioxidants supplementation was added to the diet for a period of 105 days. The antioxidants were weighed daily on precision scale (Shimadzu® AY 220), in disposable cups, so that each animal received the daily amount of antioxidant, according to its treatment in the trough. To improve consumption of the amount supplied, a small amount of feed was placed on the antioxidant, with individual supply per animal. The animals were monitored until the antioxidant and concentrate were fully ingested, preventing any other bovine from approaching the trough. The food was provided as a total diet, and the leftover food was weighed and discarded daily before the morning feeding, to determine food consumption by the group and to avoid that the leftovers did not exceed more than 10% of the total provided.

For sample collection, the cattle were kept at station and restrained in the stall. Blood samples were obtained every 28 days (0, 28, 56, 84, and 105 days) by jugular puncture in tubes containing 10% ethylenediaminetetraacetic acid, disodium salt (EDTA) and tubes with heparin.

After collection, the tubes were stored in thermal boxes at 10°C and sent to the laboratory for a maximum period of two hours to perform the erythrogram and obtain the hemolysate. After obtaining aliquots to measure TBARS and antioxidants: SOD, CAT, thiol groups and glutathione), the samples were stored in the freezer at -80°C.

The samples destined for the erythrogram were processed as they arrived at the laboratory, not exceeding six hours from the time of collection. The erythrocyte count and hemoglobin were evaluated in a semiautomatic equipment (BC 2800 VET, Mindray, Shenzhen). The hematocrit was obtained by microcentrifugation using the microhematocrit technique<sup>(30)</sup>.

TBARS analysis in the samples was determined according to the methodology proposed by Esterbauer and Cheeseman<sup>(31)</sup>. GST and GSH-Px were determined by the techniques described by Tietze<sup>(32)</sup> and Bayoumi and Rosalk<sup>(33)</sup>, respectively. SOD activity was performed according to the procedures proposed by Beutler<sup>(34)</sup>. CAT was established by the method proposed by Aebi<sup>(35)</sup>.

The data were submitted to descriptive statistics and were later analyzed by analysis of variance (ANOVA), using Tukey's test, by means of the Statistical Analysis System software (SAS v.9.3, Cary, North Carolina), with a 5% significance level.

## Results

The mean values of erythrocytes, hemoglobin, and hematocrit are described in Table 1. There was no difference in the supplementation between the experimental groups

( $p < 0.05$ ). The results from the fourth collection were discarded because the equipment was not properly calibrated on the day the laboratory was performed.

In the hematological evaluation (erythrocytes, hemoglobin and hematocrit) numerical alterations were found in the parameters measured, but these alterations did not produce differences between the different treatments. The differences were observed only with in each group, between the experimental times. At the 2nd collection, the erythrocyte count in G4 showed lower results than G1 and G3 ( $p < 0.05$ ). In the determination of hemoglobin, in this same collection, there were significant variations ( $p < 0.05$ ) in which the highest value was found in G3 and the lowest in G4. The hematocrit did not show significant variations ( $p > 0.05$ ) in the experimental period.

In the TBARS determination (Table 2) when analyzing the collections performed, it was verified that there was an interaction groups/time ( $p < 0.05$ ), in which G2 presented lower results in relation to the other groups 28, 56 and 105 days. Differences were identified ( $p < 0.05$ ) between the means of the evaluated, so that G3 showed the highest mean TBARS concentration among the treatments and G2 the lowest. and G2 the lowest value. The marginal means between collections were similar ( $p > 0.05$ ).

In the enzymatic activity of GSH-T (Table 3), no interaction was observed in relation group/time ( $p > 0.05$ ). There was a significant difference between group means of the groups ( $p < 0.05$ ), in G5 had the lowest mean value and G1 had the highest mean value. However, between collection there was no significant difference ( $p > 0.05$ ).

**Table 1.** Means X = mean at each time and Mean = general mean considering all times of erythrocytes, hemoglobin, and hematocrit of Nelore cattle fed *Brachiaria sp.* hay and supplemented with antioxidants (G1 – control group, G2 – selenium and vitamin E group, G3 – zinc group, G4 – selenium group, and G5 – vitamin E group)

Collection	Erythrocytes (x10 <sup>9</sup> /ul)		Hemoglobin (g/dL)		Hematocrit (%)	
	X̄	Means	X̄	Means	X̄	Means
G1	1 (Day 0)	7.74		11.96		36.48
	2 (28 days)	8.21 <sup>A*</sup>	7.81 <sup>a</sup>	11.95 <sup>AB*</sup>	11.37 <sup>a</sup>	39.24 <sup>A*</sup>
	3 (56 days)	8.01 <sup>A*</sup>		10.99 <sup>A*</sup>		38.84 <sup>A*</sup>
	5 (105 days)	7.27 <sup>A*</sup>		10.58 <sup>A*</sup>		36.15 <sup>A*</sup>
1 (Day 0)	7.33			11.78		
G2	2 (28 days)	7.44 <sup>AB*</sup>	7.35 <sup>a</sup>	11.06 <sup>AB*</sup>	11.05 <sup>a</sup>	36.68 <sup>A*</sup>
	3 (56 days)	7.49 <sup>A*</sup>		10.61 <sup>A*</sup>		37.99 <sup>A*</sup>
	5 (105 days)	7.12 <sup>A*</sup>		10.73 <sup>A*</sup>		36.91 <sup>A*</sup>
	1 (Day 0)	7.51				11.74
G3	2 (28 days)	8.42 <sup>A*</sup>	7.92 <sup>a</sup>	12.32 <sup>A*</sup>	11.44 <sup>a</sup>	40.95 <sup>A*</sup>
	3 (56 days)	7.85 <sup>A*</sup>		10.74 <sup>A*</sup>		38.55 <sup>A*</sup>
	5 (105 days)	7.58 <sup>B*</sup>		11.01 <sup>B*</sup>		38.60 <sup>A*</sup>
	1 (Day 0)	7.45				11.65
G4	2 (28 days)	7.33 <sup>B*</sup>	7.38 <sup>a</sup>	10.35 <sup>B*</sup>	10.68 <sup>a</sup>	34.41 <sup>A*</sup>
	3 (56 days)	7.47 <sup>A*</sup>		10.24 <sup>A*</sup>		35.96 <sup>A*</sup>
	5 (105 days)	7.26 <sup>A*</sup>		10.46 <sup>A*</sup>		36.38 <sup>A*</sup>
	1 (Day 0)	8.09				12.45
G5	2 (28 days)	7.91 <sup>AB*</sup>	7.84 <sup>a</sup>	11.06 <sup>AB*</sup>	11.42 <sup>a</sup>	36.66 <sup>A*</sup>
	3 (56 days)	8.12 <sup>A*</sup>		11.05 <sup>A*</sup>		39.11 <sup>A*</sup>
	5 (105 days)	7.56 <sup>A*</sup>		11.11 <sup>A*</sup>		37.80 <sup>A*</sup>
	Means	7.66				11.19
Probability	0.471		0.528		0.215	

Means followed by different lowercase letters in the columns indicate a significant difference between experimental groups. Means followed by different uppercase letters with an asterisk (\*) in the columns indicate a significant difference between experimental groups as a function of collection.

**Table 2.** Means of the concentration of thiobarbituric acid reactive substances (nM/gHb) in erythrocytes of Nelore cattle fed *Brachiaria sp.* hay and supplemented with antioxidants (G1 – control group, G2 – selenium and vitamin E group, G3 – zinc group, G4 – selenium group and G5 – vitamin E group)

Collection	G1	G2	G3	G4	G5	Mean	Group	Time	Interaction
1 (Day 0)	22.38	19.98	23.58	21.13	21.72	21.76 <sup>a</sup>			
2 (28 days)	21.18 <sup>A*</sup>	18.62 <sup>B*</sup>	22.88 <sup>A*</sup>	22.63 <sup>A*</sup>	21.90 <sup>A*</sup>	21.44 <sup>a</sup>			
3 (56 days)	23.54 <sup>A*</sup>	18.93 <sup>C*</sup>	23.26 <sup>A*</sup>	20.55 <sup>BC*</sup>	22.23 <sup>AB*</sup>	21.70 <sup>a</sup>	<0.0001	0.1079	0.001
4 (84 days)	21.44 <sup>A*</sup>	20.91 <sup>A*</sup>	22.68 <sup>A*</sup>	22.12 <sup>A*</sup>	22.38 <sup>A*</sup>	21.90 <sup>a</sup>			
5 (105 days)	21.80 <sup>B*</sup>	21.45 <sup>B*</sup>	23.68 <sup>A*</sup>	22.23 <sup>AB*</sup>	22.69 <sup>AB*</sup>	22.37 <sup>a</sup>			
Mean	21.99 <sup>B</sup>	19.98 <sup>C</sup>	23.12 <sup>A</sup>	21.88 <sup>B</sup>	22.30 <sup>AB</sup>				

Means followed by different capital letters in the lines indicate a significant difference between experimental groups. Means followed by different lowercase letters in the columns indicate a significant difference between collections. Means followed by different capital letters with an asterisk (\*) in the lines indicate a significant difference between experimental groups as a function of collection.

**Table 3.** Means of total glutathione ( $\mu\text{M/gHb}$ ) in erythrocytes of Nelore cattle fed *Brachiaria sp.* hay and supplemented with antioxidants (G1 – control group, G2 – selenium and vitamin E group, G3 – zinc group, G4 – selenium group and G5 – vitamin E group)

Collection	G1	G2	G3	G4	G5	Mean	Group	Time	Interaction
1 (Day 0)	42.58	42.85	44.86	44.19	41.18	43.13 <sup>a</sup>			
2 (28 days)	43.71 <sup>A*</sup>	42.63 <sup>A*</sup>	43.13 <sup>A*</sup>	42.04 <sup>A*</sup>	41.03 <sup>A*</sup>	42.51 <sup>a</sup>			
3 (56 days)	45.26 <sup>A*</sup>	40.97 <sup>A*</sup>	46.09 <sup>A*</sup>	44.27 <sup>A*</sup>	40.45 <sup>A*</sup>	43.41 <sup>a</sup>	0.0112	0.4867	0.9196
4 (84 days)	45.83 <sup>A*</sup>	42.01 <sup>A*</sup>	43.78 <sup>A*</sup>	45.48 <sup>A*</sup>	42.54 <sup>A*</sup>	43.93 <sup>a</sup>			
5 (105 days)	43.62 <sup>A*</sup>	42.91 <sup>A*</sup>	42.81 <sup>A*</sup>	44.18 <sup>A*</sup>	39.05 <sup>A*</sup>	42.52 <sup>a</sup>			
Mean	44.60 <sup>A</sup>	42.13 <sup>AB</sup>	43.95 <sup>AB</sup>	44.00 <sup>AB</sup>	40.77 <sup>B</sup>				

Means followed by different capital letters in the lines indicate a significant difference between experimental groups. Means followed by different lowercase letters in the columns indicate a significant difference between collections. Means followed by different capital letters with an asterisk (\*) in the lines indicate a significant difference between experimental groups as a function of collection.

In the determination of the enzymatic activity of GSH-Px there was an interaction regarding group/time ( $p < 0.05$ ), with a significant difference at all times of collection. There was a significant difference ( $p < 0.05$ ) between the experimental groups, with G3 showing the

lowest mean value and G2 the highest (Table 4). Considering the times of collection, the highest means were found on day 56 and the lowest on days 28 and 84 ( $p < 0.05$ ), however, without significant difference ( $p > 0.05$ ) between the beginning and the end of the study.

**Table 4.** Means of glutathione peroxidase (UI/gHb) in erythrocytes of Nelore cattle fed *Brachiaria sp.* hay and supplemented with antioxidants (G1 – control group, G2 – selenium and vitamin E group, G3 – zinc group, G4 – selenium group and G5 – vitamin E group)

Collection	G1	G2	G3	G4	G5	Mean	Group	Time	Interaction
1 (Day 0)	0.67	0.82	0.58	0.76	0.71	0.71 <sup>ab</sup>			
2 (28 days)	0.66 <sup>ABC*</sup>	0.79 <sup>A*</sup>	0.53 <sup>C*</sup>	0.63 <sup>BC*</sup>	0.67 <sup>AB*</sup>	0.66 <sup>b</sup>			
3 (56 days)	0.69 <sup>AB*</sup>	0.83 <sup>A*</sup>	0.61 <sup>B*</sup>	0.75 <sup>AB*</sup>	0.87 <sup>A*</sup>	0.75 <sup>a</sup>	<0.0001	0.0022	0.0352
4 (84 days)	0.62 <sup>B*</sup>	0.86 <sup>A*</sup>	0.52 <sup>B*</sup>	0.67 <sup>B*</sup>	0.68 <sup>B*</sup>	0.67 <sup>b</sup>			
5 (105 days)	0.72 <sup>AB*</sup>	0.82 <sup>A*</sup>	0.60 <sup>B*</sup>	0.70 <sup>AB</sup>	0.79 <sup>A*</sup>	0.73 <sup>ab</sup>			
Mean	0.68 <sup>B</sup>	0.82 <sup>A</sup>	0.56 <sup>C</sup>	0.68 <sup>B</sup>	0.75 <sup>AB</sup>				

Means followed by different capital letters in the lines indicate a significant difference between experimental groups. Means followed by different lowercase letters in the columns indicate a significant difference between collections. Means followed by different capital letters with an asterisk (\*) in the lines indicate a significant difference between experimental groups as a function of collection.

In the measurement of SOD interaction was observed in relation group/time ( $p < 0.05$ ) in the mean values, there was significant difference between the experimental groups 56, 84 and 105 days (Table 5). Differences were identified ( $p < 0.05$ ) among the groups

evaluated, in which G3 presented the mean value lower than the other treatments, and G1 and G4 presented the highest values. The marginal means between collection were similar ( $p > 0.05$ ).

**Table 5.** Means of superoxide dismutase (UI/mgHb) in erythrocytes of Nelore cattle fed *Brachiaria sp.* hay and supplemented with antioxidants (G1 – control group, G2 – selenium and vitamin E group, G3 – zinc group, G4 – selenium group and G5 – vitamin E group)

Collection	G1	G2	G3	G4	G5	Mean	Group	Time	Interaction
1 (Day 0)	276,38	280,38	267,25	264,5	265	270,70 <sup>a</sup>			
2 (28 days)	278,12 <sup>A*</sup>	265,37 <sup>A*</sup>	253,87 <sup>A*</sup>	275,12 <sup>A*</sup>	266,62 <sup>A*</sup>	267,82 <sup>a</sup>			
3 (56 days)	290,87 <sup>A*</sup>	279,87 <sup>AB*</sup>	255,00 <sup>C*</sup>	298,37 <sup>A*</sup>	266,00 <sup>BC*</sup>	278,02 <sup>a</sup>	<0,0001	0,0583	0,0125
4 (84 days)	297,00 <sup>A*</sup>	274,87 <sup>AB*</sup>	246,75 <sup>C*</sup>	297,25 <sup>A*</sup>	268,50 <sup>BC*</sup>	276,87 <sup>a</sup>			
5 (105 days)	284,50 <sup>AB</sup>	279,50 <sup>AB*</sup>	239,50 <sup>C*</sup>	304,00 <sup>A*</sup>	274,25 <sup>B*</sup>	276,35 <sup>a</sup>			
Média	287,63 <sup>A</sup>	274,90 <sup>B</sup>	248,78 <sup>C</sup>	293,69 <sup>A</sup>	268,84 <sup>B</sup>				

Means followed by different capital letters in the lines indicate a significant difference between experimental groups. Means followed by different lowercase letters in the columns indicate a significant difference between collections. Means followed by different capital letters with an asterisk (\*) in the lines indicate a significant difference between experimental groups as a function of collection.

The determination of CAT showed interaction in relation group/time ( $p < 0.05$ ) in the experimental groups only at 84 and 105 days (Table 6). It was found that in G3, the mean value was higher ( $p < 0.05$ ) than the other

treatments, while G2 presented the lowest values ( $p < 0.05$ ). The marginal means between collection were similar ( $p > 0.05$ ).

**Table 6.** Means of catalase (UI/mgHb) in erythrocytes of Nelore cattle fed *Brachiaria sp.* hay and supplemented with antioxidants (G1 – control group, G2 – selenium and vitamin E group, G3 – zinc group, G4 – selenium group, and G5 – vitamin E group)

Colheitas	G1	G2	G3	G4	G5	Média	Grupo	Tempo	Interação
1 (Day 0)	58,91	56,53	56,27	55,83	54,28	56,36 <sup>a</sup>			
2 (28 dias)	60,08 <sup>A*</sup>	54,87 <sup>A*</sup>	56,92 <sup>A*</sup>	59,28 <sup>A*</sup>	52,17 <sup>A*</sup>	56,66 <sup>a</sup>			
3 (56 dias)	59,78 <sup>A*</sup>	51,10 <sup>A*</sup>	59,36 <sup>A*</sup>	56,89 <sup>A*</sup>	56,84 <sup>A*</sup>	56,80 <sup>a</sup>	<0,0001	0,8829	0,0087
4 (84 dias)	54,32 <sup>A*</sup>	50,33 <sup>A*</sup>	68,73 <sup>B*</sup>	56,80 <sup>A*</sup>	58,95 <sup>AB*</sup>	57,82 <sup>a</sup>			
5 (105 dias)	53,13 <sup>A*</sup>	51,07 <sup>A*</sup>	66,88 <sup>B*</sup>	57,81 <sup>A*</sup>	56,33 <sup>A*</sup>	57,04 <sup>a</sup>			
Média	56,82 <sup>B</sup>	51,84 <sup>C</sup>	62,97 <sup>A</sup>	57,70 <sup>AB</sup>	56,07 <sup>BC</sup>				

Means followed by different capital letters in the lines indicate a significant difference between experimental groups. Means followed by different lowercase letters in the columns indicate a significant difference between collections. Means followed by different capital letters with an asterisk (\*) in the lines indicate a significant difference between experimental groups as a function of collection.

## Discussion

The erythrocyte profile of the animals during the whole experimental remained within the reference limits<sup>(36)</sup>. In previous studies with cattle fed *Brachiaria sp.* grass, similar results to the hematological profile for erythrocyte count<sup>(37)</sup> and hemoglobin concentration were observed for animals receiving selenium<sup>(9)</sup> and vitamin E supplementation in the feed<sup>(38)</sup>. However, divergent results were observed in the study<sup>(39)</sup> using supplementation of different selenium sources (organic and inorganic) in dairy cow diets, where mean erythrocyte, hemoglobin and hematocrit values were higher than those obtained in the control group. Assays evaluating erythrocyte oxidative damage<sup>(37)</sup> concluded that, antioxidant association promotes less erythrocytes alterations with potential benefits of treatment by antioxidants supplementation on erythrocytes.

The increase in the mean values erythrocytes in G3 could be explained by the function that zinc plays in maintaining the integrity of erythrocyte cell membranes<sup>(40)</sup>. The action of this microelement favors the association of proteins present in the membrane with other components of the cell cytoskeleton<sup>(41)</sup>, which in turn provides antioxidants protection to the membranes against lipid and protein oxidation<sup>(42)</sup> and antagonizes possible deleterious effects by ionic elements<sup>(43)</sup>.

The measurement of TBARS in the erythrocytes throughout the experiment showed that during the collection the means of the animals did not present significant difference. However, there was a significant difference between the proposed treatments ( $p < 0.05$ ). Malondialdehyde (MDA) is one of the main markers of lipid peroxidation and it is used to measured TBARS. The increased blood concentration of this marker is related to

an increase lipid peroxidation and oxidative damage<sup>(44)</sup>. Animals that received selenium and vitamin E supplementation showed higher lipid stability. The animals that received selenium and vitamin E showed higher lipid stability. In an experiment with isolated and associated supplementation of selenium and vitamin E in cattle<sup>(45)</sup>, an improvement in the redox state of the blood was observed when association of antioxidants occurred.

The values obtained in the measurement of TBARS in the supplemented groups showed results similar to those observed in a study with Simmental cattle<sup>(46)</sup> aged around 20 months fed with feed and roughage. Tests on the influence of some antioxidants (zinc and vitamin E, isolated and associated) on lipid peroxidation in the blood of cattle<sup>(47)</sup>, it was observed that the association of antioxidants resulted in lower lipid peroxidation indices. However, the results obtained in the present study were higher than those found in the literature in animals supplemented with antioxidants when evaluating lipid peroxidation in bovines<sup>(38,48-50)</sup>. This fact probably occurred because different methodologies and concentration units to measure MDA as an indicator of oxidative stress.

It is also worth mentioning that the maintenance of GSH-t levels was due to the fact that the glutathione in higher concentration in most cells, including mammalian erythrocytes, is in the reduced form (GSH). The depletion of GSH levels can occur directly, by conjugation with free radicals, and indirectly by inhibitors of its synthesis and regeneration<sup>(51,52)</sup>.

As the consumption of GSH probably occurred only because of its antioxidant property, the permanence of high GSH concentrations in erythrocytes contributed to the maintenance of GSH-T levels. Allied to this, the maintenance of GSH-T values may have also occurred due to the "compensatory increase" in GSH production by other organs, in an attempt to help the body, combat the increase in ROS production<sup>(53)</sup>. This excessive generation of ROS may increase the degree of lipid peroxidation and cell damage. Similar behavior to what occurred in this study was reported by other authors<sup>(45)</sup>, when they described that the associations of selenium and vitamin E provided to the analyzed bovines a synergism between the effects of antioxidants administered resulting in less deleterious effects on erythrocyte cells.

Also regarding GSH, the results obtained in this assay for the isolated supplementation of selenium and vitamin E were lower than those found in an evaluation of the benefits of selenium supplementation in cattle<sup>(9)</sup> on the erythrocyte membrane stability. In an experiment to analyze the effects of vitamin E supplementation in cows in transition<sup>(38)</sup>, they found higher results and a negative correlation between enzyme activity and lipid peroxidation marker. It is believed that this divergence occurred due to the gender, the not concentration of

antioxidant supplied and the physiological state of the animals, besides the methodology employed, which the use of commercial reagents different from those employed in this study.

The means of SOD between the collection showed significant difference ( $p < 0.05$ ) for the analyzed groups due to the variation in the concentration of  $H_2O_2$  caused by the action of SOD, indicating that there was activation of the glutathione-reducing cycle, in which the groups supplemented with selenium and vitamin E (G2), selenium (G4), and vitamin E (G5) showed increasing values throughout the collection. Researchers working with measurements of oxidative stress in cattle<sup>(54)</sup> on hay-based diet, verified through activities of SOD and CAT similar results to this study, which demonstrates that high concentrations of  $H_2O_2$  do not produce an imbalance with the antioxidants. In an evaluation of the influence of metabolic disorders on oxidative stress<sup>(55)</sup>, they verified that the inclusion of vitamin E did not provide alterations in SOD and GSH-Px.

The evaluation of the enzymatic activity of CAT promoted an increase in the means of the group that received zinc supplementation (G3) at 84 a 105 days. This enzyme plays an important role in the evaluation of oxidative stress, as it acts to neutralize  $H_2O_2$  at high concentrations, formed by the dismutation of the  $O_2^-$  radical promoted by SOD. However, in low concentrations of this free radical, GSH acts in the neutralization of  $H_2O_2$ <sup>(51)</sup>. Some authors<sup>(56)</sup> have reported the importance of determining the interrelationship between the activities of these enzymes to evaluate the defense mechanisms of antioxidants present in the body. The measurement of the activity of the enzymes SOD, GSH-Px, and CAT is commonly used to assess the defense capacity of the organism against the action of ROS<sup>(57)</sup>. The observations of variations between collection for CAT were analogous to the study for ruminants<sup>(50)</sup>, indicating that this variation is probably attributed to the stress condition.

The joint evaluation of the oxidative stress biomarkers evaluated in this study reveals that erythrocytes did not under go lipid peroxidation, which was mainly confirmed by the no change in TBARS values in erythrocyte cells.

## Conclusion

The supplementation of confined Nelore cattle fed with *Brachiaria sp.* hay with, containing antioxidants, in form isolated (zinc, selenium, and vitamin E) or associated (selenium and vitamin E), does not alter the oxidative stress in erythrocytes.

## Conflict of interest declaration

The authors declare that there is no conflict of interest.

## Author Contributions

*Data curation:* R. D. da S. Cunha, G. L. Costa, U. R. C. Pinto, J. J. S. Ferezin; *Formal Analysis:* R. D. da S. Cunha, P. H. J. da Cunha, M. C. S. Fioravanti; *Funding acquisition:* M. C. S. Fioravanti; *Investigation:* R. D. da S. Cunha, G. L. Costa, U. R. C. Pinto, J. J. S. Ferezin; *Project administration:* R. D. da S. Cunha, G. L. Costa; *Resources:* P. H. J. da Cunha, M. C. S. Fioravanti; *Supervision:* P. H. J. da Cunha, M. C. S. Fioravanti; *Validation:* R. D. da S. Cunha, J. J. S. Ferezin; *Visualization:* R. D. da S. Cunha, P. H. J. da Cunha, M. C. S. Fioravanti. *Writing – original draft:* R. D. da S. Cunha, P. H. J. da Cunha, M. C. S. Fioravanti.

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