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ANIMAL PRODUCTION

Changes in hematological biomarkers of Nellore cows at different reproductive stages

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ABSTRACT. This study was conducted to evaluate changes in hematological biomarkers of Nellore cows at different reproductive stages. Fifteen multiparous cows with 4 ± 1 years of age and live weight of 400 ± 50 kg were used at different stages (non-pregnant, in the initial, middle and late pregnancy, at birth, one day postpartum, 30 and 60 days postpartum). Blood collections were performed every 30 days and assayed for the following hematological biomarkers: hemogram (red blood cells, globular volume, hemoglobin, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)) and leukogram (total and differential counts). The erythrogram was influenced ($p < 0.001$) by the reproductive stages, with variation in the results obtained for the total red cell count, hemoglobin content, globular volume, MCV, MCHC and total plasma proteins. The reproductive stages influenced the leukocyte profile with variation in the total leukocyte count and in the absolute values of neutrophils, eosinophils, lymphocytes and monocytes. Hematological biomarkers of Nellore cows showed significant changes due to the physiological adjustments required in response to the metabolic requirements imposed in the final stage of pregnancy and in the beginning of lactation, ensuring the nutritional contribution in energy and oxygen transported through the blood.

Keywords: blood; bovine; erythrogram; leukogram; postpartum; prepartum.

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Introduction

The adoption of specialized breeds in meat production by farmers is one of the main determining factors for the development of beef cattle in Brazil. In this context, the Nellore breed emerges as the most adopted by Brazilian breeders for meat production, satisfying the needs of the production systems, presenting particular characteristics as high productive and reproductive indexes in the different conditions of production, linked to good adaptability and rusticity (Ferraz & Felício, 2010). However, the performance of the animals does not depend only on the adaptive factors, since the reproductive period especially during the pregnancy can cause important physiological changes in biomarkers, mainly, in situations of high metabolic demand in detriment to the high blood volume required by the uterus during the pregnancy period (Abuelo, Hernández, Benedito, & Castillo, 2015). During pregnancy in mammals, mainly in the final third of pregnancy, is characterized by high foetal growth (approximately 80%). Still, close to parturition, more specifically, the transition period (last three weeks of pregnancy and the first three weeks of lactation) may occur many physiological changes such as preparation for calving, preparation of the mammary gland for the production of colostrum and peak of milk production. The physiological changes occur generating high energy and protein demand for maternal maintenance and fetal development, increasing the nutrients requirements that promote changes in blood parameters (Gravena et al., 2010).

In the last years, researches have been directed to the field of veterinary hematology in the search for solutions related to the clinical problems pertinent to the different animal species, in the detection of tissue pathological changes in different physiological stages of the animal's life, as well as the factors causing its variations. Studies of blood components besides demonstrating the health state can be used as a tool for monitoring physiological changes occurring in animals. Thus, among these factors that cause changes in hematological biomarkers of domestic species, the pregnancy, birth and postpartum period correspond to the physiological phases in which hematological indices may undergo changes in the reference values (Bezerra et al., 2008), being necessary to carry out researches, since few studies with Nellore cows in different physiological reproductive stages are available in the literature.

In this way, it was hypothesized that the advancement of reproductive stages promotes changes in hematological values of Nellore cows and that the knowledgement of these values may help to maintain nutritional status and to avoid modifications that may lead to disorders in this period in both female and foetus. Therefore, the objective of this study was to evaluate the influence of gestation, birth and postpartum on the hematological biomarkers (erythrogram and leukogram) of Nellore cows.

Material and methods

Study location and ethical committee

The experiment was carried out at the experimental bovine farm of Campus Professora Cinobelina Elvas - Federal University of Piauí (UFPI), located in the city of Alvorada do Gurguéia, State of Piauí. This study complied with all ethical principles in research involving animals and it was approved by Bioethics Committee of the UFPI (number 048/2012).

Animals, estrus synchronization, handling, and feeding

Thirty Nellore multiparous cows were used. Before starting the experimental period (specifically three months before), the cows were dewormed using Ivermectin (Ivomec - Merial®, São Paulo, Brasil) and vaccinated against foot-and-mouth disease and Clostridiosis (Bioxell-Vallee® and Sintoxan T-Merial®, São Paulo, Brazil) respectively.

For this study, the cows were submitted to oestrus synchronization and artificially inseminated at fixed time. After 30 days, the pregnancy diagnosis was performed by trans-rectal ultrasonography. Fifteen pregnant animals with 4 ± 1 years of age, and body condition score (BCS) of 7 ± 1 and live weight of 400 ± 50 kg were selected. During the experimental period, the cows remained in paddocks with an area of 8 hectares, formed by pastures of *Panicum maximum* cv. Mombaça and *Brachiaria brizantha* cv. Marandu and Xaraés. In the late afternoon, the cows were collected to provide concentrated supplementation (ground corn, soybean meal and mineral supplement – Fosbovi 20®, Tortuga, São Paulo, Brasil) based on dry matter (Table 1), once daily in a collective trough at the rate of 2 kg for each animal.

Blood collection and analysis

Blood collections were performed every 30 days, totalling 14 collections, being one of those collections was to form the control group (non-pregnant) and the others were performed for evaluation and monitoring of blood parameters: in the pregnancy period (initial, middle and late pregnancy), at birth, one day postpartum, 30 and 60 days postpartum. The blood collection was always performed in the morning before the animals were released to the pasture, by jugular venipuncture, using disposable needles (25 x 0,8 mm, Greinerbio-onne®, Americana, São Paulo, Brasil) and vacutainer glass tubes containing 0.05 mL of ethylenediamine tetraacetic acid (EDTA) to five ml of blood collected. The blood samples were kept in iceboxes until the arrival at the Laboratory of Veterinary Clinical Pathology of the Veterinary Hospital (HVU-CPCE). Being performed the hemogram (global count the number of red blood cells, determination of globular volume, hemoglobin content, Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC), total and differential counts of leukocytes) within 24 hours post collection. The counting of the total number of red blood cells was performed in a Neubauer chamber, with dilution of the cells made in a ratio of 1:200, using a 20-microliter semiautomatic pipette. To determine the globular volume, the microhematocrit technique was used. The determination of the hemoglobin content was performed by the cyanometa-hemoglobin method with previous dilution in Drabkin's solution.

The values obtained by counting the number of red cells, the globular volume and determining the hemoglobin content were used to establish the values of the absolute hematimetric indexes (MCV and MCHC). Regarding to leukogram, the count of the total number of leukocytes was carried out using a Neubauer Chamber, being the blood samples diluted in Turk's liquid at the ratio of 1:20, following the recommendations of Viana et al. (2003). Two blood smears for each sample was performed to differential leukocyte count with the use of Romanowsky-type stains (Panótico Rápido – LABORCLIN® LTDA, Pinhais, Paraná, Brazil), according to standard technique for the animals described by Viana et al. (2003). In each blood smear 100 leukocytes were identified, classified and read in a 1000x magnification microscope, according to their morphological and color characteristics, in neutrophils, eosinophils, basophils, lymphocytes and monocytes. The determination of the Total Plasma Protein (TPP) was performed by refractometry, after centrifugation at 12000 g of capillary blood of micro hematocrit.

Statistical analysis

The experiment was conducted in a completely randomized design, considering as treatment effect the physiological phases (not pregnant, initial third, middle, final of pregnancy, birth, one day after birth and two months postpartum) with 15 repetitions over time. Statistical analyses were performed with SAS Software version 9.1 (Cary, North Carolina), being the means compared by Tukey test at 5% of probability.

Results

The erythrogram was influenced ($p < 0.001$) by the reproductive stages, with variation in the results obtained for the total red cell count, hemoglobin content, globular volume, MCV, MCHC and TPP (Table 2). A high number of red blood cells were observed in the immediate postpartum period (11.19 x 106 uL) compared to the other stages.

The hematocrit differed ($p < 0.001$) among reproductive stages, with lower (31%) and higher (41%) percentage in non-pregnant cows and cows in the first month of postpartum, respectively. The hemoglobin levels were influenced by the reproductive stage ($p < 0.001$) with high values in the final third of pregnancy.

The reproductive stages affected the hematimetric indexes (MCV and MCHC), which the VCM presented high values during pregnancy, at birth and one day postpartum. CHCM was higher during the birth. There was a significant reduction in TPP one day postpartum.

Table 1. Chemical composition and amount of ingredients in concentrate supplied to animals.

Ingredients (g kg^{-1} of DM)	
Ground corn	700
Soybean meal	250
Mineral Supplement[1]	50.0
Concentrate composition (g kg^{-1} of DM)	
Dry matter	83.56
Crude protein	18.56
ether extract	3.36
Neutral detergent fiber	11.90
Acid Detergent Fiber	7.37
Calcium	10.00
Phosphorus	5.00

[1]Supplement mineral: zinc: 1300 mg kg^{-1}; copper: 1530 mg kg^{-1}; manganese: 1300 mg kg^{-1}; iron: 1800; iodine: 75.0 mg kg^{-1}; sulfur: 12.0 g kg^{-1}.

Table 2. Influence of the reproductive stage on the erythrogram of Nellore cows.

Reproductive stage	Erythrogram					
	RBC (x10^6 μL^{-1})	Ht (%)	Hgb (g dL^{-1})	MCV (fL)	MCHC (%)	TPP (g dL^{-1})
Non-pregnant	7.31^{c}	31.00^{c}	11.14^{d}	37.32^{bc}	36.24^{bcd}	7.62^{abc}
Initial pregnancy	8.82^{b}	36.00^{b}	11.69^{cd}	41.54^{ab}	32.01^{e}	7.60^{abc}
Middle pregnancy	8.80^{b}	36.00^{b}	12.56^{bc}	41.74^{ab}	34.26^{cde}	7.72^{ab}
Late pregnancy	8.78^{b}	38.00^{ab}	14.43^{a}	43.67^{a}	37.91^{bc}	7.97^{a}
Parturition	9.11^{b}	40.00^{a}	13.45^{ab}	43.29^{a}	43.68^{a}	7.30^{bc}
1 day postpartum	9.21^{b}	39.00^{a}	13.75^{ab}	43.15^{a}	39.20^{b}	7.15^{c}
30 days postpartum	11.19^{a}	41.00^{a}	13.34^{ab}	35.81^{c}	36.41^{bcd}	7.46^{abc}
60 days postpartum	10.00^{ab}	37.00^{ab}	12.60^{bc}	34.15^{c}	33.17^{de}	7.28^{bc}
Reference[1]	5-10	24-46	8-15	40-60	30-36	7-8,5
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
MSE[2]	1.4920	0.2836	0.1432	0.4803	0.4339	0.0463

*Means followed by different letters in the same column differ statistically ($P < 0.05$) by Tukey test; RBC-Red blood Cells, Ht-Hematocrit, Hgb- Hemoglobin, MCV-Mean corpuscular volume, MCHC-Mean corpuscular hemoglobin concentration, TPP- Total plasma protein, PP-Postpartum. [1]Reference range for adult cattle Weiss and Wardrop (2010). [2]MSE = Mean standard error.

The reproductive stages influenced the leukocyte profile (Table 3) with variation ($p < .001$) in the total leukocyte count and in the absolute values of neutrophils, eosinophils, lymphocytes and monocytes.

Table 3. Influence of the reproductive stage on the leukocyte profile of Nellore cows.

Reproductive stage	Leukogram					
	Leu ($x10^3 \mu L^{-1}$)	Neu (mm^{-3})	Eos (mm^{-3})	Bas (mm^{-3})	Lin (mm^{-3})	Mon (mm^{-3})
Non-pregnant	9363.18^b	4373.2^b	106.67^b	0^a	4603.9^b	279.41^b
Initial pregnancy	13253.31^a	5967.1^a	211.90^b	0^a	6794.4^a	520.50^a
Middle pregnancy	11289.04^b	3876.0^b	226.55^b	0^a	6907.4^a	279.09^b
Late pregnancy	11580.02^{ab}	5397.3^{ab}	606.00^a	0^a	5136.1^{ab}	440.62^{ab}
Parturition	8527.4^b	4149.7^b	112.69^b	0^a	3913.3^b	351.71^{ab}
1 day postpartum	9222.15^b	4087.8^b	133.50^b	0^a	4535.9^b	464.95^{ab}
30 days postpartum	8884.12^b	3884.5^b	164.45^b	0^a	4511.7^b	323.47^{ab}
60 days postpartum	9684.43^b	3951.1^b	86.44^b	0^a	5304.6^{ab}	255.85^b
Reference[1]	4000-12000	600-4000	0-2400	0-200	2500-7500	25-840
P-Value	<.0001	<.0001	<.0001	0	<.0001	0.0023
MSE[2]	285.61	164.25	17.88	0	178.25	20.47

*Means followed by different letters in the same column differ statistically ($p < 0.05$) by Tukey test Leu-Leukocytes, Neu-Neutrophils, Eos-Eosinophils, Bas-Basophils, Lin-Lymphocytes, Monocytes. [1]Reference range for adult cattle Weiss and Wardrop (2010). [2]MSE= Mean standard error.

The total number of leukocytes and the absolute neutrophil values were elevated in the initial and final third of pregnancy. In the final phase of pregnancy, a high number of circulating eosinophils were observed. In the initial (6794.4 mm^{-3}) and medium third (6907.4 mm^{-3}) of pregnancy, a higher number of lymphocytes were observed. Monocyte counts were influenced by reproductive stages ($p < 0.002$**)** with higher absolute values in the initial phase of pregnancy (520.5 mm^{-3}) and one day postpartum (464.9 mm^{-3}). Lowest values were observed in non-pregnant cows (279.4 m^{-3}) and in the second month postpartum (255.9 mm^{-3}).

Discussion

Many hematologic changes were observed with the transition of the reproductive stages. In the immediate postpartum there was increased production of red blood cells, which was above the reference limits. Weiss and Wardrop (2010), demonstrating erythrocytosis at the beginning of uterine involution. However, in the pregnancy and lactation, there was higher production of red blood cells, a consequence of fetal growth and milk production, respectively. The result evidenced for the total RBC count was probably related to the circulatory and endocrine changes, in which cows are imposed in periods of higher metabolic demands, since during the final third of pregnancy increases the uterine demand for the blood supply to guarantee fetal nutrition, expanding the nutritional requirements mainly of oxygen used to the gas exchanges via placental (Abuelo et al., 2015; Gravena et al., 2010).

According to Cunningham (2011), the total RBC count is associated with the need for oxygen in the body, i.e, as demand increases in the body increases the amount of red blood cells, resulting in release of erythropoietin by the kidney tissue. It is stimulated by the action of progesterone and placental chorionic somatotropin, which during late pregnancy increasing the synthesis of circulating RBC (Fonteque et al., 2010; Longo, 1983). Another explanation that fits the situation evidenced for the RBC counts at birth is the possible hemoconcentration not only by the reduction in water intake but also by the greater spleen contraction (Van Soest & Blosser, 1954).

The hematocrit changes showed higher flow of red blood cells cows during the transition period, mainly, in the postpartum. However, these changes were within the reference range for the specie (Weiss & Wardrop, 2010). The values found for the globular volume are associated with high metabolic requirement for the foetus, which intensifies in the final third of pregnancy, where the demand for RBC becomes more pronounced, influencing the hematocrit percentage (Ferreira et al., 2009; Mungen, 2003; Sousa, Sousa, Paula, & Barrêto Júnior, 2011). Besides foetal development, in the third final of pregnancy, intense changes occur in the postpartum caused by the high metabolic requirement that aims to guarantee the supply of nutrients to the mammary tissue for the synthesis of colostrum. This mechanism promotes alterations in physiological responses through the release of cortisol in these phases in detriment of the circulatory changes, leading the pregnant cows to reduce the blood plasma volume increasing hematocrit concentration (Ingvartsen & Andersen, 2000; Sousa et al., 2011).

Increased hemoglobin levels in the final third of pregnancy may be related to increased oxygen flow due to foetal growth and cow requirements (Abud et al., 2016). Changes in hemoglobin concentrations did not promote drastic changes in the values found for this blood variable, remaining in all reproductive phases within the normality for the specie (Weiss & Wardrop, 2010), probably due to the supplementation offered. According to Bezerra et al. (2013), diets deficient in energy are related to low hemoglobin concentrations. However, increased hemoglobin concentrations in the final third of pregnancy in Holstein cows were also reported by Oliveira, Barbosa, Pfeifer, and Cardoso (2003) and Saut and Birgel Junior (2012) in a study with buffaloes. This high blood flow seeking better oxygenation of the maternal and mammary tissues may be evidenced by the higher MCHC (Müller, Kesser, Koch, Helfrich, & Rietz, 2019; Polizopoulou, 2010) both in the final third of pregnancy (80% of foetal growth and development of the mammary gland) as well as in birth and one day postpartum. However, the MCV in the transition period was increased compared to the non-pregnant phase, but within the normal range for the specie (Weiss & Wardrop, 2010).

Anatomical and physiological modifications in the uterus are induced by fetal and placenta growth, stimulating the bone marrow to produce red cells in the blood stream, raising maternal blood volume to meet maternal-fetal needs. This occurs due to that, in the final phase of pregnancy, the hypervolemia is necessary to protect the mother and the foetus against the harmful effects of decreased venous return, since at parturition there is blood loss (Baimishev, Eremin, Baimishev, & Baimisheva, 2019; Brito et al., 2006; McMullin, White, Lappin, Reeves, & MacKenzie, 2003).

In relation to total leukocytes and neutrophils, the changes were probably influenced by physiological events present in the early stages of pregnancy development. The events are inflammatory characteristics with the predominance of cells of the maternal immune system in response to the chemical substances (cytokine) produced by the concept during the endometrial implantation and at the moment of placentation. These changes can be related to stress during the birth, occurring the release of adrenocortical hormones in the blood stream. This promotes increase of circulating leukocytes, changing the cellular proportions in the differential count at the birth, triggering leukocytosis by neutrophilia at this stage, and with the evolution of the puerperal periods occurred the leukocyte reduction (Müller et al., 2019; Saut et al., 2009).

Variations in lymphocyte counts in the middle third of pregnancy were related to the progesterone production by the *corpus luteum* and placenta, mainly, in the initial and middle third of pregnancy, causing changes in lymphocyte values. On the other hand, the evident reduction in the lymphocytic in the final third of pregnancy and at birth are conditioned by the estrogenic peak at parturition, one of the moments of high stress for the cow, due to the large amount of adrenocorticoids synthesized not only by the cows, but also by the foetus. Monocyte counts showed that these cells are little influenced by pregnancy, birth and postpartum (Iriadam, 2007).

Conclusion

Hematological biomarkers of Nellore cows showed significant changes due to the physiological adjustments required in response to the metabolic requirements imposed in the final stage of pregnancy and in the beginning of lactation, ensuring the nutritional contribution in energy and oxygen transported through the blood.

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