RENAN BRAGA PAIANO

Effects of anemia on periparturient cows

São Paulo

2018

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Dissertation submitted to the Postgraduate Program in Anatomy of Domestic and Wild Animals of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Master's degree in Sciences

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Advisor:

Prof. Eduardo Harry Birgel Junior

De acordo:________________________

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CERTIFICADO

Certificamos que a proposta intitulada "Avaliação do perfil metabólico de fêmeas bovinas durante o período de transição e puerpério tardio ", protocolada sob o CEUA nº 8022150216, sob a responsabilidade de **Eduardo Harry Birgel Junior** *e equipe; Renan Braga Paiano* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 31/01/2018.

We certify that the proposal "The metabolic profiles in cows during the transition period and late postpartum", utilizing 50 Bovines (50 females), protocol number CEUA 8022150216, under the responsibility of **Eduardo Harry Birgel Junior** *and team; Renan Braga Paiano* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 01/31/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 05/2017 a 10/2017 Área: Anatomia dos Animais Domésticos E Silvestres

Origem:	Prefeitura do Campus da USP de Pirassununga						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	2 a 10 anos	N:	50
Linhagem:	Holandês Preto e Branco			Peso:	400 a 690 kg		

Resumo: O período de transição nos bovinos é uma fase no qual a fêmea passa por diversas alterações fisiológicas e metabólicas. Nesse período
é importantíssimo realizar o acompanhamento do escore de condição corporal desses animais que tendem a perder peso durante essa época
devido à mobilização de gordura e a diminuição de ingestão de alimentos. É fundamental avaliar nessa fase por meio do exame de cardiotocografia a reatividade do feto. Durante a parturição é necessário monitorar e intervir imediatamente caso seja necessário através de
manobras obstétricas e reanimação do bezerro. Após o parto é preciso avaliar a condição de saúde uterina. Neste sentido o presente trabalho tem
como objetivo monitorar o período de transição, assim como as condições de parto, puerpério e o acompanhamento da involução uterina de vacas
e novilhas da raça Holandês Preto e Branco, assim como a monitoração das condições fisiológicas ao nascimento de seus respectivos bezerros

Local do experimento: USP-PIRASSUNUNGA

São Paulo, 02 de fevereiro de 2018

Profa. Dra. Anneliese de Souza Traldi
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretária Executiva
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

EVALUATION FORM

Author: PAIANO, Renan Braga

Title: Effects of anemia on periparturient cows

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Date: _____/_____/_____

Committee Members

Prof. ____

Institution:_________________________ Decision: ________________________

Prof. ____

Institution: _________________________ Decision: ________________________

Prof. ____

Institution: _________________________ Decision: ________________________

DEDICATION

I would like to dedicate this dissertation to the reason of my life. People who have always believe in me, have always supported me and are the foundation of everything.
I dedicate this dissertation to my family.

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I would like to thank God first for having blessed me and guided me every day of my life.

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My grandfather Lázaro Braga, who taught me to love and care for animals, all my professional victories, will be dedicated to your sweat and dedication to me always.

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My stepfather João who always supported me. All my family members are very grateful.

My dear wife Maria Jeannine Bonilla Lagos Paiano, a woman who will be with me every day of my life, her advice has always helped me in everything, to be a better person or to be a better professional. Your effort, affection, love and patience make me face life with happiness and love in everything I do. I love you in essence.

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EPIGRAPH

"God is great, God is strong, when he wants there is no one who does not want to. Whoever you are, whatever your social position you have in life, the highest or the lowest, always have as goal a lot of strength, a lot of determination and always do everything with a lot of love and with a lot of faith in God, that one Day you get there. Somehow you get there"

Ayrton Senna

RESUMO

PAIANO, R. B. **Efeitos da anemia em vacas periparturientes.** [Effects of anemia on periparturient cows]. 2018. 77 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2018.

Os objetivos deste estudo foram caracterizar o padrão hematológico e produtivo durante o periparto em vacas com e sem anemia, assim como avaliar o eritrograma em animais que apresentaram metrite puerperal aguda (APM), claudicação, acetonemia, valores de ácidos graxos não esterificados aumentados (AGNE) e vacas com diferentes categorizações de escore de condição corporal (ECC). No estudo 1, foram utilizadas 50 vacas Holandesas (29 multíparas e 21 primíparas), foram realizadas colheitas sanguíneas e exame físicos em 13 momentos diferentes: 18 ± 3, 12 ± 2, 5 ± 1, e 2 ± 1 dias antes do parto, parto, e 1, 7, 14, 21, 30, 45 e 60 dias após o parto. Foram realizadas avaliações do eritrograma sendo mensurado a contagem de hemácias (RBC), concentração de hemoglobina, volume globular, além dos valores hematimétricos absolutos do volume corpuscular médio (VCM), concentração hemoglobínica corpuscular média (CHCM), hemoglobina corpuscular média (HCM) e amplitude de distribuição dos glóbulos vermelhos (RDW), para as análises bioquímicas foram determinadas a concentração sérica de ferro, betahidróxidobutirato (BHBA), AGNE e bilirrubina total (TBIL). Anemia foi classificada de acordo com os valores de hemoglobina < 8 g/dL e o volume globular < 24 %. A prevalência de anemia foi maior no período 60 dias após o parto afetando 18,3% dos animais, o padrão da anemia apresentado foi normocítica, normocrômica e regenerativa. Os valores das contagens de hemácias, volume globular e hemoglobina foram menores ($P < 0,05$) para os animais com anemia. Enquanto os animais com APM e com claudicação, o valor do eritrograma foi muito semelhante ao longo do pós-parto. Não foi observado diferença entre os grupos de acordo com os valores de AGNE, ECC e perda de ECC durante as coletas realizadas no pós-parto. Em conclusão os animais anêmicos apresentaram os valores do exame físico de acordo com os limites fisiológicos, a anemia não provocou perdas produtivas nos animais afetados. Não foi evidenciado que animais com APM e claudicantes apresentassem maior redução da crase sanguínea, excluindo a ocorrência de anemia inflamatória, sendo que embora a prevalência de anemia aumentasse durante o pós-parto não foi possível caracterizar nenhuma associação com a redução dos valores hematológicos entre as categorizações realizadas. No estudo 2, foram realizadas coletas sanguíneas em 336 animais (252 multíparas e 84 primíparas) entre 21 e 30 dias em lactação (DEL) em sete fazendas no Estado de São Paulo com objetivo de caracterizar a prevalência de anemia nos animais, prevalência de anemia em diferentes categorizações realizadas como: distocia, retenção dos anexos fetais, mastite, problema digestivo, claudicação, acetonemia, ECC no momento da coleta e número de parto, assim como a análise do eritrograma, bioquímico e ECC e produção de leite entre os animais com e sem anemia. Em conclusão a prevalência de anemia foi baixa (16,3%) e nenhuma associação com as categorizações realizadas nesse estudo foi evidenciada.

Palavras-chave: Anemia. Eritrograma. Hematologia. Vaca leiteira.

ABSTRACT

PAIANO, R. B. **Effects of anemia on periparturient cows.** [Efeitos da anemia em vacas periparturientes]. 2018. 77 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2018.

The objectives of this study were to characterize the hematological and productive pattern during the peripartum in cows with and without anemia, as well as to evaluate the erythrogram in animals that presented acute puerperal metritis (APM), lameness, acetonemia, increased non-esterified fatty acids (NEFA) and cows with different categorization of body condition score (BCS). In study 1, 50 Holstein cows (29 multiparous and 21 primiparous) were used. Blood samples and physical examination were performed at 13 different times: 18 ± 3, 12 ± 2, 5 ± 1, and 2 ± 1 before calving, and 1, 7, 14, 21, 30, 45 and 60 days postpartum. Erythrogram evaluations were performed, and red blood cell count (RBC), hemoglobin concentration, packed cell volum (PCV), and absolute hematimetric values of mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin, red cell distribution width (RDW), serum concentrations of iron, betahydroxybutyrate (BHBA), AGNE and total bilirubin (TBIL) were determined for the biochemical analyzes. Anemia was classified according to hemoglobin values <8 g / dL and globular volume <24%. The prevalence of anemia was higher in the period 60 days postpartum affecting 18.3% of the animals, the pattern of anemia presented was normocytic, normochromic and regenerative. RBC, PCV and hemoglobin were lower ($P < 0.05$) for animals with anemia. While the animals with APM and lameness the value of the erythrogram was very similar throughout the postpartum period. No difference was observed between the groups according to the values of NEFA, BCS and loss of BCS during the periods performed in the postpartum period. In conclusion the anemic animals presented the values of the physical examination according to the physiological limits, the anemia did not cause productive losses in the affected animals. It was not evidenced that animals with APM and claudicants presented a greater reduction of blood crass, excluding the occurrence of inflammatory anemia, and although the prevalence of anemia increased during postpartum, it was not possible to characterize the main association of the reduction of hematological values between the categorizations. In the second study, blood was sampled from 336 animals (252 multiparous and 84 primiparous) between 21 and 30 days in lactation (DEL) on 7 farms in the State of São Paulo with the objective of characterizing the prevalence of anemia in dairy cows, prevalence of anemia in different categorizations such as: lactation number, BCS at the time of collection, dystocia, retention of fetal membranes, mastitis, digestive problem, lameness and acetonemia, as well as the analysis erythrogram, biochemical profile, BCS, and milk production among animals with and without anemia. In conclusion, the prevalence of anemia was low (16.3%) and no association with the categorizations performed in this study were observed.

Keywords: Anemia. Erythrogram. Dairy cow. Hematology.

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SUMMARY

1. INTRODUCTION

According to the Food and Agriculture Organization (FAO, 2013), bovine milk production in the world in 2012 was 600 million tons, Brazil contributing with 32.2 billion tons. This production is achieved due the intensification of production systems to obtain higher profitability (RAMIRES, 2013). However, the increased of productivity, when realized in an unplanned manner, can predispose the increase of diseases in the herd.

In this context, an illness that causes reduced productivity of animals is anemia, often resulting in culling of the animals (BURKE, et al., 2007). The hematological exams, associated to the clinical examination, help in the early diagnosis of this disease, avoiding economic loss as caused by the death of the animal. Few scientific studies evaluated the occurrence of anemia during the peripartum in dairy cows. Thus, the realization of the present study meets the need to have more information about this affection in Holstein cows.

2. LITERATURE REVIEW

2.1 Peripartum

The peripartum also known as the transition period is defined as the transition from the gestational-non-lactating state to the non-gestational-lactating (DRACKLEY, 1999; GRUMMER, 1995). This period occurs chronologically between three weeks prior to parturition until three weeks postpartum (ALLEN et al., 2005; DRACKLEY, 1999; GRUMMER, 1995; GRUMMER et al., 2004; SORDILLO; RAPHAEL, 2013), being a process of homeorhesis regulation of metabolic functions to accommodate parturition and lactogenesis (BAUMAN; CURRIE, 1980). These changes are related to increased nutritional demand due to energy mobilization for colostrogenesis, fetal growth and animal maintenance. During this phase occurs the reduction of dry matter intake may be greater than 30% (HAYIRLI et al., 2002).

Thus, the transition from the end of gestation to the beginning of lactation is characterized by changes in animal metabolism, predisposing to an increase in the incidence of diseases such as fatty liver, ketosis, hypocalcemia, retained placenta, metritis, endometritis, abomasum displacement, mastitis, laminitis among other diseases (GONZÁLEZ; SILVA, 2014; GOFF; HORST, 1997; INGVARTSEN et al., 2003; KELTON et al., 1998; MALLARD et al., 1998; MANN et al., 2015).

2.2 Puerperium

The puerperium starts from the release of fetal membranes, which normally occur between 6 and 8 hours after the parturition. The puerperium is divided into recent puerperium up to the 10th day, clinical puerperium up to the 21st day and the last phase is known as a late puerperium occurring between the 21st and 42nd days (GRUNERT; BIRGEL; VALE, 2005). This period is marked by uterine contamination by microorganisms, followed by elimination of the loquia, associated with an intense reduction of uterine size, and at the end of the physiological puerperium the uterus must be completely involuted (GRUNERT, 1979).

2.3 Body condition score

During the peripartum and the puerperium, mobilization of body reserves occurs, mainly due to the negative energy balance, to meet the increase in energy demand (HAYIRLI et al., 2002). The body condition score (BCS) is a form of evaluation to estimate the nutritional status of dairy cows (EDMONSON et al., 1989), being related to the metabolic profile of the animals (BUSATO et al., 2002).

Excess weight loss, as well as elevated BCS, can cause several reproductive disorders such as anestrous with inactive ovaries, ovarian cysts and increased voluntary waiting period (STUDER, 1998). The animals may also present alterations in the development of follicular waves, as well as in the interval for the first ovulation, resulting in a higher incidence of anovular cows and reduced conception rate (SANTOS et al., 2004; STAPLES et al., 2000).

The ideal BCS at parturition is 3-3.5 with a maximum loss of 0.5 point during the lactation period (MULLIGAN; DOHERY, 2008). Animals with high (> 4) or low (<2) BCS on the 1-5 scale are more likely to present immunosuppression, thereby increasing the risk of diseases such as ketosis, hepatic lipidosis, and hypocalcemia during the transitional period (ROCHE et al., 2009; INGVARTSEN et al., 2003; MULLIGAN; DOHERTY, 2008).

2.4 Energy profile

The most used indicators for the evaluation of the energy profile are the non-esterified fatty acids (NEFA), betahydroxybutyrate (BHBA), cholesterol and triglycerides (HERDT, 2000), which can assist in monitoring mainly the intensity of mobilization of body reserves, and the negative energy balance (LEBLANC, 2005). During the peripartum, there is an increase in these metabolites, mainly due to the reduction of dry matter intake by the animals in the moment of the parturition (GRUMMER, 1995).

2.5 Protein profile

Protein metabolism may change due to parturition, nutrition, lactation, as well as the seasons (CONTRERAS, 2000). The main indicators of the protein profile are the total proteins, albumin and globulins that are produced by the liver (GONZÁLEZ; SILVA, 2006). During the peripartum, migration of globulins and total protein towards the mammary gland occurs due to colostrogenesis (SAUT; BIRGEL JUNIOR, 2008).

2.6 Mineral profile

Mineral metabolism is influenced in the days before calving due to the migration of calcium mainly towards the mammary gland, due to the production of colostrum, associated with a decrease in food intake, leading to a reduction in the concentration of this mineral (DRACKLEY, 1999; GOFF, 1997).

2.7 Enzymatic profile

The major enzymes used to monitor hepatic function are alanine aminotranferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT), and may be increased during the peripartum due to intense liver activity, mainly caused by fat metabolization (GONZÁLEZ; SILVA, 2006).

2.8 Erythrocyte profile

Erythrogram parameters can be measured by the packed cell volume (PCV), hemoglobin content, as well as by red blood cell (RBC) count. (JONES; ALISSON, 2007). Anemia is the main condition involving red cells in cattle, being a consequence of the inability of erythropoietic tissue to restore red blood cells so that the values are not lower than the biological limit (BENESI, 1985). Anemia is defined as RBC counts less than 5 x 10^6cells/µL, PCV less than 24%, or hemoglobin concentration less than 8 g/dL (JAIN, 1986).

Anemias can be classified according to the bone marrow response in regenerative or non-regenerative, the regenerative represent a good bone marrow response in producing new cells, indicating that the anemia may be being caused by extra-medullary factor, occurring in condition of hemolysis, or blood loss (SANTOS, 2013; JONES; ALISSON, 2007).

Blood loss may be internal (inside a body cavity) such as the hemoabdomen or hemothorax, external blood loss (outside the body cavity) may occur under conditions of abomasal ulcers, also in surgeries and trauma (JONES; ALISSON, 2007). Another cause of regenerative anemia is hemolysis, which may be a normal process in cases of removal of old red blood cells from the spleen, or pathological in cases of hemoparasitism. Nutritional deficiencies such as lack of phosphorus is also a cause of regenerative anemia (JONES; ALISSON, 2007). When there is extravascular hemolysis, icteric, bilirubinemia or bilirubinuria may occur. While hemoglobinemia or hemoglobinuria occurs in cases of intravascular hemolysis. In cattle, the presence of polychromasia or reticulocyte is indicative of regeneration, because immature RBC are not normally present in circulation.

Another classification of anemia involves absolute hematimetric indexes, mainly the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC). When the value of MCV is within normal range anemia is known as normocytic, MCV increased anemia is classified as macrocytic, in case of MCV decrease microcytic anemia, microcytic anemia reflect defects in hemoglobin synthesis, or iron deficiency (CASCIO; DELOUGHERY, 2017). As for CHCM values within normality anemia is classified as normocromic, values reduced to anemia is classified as hypochromic (BIRGEL, 1982; BENESI, 1985).

In the case of non-regenerative anemias, the bone marrow is not responding to anemia, indicating that the cause may be located directly in the bone marrow, or in cases secondary to hepatic abscess, endocrine diseases, nutritional deficiency and inflammatory diseases (JONES, ALISSON, 2007).

Due to inflammation, the animal's immune system produces some proteins that help in a series of responses to protect the animal organism, this action involves mainly lethargy, fever, neutrophilia and production of new proteins (TIZARD, 2002). This response is mediated mainly by IL-1, IL-6 and TNF-α cytokines originating from macrophages (TIZARD, 2002). Erythropoiesis may be impaired due to inflammatory disease, mainly due to the action of inflammatory cytokines that can cause direct toxicity in erythroid precursor cells (CHIKAZAWA; DUNNING, 2016). In chronic inflammatory diseases, the proliferation and differentiation of erythroid precursors are impaired because of an altered response to erythropoietin (WEISS; GOODNOUGH, 2005). The main effect of cytokine production in the liver is the acute phase response stimulus, characterized by the induction of production of positive acute phase proteins such as haptoglobin and ceruloplasmin and the decrease of the production of negative acute phase proteins such as albumin , the retinol binding protein (FLECK, 1989) and apolipoproteins (BRUSS, 1997). In ruminants, the main acute phase proteins are haptoglobin and serum amyloid A (SAA) (TIZARD, 2002). Haptoglobin is related to diseases and severe inflammation in cattle, its main function is related to the binding with iron molecules reducing the availability of iron for bacterial growth, another characteristic of haptoglobin is binding with free hemoglobin preventing the oxidation of lipids and proteins (TIZARD, 2009). According to Trevisi et al., (2012) the increase in inflammation before the parturition is related to the low liver function index, represented by changes in albumin, cholesterol and bilirubin levels at the beginning of lactation, being associated with an increase in the circulation of IL-6 and haptoglobin after calving.

The reduction of the amount of red blood cells in the body resulting in reduced amount of oxygen in the tissues (THRALL, 2004; JONES; ALISSON, 2007). The main mechanisms of physiological compensation for anemia involve the increased cardiac output, and increased plasma volume that allow the remaining red blood cells to travel more efficiently due to decreased viscosity (CASCIO; DELOUGHERY, 2017), these mechanisms aid in the greater distribution of oxygen to tissues.

According to Benesi, (1985) the symptomatic manifestations of anemia are variable and dependent on the etiology and pathogenesis of the process, degree / intensity, changes in total circulating blood volume and requirements and / or management to which animals are subjected affected. Animals in the condition of anemia may present tachycardia with heart murmurs, cold extremities, tachypnea, superficial respiration, dyspnea, exercise intolerance, weakness, mental depression or aggression, (BENESI, 1985; JONES; ALISSON, 2007).

Tennant et al., (1975) found that 8% of calves up to 7 days of age had anemia. The occurrence of anemia in cattle was observed by several authors, being the greatest occurrence among calves, mainly during the first days of life (KANEKO; MILLS, 1970; BIRGEL, 1972; TENNANT et al., 1975; BENESI et al., 2000). One of the main causes related to anemia in calves is iron deficiency (TENNANT et al., 1975). This deficiency may occur due to the low concentration of iron in the colostrum of cows (RADOSTITS et al., 2002), and due the gastrointestinal endoparasites, hematophagous ectoparasites, inflammatory bowel diseases, among others (THRALL, 2004). Iron has an action on the activation of the immune response (ATYABI et al., 2006), due to its importance this mineral when its concentration is reduced below the physiological values can compromise important physiological functions that require the iron to be able to act, as responses immune or enzymatic actions (HENTZE et al., 2004). About 65-70% of the body's iron is found in hemoglobin, while 20% is stored in the liver and spleen, and this is a fundamental demand in the case of blood loss (CANÇADO; CHIATTONE, 2002; ANDERSON et al., 2007; STORILLO, 2016).

Lactating cows may suffer significant physiological and hematological changes during the transition from parturition for lactation becoming more prone to uterine and mammary infections (MATEUS et al., 2002; SAUT, 2008). The value of hemoglobin may decrease in dairy cows during postpartum due to protein catabolism that occurs during the transition period to supply amino acids for milk production (SEKER; UNSUREN, 1989; KIDA, 2002). Hormonal and nutritional disorders can cause hematological changes, and blood tests help in the diagnosis of these changes, which may predispose to the occurrence of diseases such as anemia (JONES; ALLISON 2007).

2.9 Main diseases related to the puerperium

2.9.1 Fatty liver

Fatty liver, also known as hepatic steatosis, occurs due to intense lipomobilization which is characterized by increased blood NEFA, which can accumulate in the form of triglycerides in the hepatocyte cytosol due to the decrease in the production of very low density lipoproteins (VLDL) (BOBE et al., 2004; DRACKLEY et al., 1999; HERDT et al., 1983; HOLTENIUS, 1993; SEVINC et al., 2003; VAZQUEZ-ANON et al., 1994; VEENHUIZEN et al., 1991). The lipoprotein VLDL is the main responsible for the transport of triglycerides (BRUSS, 1997). The production of VLDL in ruminants is lower when compared to primates and rodents, and this factor is associated with an increase in the incidence of fatty liver in milk cows with an increase in NEFA in the blood circulation (BOBE et al., 2004).

Fat mobilization results in incomplete oxidation products such as BHBA (VAZQUEZ-AÑON et al., 1994). Increased blood concentrations of NEFA and BHBA are associated with disorders in dairy cows' health and decreased milk production (MANN et al., 2016; OSPINA et al., 2010). Hepatic lipidosis and hepatocyte degeneration involve cell membrane damage, hepatocyte destruction, and the release of enzymes into the cytoplasm, such as AST and GGT (JORRITSMA et al., 2001; LUBOJACKA et al., 2005; PECHOVA et al., 1997).

During the transition period, hormonal changes and stress release cortisol and catecholamine that can induce hepatic steatosis (GOFF; HORST, 1997). Increased accumulation of fatty acids in hepatocytes and ketone bodies causes changes in hepatic morphology and physiology (GONZÁLEZ et al., 2011; VAZQUEZ-ANON et al., 1994; VEENHUIZEN et al., 1991).

Cows suffering from hepatic lipidosis present reduced endogenous liver synthesis, causing a decrease in the serum concentration of glucose, total protein, albumin, globulins, cholesterol, triglycerides and urea. Under these conditions, the hepatocyte excretion function may be reduced, leading to increased blood concentration of total bilirubin, ammonia, and bile acids (LUBOJACKA et al., 2005; PECHOVA et al., 1997).

Cows with NEFA values > 0.3 mEq / L one week before calving are twice as likely to develop metritis (CHAPINAL et al., 2011). Ospina et al., (2010) noted that the NEFA value > 0.3 mEq / L from 1 to 2 weeks before the parturition is associated with an increased risk of retained placenta, abomasal displacement, and metritis during the puerperium. The use of NEFA in both pre and postpartum periods may help to monitor dairy cow health (CHAPINAL et al., 2011).

2.9.2 Ketosis

During the transition period, a major metabolic disease in bovine health is ketosis, which occurs most frequently during the puerperium, especially after the second lactation (OETZEL, 2004; KIROVSKI, 2008). Ketosis begins when the cows are undergoing a negative energy balance, occurring usually two weeks before calving or at the beginning of lactation.

Physiologically ketone bodies are produced by the liver and exported to the other tissues to provide energy, without their accumulation in the body; in situations of intense lipomobilization the ketone bodies accumulate harming the animal's sanity (ORTOLANI, 2002). The pathogenesis involves increased mobilization of fat due to inadequate hepatic metabolism causing an increase for NEFA (result of incomplete oxidation of ketone bodies, increased liver triglyceride stores and decreased VLDL secretion (ORTOLANI, 2002; RAMIRES, 2013).

According to the origin, ketosis is classified as primary or secondary (GEISHAUSER et al., 1998). Primary ketosis is a syndrome that develops independently of another disease (KRONFELD, 1982). While the secondary disease is triggered by a primary disease causing reduced food intake and fat mobilization, it will reduce the concentration of plasma glucose and increase the concentration of ketone bodies, NEFA, exacerbating the negative energy balance, triggering the disease (KRONFELD, 1982).

For the diagnosis of ketosis in dairy cows, blood BHBA values are measured as greater than 1.0 mmol / L (GOLDHAWK et al., 2009; KINOSHITA et al., 2010), greater than 1.2 mmol / L (GEISHAUSER et al., 2000; SEIFI et al., 2011) and greater than 1.4 mmol / L (GEISHAUSER et al., 2000). The values of serum BHBA greater than 1.2 mmol / L during the first or second week postpartum are associated with a 3-fold increase in the risk of metritis and abomasal displacement (GEISHAUSER et al., 2000). The main consequences of this disease are economic losses, reduction of milk production, increase of reproductive disorders, displacement of abomasum, mastitis, reduction of the pregnancy rate and increase of the period of service (LEBLANC et al., 2005; MCART et al., 2013; SUTHAR et al., 2013; WALSH et al., 2004).

2.9.3 Hypocalcemia

Hypocalcemia of the dairy cow is also known as vitular fever or hypocalcemic puerperal paresis. This disease determines motor incoordination, paresis and permanent decubitus, when the serum concentration is lower than 6 mg / dL (ALLEN, 1993; SMITH; RISCO, 2005). Reduction of serum calcium levels to values between 6.2 and 7.5 mg / dL were reported as a common condition in milk cows, and could affect up to 50% in dairy cows during early lactation (MELENDEZ; RISCO, 2005).

This condition can cause reduced milk yield and loss of fertility (GOFF; HORST, 1997), reducing ruminal movement, as well as rumination, affecting ruminal filling and matte particle passage rate (MARTINEZ, 2014), as well as being associated with alterations in energy metabolism, thus reducing the immune function (LARSEN et al., 2001; MARTINEZ et al., 2014). Kimura et al., (2006) have observed that hypocalcemia is associated with the reduction of intracellular calcium, thereby impairing the function of mononuclear cells in peripheral blood.

2.9.4 Retention of fetal membranes

Retention of fetal membranes or retained placenta is classified as failure to expel the placenta after the first 12 or 24 hours (LEBLANC, 2008). It is related to the collagen dissolution failure between the caruncle and the cotyledon (EILER; HOPSKINS, 1992). Being that the non-separation of the fetal connection with the maternal connection in the placenta is one of the predominant factors for the onset of placental retention. This condition is considered one of the main risk factors for puerperal metritis (KÖNYVES et al., 2009; SMITH; RISCO, 2002). Other risk factors for placental retention are abortion, stillbirth, twin birth, dystocia and fetotomy (LEBLANC, 2008; RADOSTITS et al., 2002). The reduction in the phagocytic activity of neutrophils, and the inability to cause oxidative metabolism to kill pathogenic microorganisms is associated with the risk of retained placenta (GUNNINK, 1984; KIMURA et al., 2002).

2.9.5 Acute puerperal metritis

Acute puerperal metritis (APM) is defined as a severe inflammatory reaction involving all layers of the uterus (BONDURANT, 1999). The symptomatology is marked by fetid aqueous purulent uterine discharge with presence of fever (body temperature ≥39.5) (SHELDON, 2004; LEBLANC, 2010; LIBOREIRO et al., 2015), with signs of toxemia or septicemia, depression and anorexia, and reduction of milk production (SHELDON, 2006). APM occurs within the first 2 weeks after the calving, with approximately 50% of cases diagnosed within the first seven days after parturition (SHELDON, 2004; LEBLANC, 2008). The prevalence can vary between 25-40% (SHELDON et al., 2008) and reported incidence was 18.5% (DRILLICH et al., 2001), and may vary between farms (SHELDON, 2004).

The main risk factors associated with APM are retention of placenta, hypocalcemia, ketosis, and other problems during parturition such as dystocia, twin birth, stillbirth, abortion, and reduction of dry matter intake near parturition, number of lactations, between other factors (FÖLDI et al., 2006; MALINOWSKI et al., 2010; OSPINA et al., 2010; GIULIODORI et al., 2013).

2.9.6 Mastitis

Mastitis is defined as inflammation of the mammary gland and milk changes (FUENZALIDA et al., 2015). The main agents that cause mastitis are bacteria, fungi, yeast and algae (BRADLEY, 2002).

Initially, microorganisms such as environmental pathogens enter the teat, multiply inside the mammary gland, infiltrating the defense cells and stimulating an immune response (BRADLEY, 2002). The major environmental pathogens, such as *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Escherichia coli*, while the main contagious agents are *Streptococus agalactiae*, *Staphylococcus aureus* and *Mycoplasma* sp. (GONÇALVES, 2017).

The occurrence of mastitis is mainly related by environmental and sanitary conditions of the farm and the pathogen, the incidence can vary between 5 to 22% (ALERI et al., 2016; FLEISCHER et al., 2001).

The main risk factors are the stage of lactation, the increase in the number of lactations and the condition of environmental hygiene. It has been reported that immunosuppression caused by negative energy balance may increase the susceptibility of mastitis in milk cows (DRACKLEY, 1999). The main consequences of mastitis are related to altered and reduced milk production, drug spending, negatively influencing the dairy economy (FOURICHON et al., 1999; HARMON, 1994)

2.9.7 Lameness

Lameness it is one of the most important welfare problems in dairy cattle (GALINDO; BROOM, 2002). This condition generates economic loss in the dairy sector and is related to the welfare of dairy cows, causing a negative impact on animal production, mainly due to discomfort caused by pain (ESPEJO; ENDRES; SALFER, 2006; BICALHO et al., 2007; GARGANO, 2015). Due to these factors, claudication was reported as an indicator of well-being degradation (WHAY et al., 2003). The animal may fail to produce 270 to 574 kg of milk (AMORY et al., 2008; ARCHER; BELL; HUXLEY, 2010; GUDAJ et al., 2012). Fertility is also reduced in cows with lameness, when associated with prematurity early culling causes a negative economic impact on dairy farms (OLECHNOWICZ; JASKIWSKI, 2011; SILVA, 2017). The incidence of lameness related was 30% with the highest rates described in herds housed in free-stalls (ITO et al., 2010).

3. OBJECTIVES

The objectives of this study were:

- To evaluate the health of the animal during the peripartum by clinical and laboratorial exams;
- To characterize the hematological and productive parameters in cows with and without anemia;
- To evaluate the erythrogram analyses for animals: with and without disorders;
- To evaluate the erythrogram analyses for animals according to the BCS and BCS change;
- To evaluate the prevalence of anemia in periparturient cows
- To evaluate the prevalence of anemia in postpartum dairy cows.

4. CHAPTER 1 - HEMATOLOGICAL AND CLINICAL EVALUATION IN DAIRY COWS DURING THE PERIPARTUM

4.1 INTRODUCTION

The peripartum begins three weeks before calving until three weeks after parturition (DRACKLEY, 1999; GRUMMER et al., 2004; ALLEN et al., 2005). During this phase, there are endocrine changes in hormonal concentrations such as growth hormone, insulin, prolactin, estrogen, progesterone (GARDINAL, 2016), also physiological changes (MEGLIA et al., 2005), metabolic (POGLIANI; BIRGEL JUNIOR, 2007), hematological (SAUT; BIRGEL JUNIOR, 2012), and immunological (SANTOS, 2015). These changes may increase the risk of animals developing metabolic diseases as fatty liver, hypocalcemia and ketosis, puerperal diseases as retained fetal membranes (RFM) and acute puerperal metritis (APM) (GOFF; HORST, 1997, KELTON et al., 1998, INGVARTSEN et al., 2003, GONZÁLEZ; SILVA; 2006, MANN et al., 2015).

Anemia is a hematological disease main related on bovine with parasitism as ticks (RAJPUT et al., 2006), worms (KEYYU et al., 2005) and intra erythrocyte parasitism as babesiosis and anaplasmosis disease (SOUZA et al., 2013). When anemia is not recently detected and treated certainly economic loss will occur as a decrease in milk production, or early culling.

Therefore, the objectives of this study were to evaluate the erythrogram during the peripartum and to evaluate the prevalence of anemia in periparturient cows, as well as the hematological and productive parameters of the animals.

4.2 MATERIALS AND METHODS

4.2.1 Animals

This study was approved by the Ethical Committee on the Use of Animals of the School of Veterinary Medicine and Animal Sciences (University of São Paulo, Brazil, protocol number 8022150216). The experiment was carried out at the Dairy Cattle Farm, University of São Paulo, Pirassununga, Brazil from May 2017 to October 2017. Fifty Holstein cows (29 multiparous and 21 primiparous) were used in this study.

Feeding of the prepartum animals was composed of 14.5 kg of roughage and 4 kg of concentrate, while for the lactating animals the silage supply was 27.5 kg of roughage and 8.5 kg of concentrate. The diets were formulated according to the National Research Council (2001). The composition of the ingredients according to the dry matter (DM) used for the formulation are shown in Table 1 and the bromatological composition of the diet is shown in Table 2.

Table 1 - Composition of ingredients of prepartum and lactation diets

Ingredient (%DM)	Prepartum	Lactation
Corn silage	58.71	56.16
Ground corn	27.63	26.59
Soybean meal	08.09	14.81
Urea	0.92	0.47
Mineral and vitamin mix salt [1]	04.62	-
Mineral and vitamin mix salt [2]	-	1.04
Limestone	-	0.29
Dicalcium phosphate	-	0.17
Common salt	-	0.47

[1] Contained per kilogram: Na 2.4g, Ca 22.8g, P 2.2g, Mg 0.9 g, S 4.8 g; Co 1.4 mg, Cu 45.4 mg; Mn 135 mg; I 2.7 mg, Se 1.4, Zn 181.4 mg, Vitamin A, 18.000 UI, Vitamin D, 1.800 UI, Vitamina E, 65.3 mg, monensin 50 mg. [2] Contained per kilogram: Na 9.3g, Ca 16.6g, P 2.3g, Mg 1.2 g, S 4,8 g, Co 1.8 mg, Cu 60.5 mg, Mn 179.9 mg, I 3.6 mg, Se 1.8, Zn 241.9 mg, Vitamin A, 24.000 UI; Vitamin D, 2.400 UI, Vitamin E, 87 mg, monensin 66 mg.

Table - 2 Nutritional composition of silage and concentrate diets

Nutrient	Prepartum	Lactation
Dry matter (%NM)	46.71	47.64
Total digestible nutrients (%DM)	67.51	70.57
Crude protein (%DM)	14.71	28.53
Neutral detergent fiber (%DM)	42.36	41.78
Acid detergent fiber (%DM)	22.52	22.29
Ether extract (%DM)	03.03	03.04
DCAD[1] (mEq/kg MS)	-99.18	158.22

[1]Dietary cation-anion difference.

4.2.2 Blood sampling, analyses and clinical examination

The animals were followed during the last 21 days prior to calving, until to 60 days postpartum. Blood samples and clinical examination were performed on 13 samples in the following moments: 18 ± 3, 12 ± 2, 5 ± 1, and 2 ± 1 days before calving, parturition, 1, 7, 14, 21, 30, 45 and 60 days postpartum.

Blood was performed by coccygeal venipuncture into Vacutainer ® systems. Two blood were sampled from each animal, one tube without anticoagulant and other with EDTA, to determine biochemical profile and hematological analysis respectively. The analysis were be realized at Multiuser Laboratory in Clinical Veterinary Analysis of University of São Paulo. The hematological analyses were performed using BC-2800 Vet Mindray ® to measure a count of red blood cell, hemoglobin, packed cell volume, and mean corpuscular volume, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration and red cell distribution width (RDW). This equipment uses the impedance method to determine red blood cells and colorimetric methods to determine hemoglobin, MCV, MCH and MCHC.

Serum iron, total phosphor, BHBA, NEFA, and total bilirubin (TBIL) were determined using commercial kits of the Randox Laboratories brand, Crumlin, UK, in an automatic biochemistry system (RX Daytona® - Randox Laboratories, UK).

Clinical examination was performed to monitor the vital parameters as heart rate, respiratory rate, ruminal movements and body temperature were measured according to Dirksen et al., (1993).

4.2.3 Diagnosis of health problems

Cows were considered anemic if the hemoglobin and PCV values were lower than 8.0 g/dL and 24% respectively (JAIN, 1986). Acute puerperal metritis was characterized by the presence of fewer (≥ 39.5) and watery, fetid uterine secretion during the first 21 days after the parturition (SHELDON et al., 2006). Lameness was classified, as claudication was evident (>2 score) according to the scale 1-to5-point of Sprecher, Hostetler and Kaneene (1997).

Acetonemia was established by BHBA values > than 1.2 mmol/L in blood samples from the parturition up to 7 days in lactation (OETZEL, 2004). NEFA elevated was characterized by NEFA concentration higher than 0.3 mmol/L during the last 7 days prior to gestation, or > than 0.7 mmol/L during the first 7 days in milk (DIM) (WHITAKER, 2004).

4.2.4 BCS and milk yield

BCS was recorded using 1-to5-point scale of Ferguson et al., (1994). Milk yield were measured when the sample were obtained during the postpartum by software Alpro®. The BCS classification was performed according to the value in first evaluation: (≤ 3 BCS; 3.0-3.5 and BCS ≥ 3.75). The change in BCS from the first evaluation in prepartum until parturition was classified as without BCS loss, low BCS loss (if loss was ≤ 0.50), and high BCS loss (if loss was ≥0.75).

4.2.5 Statistical analysis

The effects of anemia, APM, lameness, acetonemia, NEFA elevated, BCS and change of BCS were analyzed by logistic regression using PROC GLIMMIX of SAS (version 9.3 SAS/STAT; SAS Institute Inc., Cary, NC) fitting binary distribution.

The hematological and biochemical analyses (iron, phosphor and TBIL), body temperature, milk yield and BCS, were analyzed by the GLIMMIX procedure of SAS for animals with and without anemia. Also, the erythrogram analyses (RBC, hemoglobin and PCV) were analyzed by the GLIMMIX procedure of SAS for animals: with and without disorders (APM, lameness, acetonemia, NEFA elevated); the categorization of BCS according to the value in first evaluating: (≤ 3 BCS; 3.0-3.5 and BCS ≥ 3.75) and BCS change from prepartum until parturition (without BCS loss; low BCS loss and high BCS loss). Models also included the effects in the periods. Differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were considered tendencies.

4.4 RESULTS

The overall prevalence of anemia during the evaluated periods was 44 % (22/50), of these 13.6 % (3/22) presented an intense anemia (reduction of PCV ≤ 19.9 %), 22.7 % (5/22) had a moderate anemia (reduction of PCV between 20-21.9 %) and 63.4 % (14/22) presented mild anemia (reduction of PCV between 22.0-23.9 %). The animals that developed anemia in only one period were 41 % (9/22), 45 % (10/22) had anemia during two periods and 14 % (3/22) in three or more periods.

Table 3 shows the prevalence of anemia during the evaluated periods, the highest prevalence of anemia was observed between the periods of 21 to 60 days postpartum with 18.3 % (9/49) of the animals. This anemia could already be seen in 2.1 % and 8 % of the anemias between 1 and 14 days postpartum, 17% of the animals on the 21st day postpartum, 16.3 % on the 30th day, and 10.4% on the 45th day postpartum and in 18.3% of the animals on the 60th day postpartum.

The count of red blood cell was lower for animals with anemia between 1 to 60 days postpartum ($P < 0.05$) (Figure 1). The concentration of hemoglobin and the percentage of packed cell volume were lower for anemic cows on 1 and 21 to 60 days postpartum ($P < 0.05$) (Figure 2 and Figure 3). The absolute hematimetric indices MCV, MCHC, MCH and RDW did not differ ($P < 0.05$) between the groups (Table 4), showing normocytic and normochromic anemia.

The milk yield (Figure 4) and the BCS (Figure 5) did not differ between the groups. The body temperature was higher ($P < 0.01$) at parturition for anemic cows (Table 5). Serum iron concentration was smaller ($P < 0.05$) in anemic cows at 7 days postpartum, and the phosphor concentration was higher ($P < 0.05$) in non-anemic animals at 1 day postpartum (Table 6). Total bilirubin were lower ($P < 0.05$) in anemic cows at 14 and 30 days postpartum, (Table 7).

For animals with and without acute puerperal metritis packed cell volume, hemoglobin and red blood cell did not differ among the groups (Table 8). Throughout the experimental period, the number of RBC, PCV and hemoglobin level in animals without APM oscillated respectively between 4.90-5.91 X 10^6/mm^3, between 25.99-32.22 %, and between 8.23-10.04 g/dL respectively. In the group of animals with uterine involution disorders the RBC ranged from 5.07-6.20 X 10^6/mm^3, the PCV ranged from 26.12-33.85 % and the hemoglobin level ranged from 8.30-10.54 g/dL.

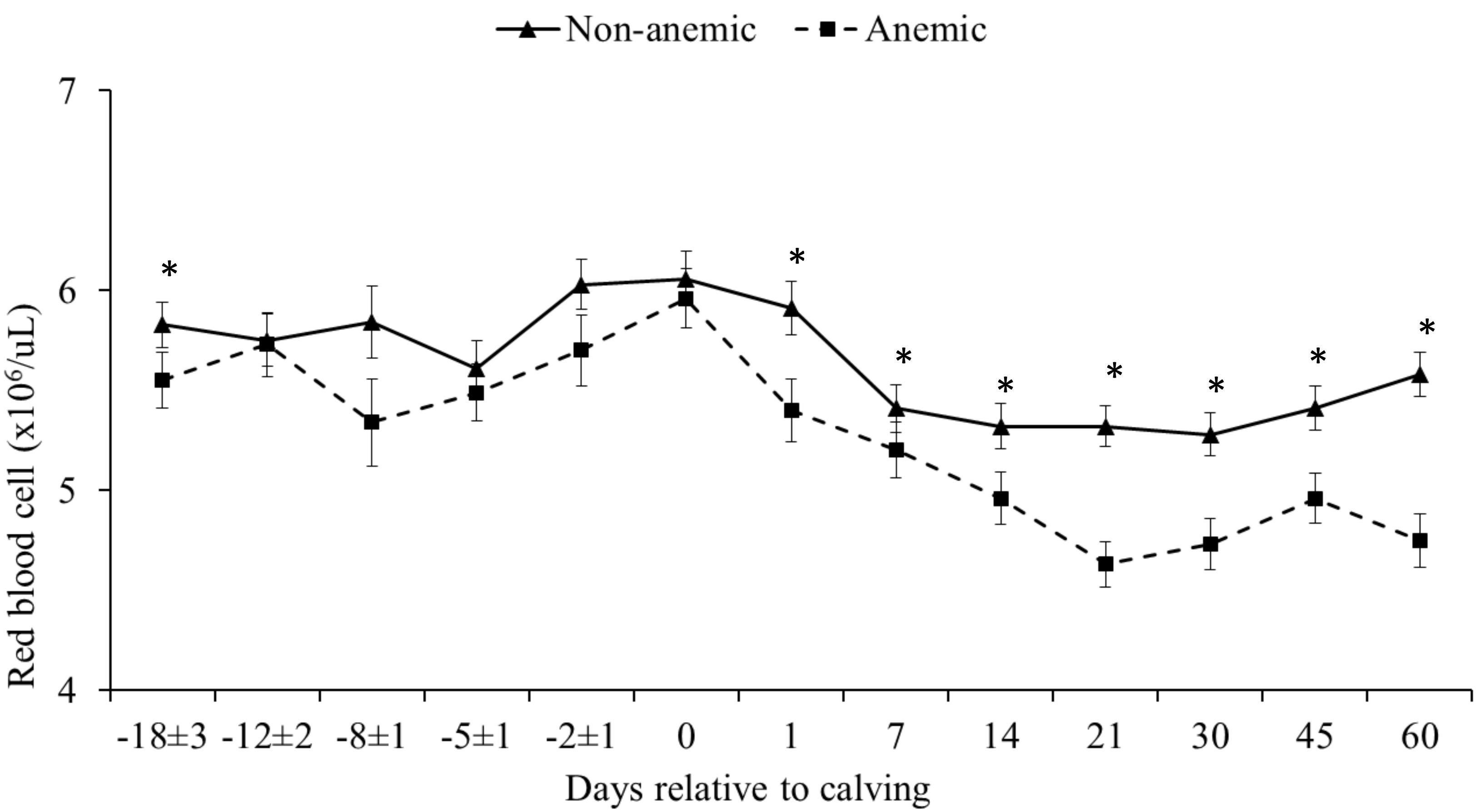
For cows with and without lameness the packed cell volume, hemoglobin and red blood cell were higher ($P < 0.01$) on day -12, and were lower (P <0.01; 0.02) on day 21 postpartum in lame cows (Table 9). Cows with acetonemia had higher ($P < 0.02$; 0.03) packed cell volume, hemoglobin and red blood cell on 1 day after calving, and had smaller ($P < 0.01$) on 60 postpartum (Table 10). The packed cell volume, hemoglobin and red blood cell did not differ between the groups according to the NEFA value (Table 11).

There was no difference between the groups according to categorized BCS during the prepartum, on hematological parameters (Table 12). Animals with high BCS loss had higher ($P < 0.05$) values of packed cell volume, hemoglobin and red blood cell on days 12 and 8 prior to calving, than animals with low BCS loss and without BCS loss (Table 13).

Table 3 - Prevalence of anemia according to the evaluation period

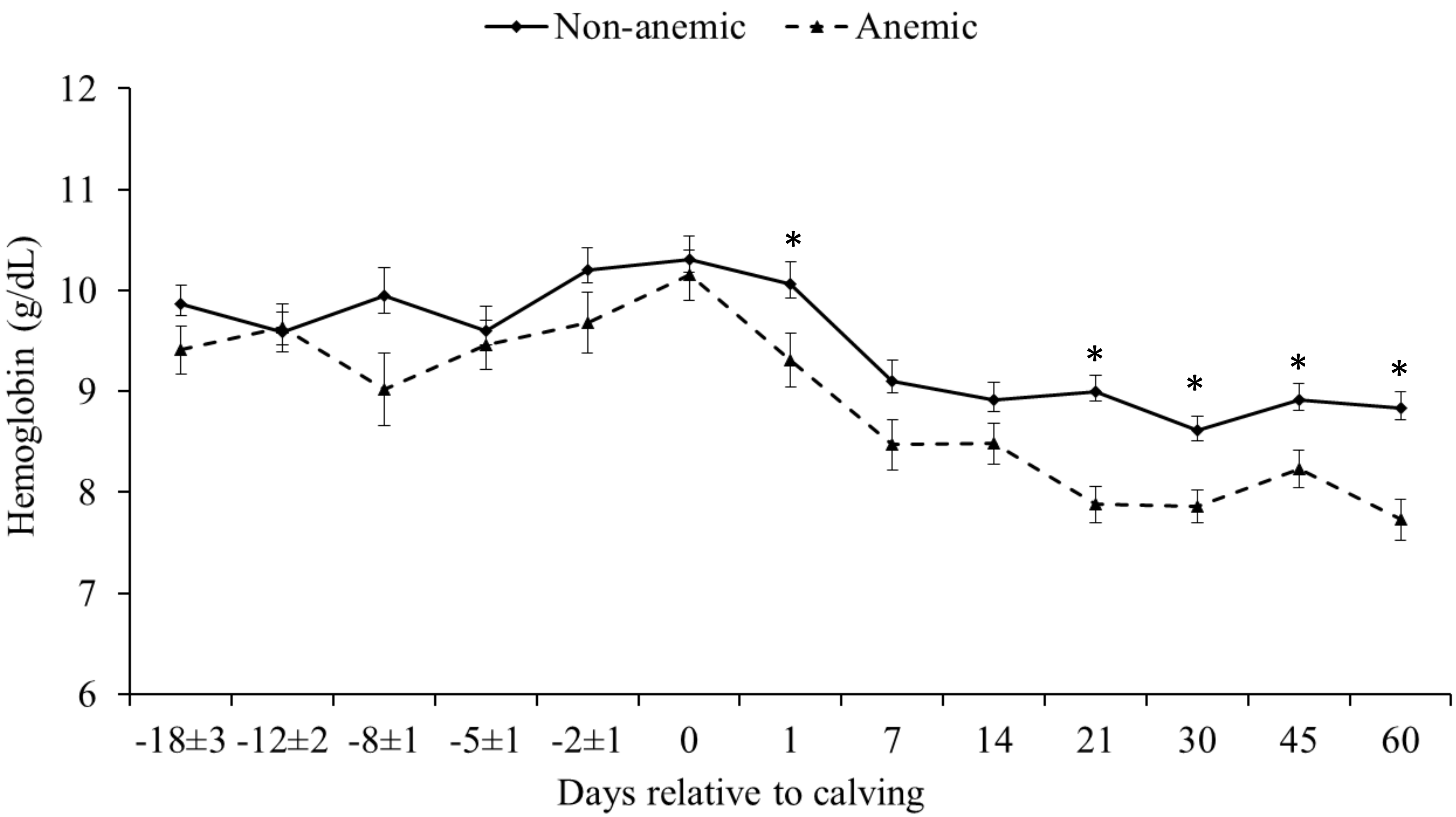
Period	Prevalence, %	N
-18±3	0.0	40
-12±2	0.0	39
-8±1	0.0	40
-5±1	4.8	42
-2±1	2.1	48
Parturition	0.0	44
1	2.1	47
7	8.0	50
14	4.1	49
21	17.0	47
30	16.3	49
45	10.4	48
60	18.3	49

Figure 1 - Mean and standard error of mean obtained for red blood cell during 18 days before to calving up to 60 days postpartum



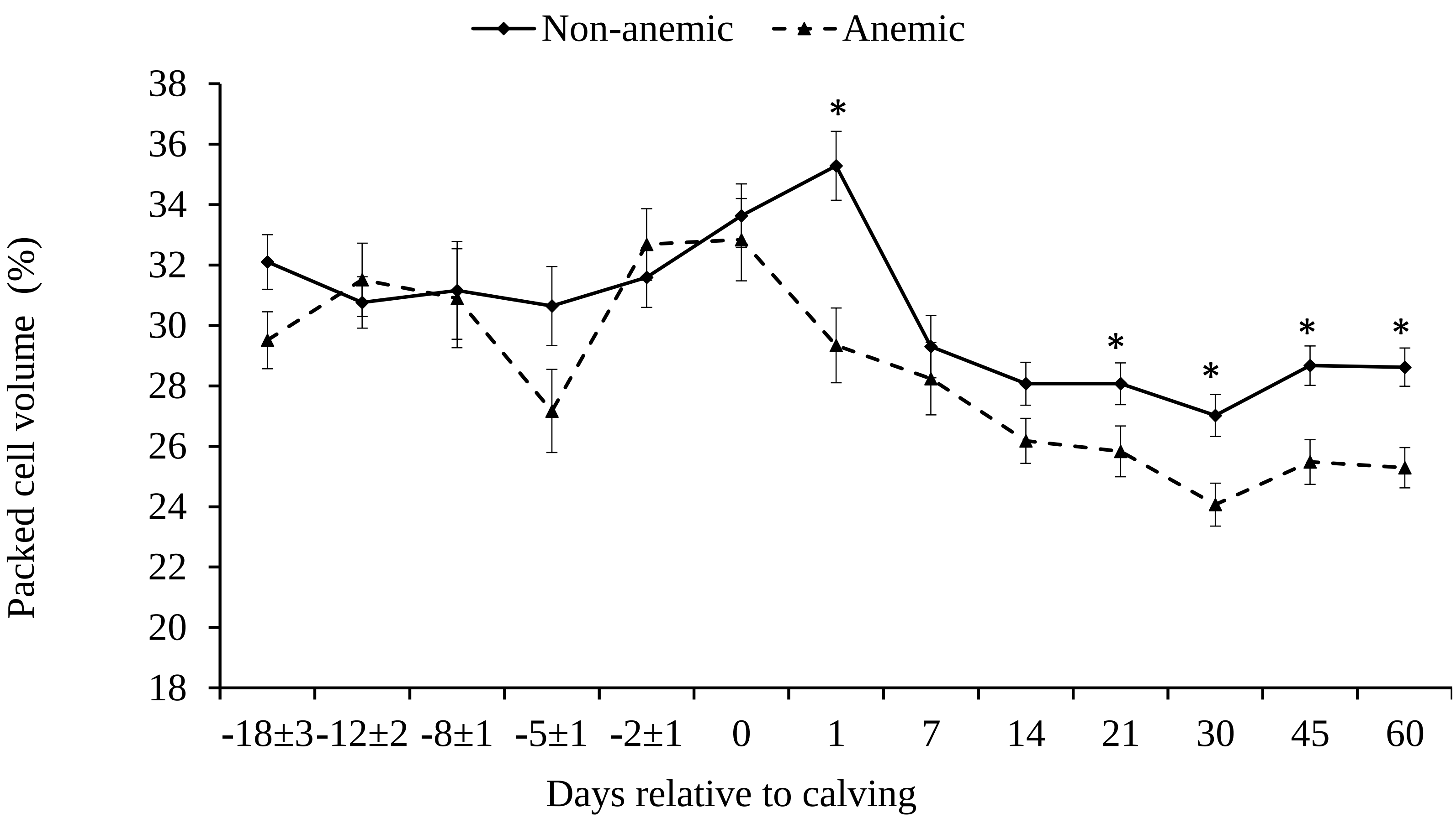
* Significant difference between anemic and non-anemic (P < 0.05).

Figure 2 - Mean and standard error of mean obtained for hemoglobin during 18 days before to calving up to 60 days postpartum



* Significant difference between anemic and non-anemic (*P* < 0.05).

Figure 3 - Mean and standard error of mean obtained for packed cell volume during 18 days before to calving up to 60 days postpartum



* Significant difference between anemic and non-anemic ($P < 0.05$).

Table 4 – Hematological parameters in dairy cows with and without anemia during 18 days before to calving up to 60 days postpartum [1]

	MCV (fL)		MCHC (g/dL)		MCH (pg)		RDW (%)	
Period	Non-anemic	Anemic	Non-anemic	Anemic	Non-anemic	Anemic	Non-anemic	Anemic
-18±3	53.30±1.11	53.18±1.46	31.11±0.62	30.94±0.76	16.88±0.22	17.09±0.28	16.37±0.20	16.57±0.24
-12±2	53.09±0.87	54.75±1.04	31.41±0.19	31.23±0.22	16.65±0.23	16.91±0.28	16.39±0.18	16.45±0.22
-8±1	53.95±0.70	55.17±0.80	31.70±0.22	31.48±0.27	17.16±0.22	17.20±0.25	16.24±0.18	16.14±0.21
-5±1	54.03±0.67	55.29±0.77	31.73±0.22	31.45±0.25	17.17±0.22	17.18±0.25	16.23±0.18	16.18±0.20
-2±1	54.76±0.66	55.56±0.77	31.18±0.21	31.28±0.24	17.10±0.21	17.15±0.25	16.37±0.17	16.14±0.20
Parturition	55.11±0.68	55.21±0.78	31.42±0.19	30.93±0.21	17.33±0.22	16.94±0.25	16.24±0.19	16.02±0.22
1	55.28±0.69	55.37±0.79	31.17±0.19	31.15±0.23	17.20±0.22	17.12±0.25	16.12±0.20	15.84±0.23
7	54.06±0.68	54.46±0.78	31.25±0.19	31.09±0.22	16.91±0.22	16.76±0.25	15.91±0.19	15.73±0.22
14	53.64±0.68	54.15±0.79	31.38±0.18	31.57±0.20	16.88±0.22	17.01±0.25	16.02±0.19	16.13±0.23
21	52.96±0.68	54.18±0.79	32.03±0.18	31.64±0.21	16.89±0.22	17.03±0.25	16.04±0.19	16.56±0.22
30	52.02±0.72	52.83±0.84	31.71±0.18	41.59±0.22	16.42±0.24	16.42±0.27	16.11±0.21	16.67±0.25
45	51.41±0.78	51.70±0.92	32.20±0.19	32.04±0.21	16.57±0.24	16.51±0.27	16.15±0.22	16.58±0.24
60	50.23±0.78	50.81±0.90	31.79±0.18	31.70±0.22	15.98±0.24	15.74±0.29	16.25±0.20	16.53±0.22

[1]Values are mean ±SEM.

Figure 4 - Mean and standard error of mean obtained for body condition score during 18 days before to calving up to 60 days postpartum

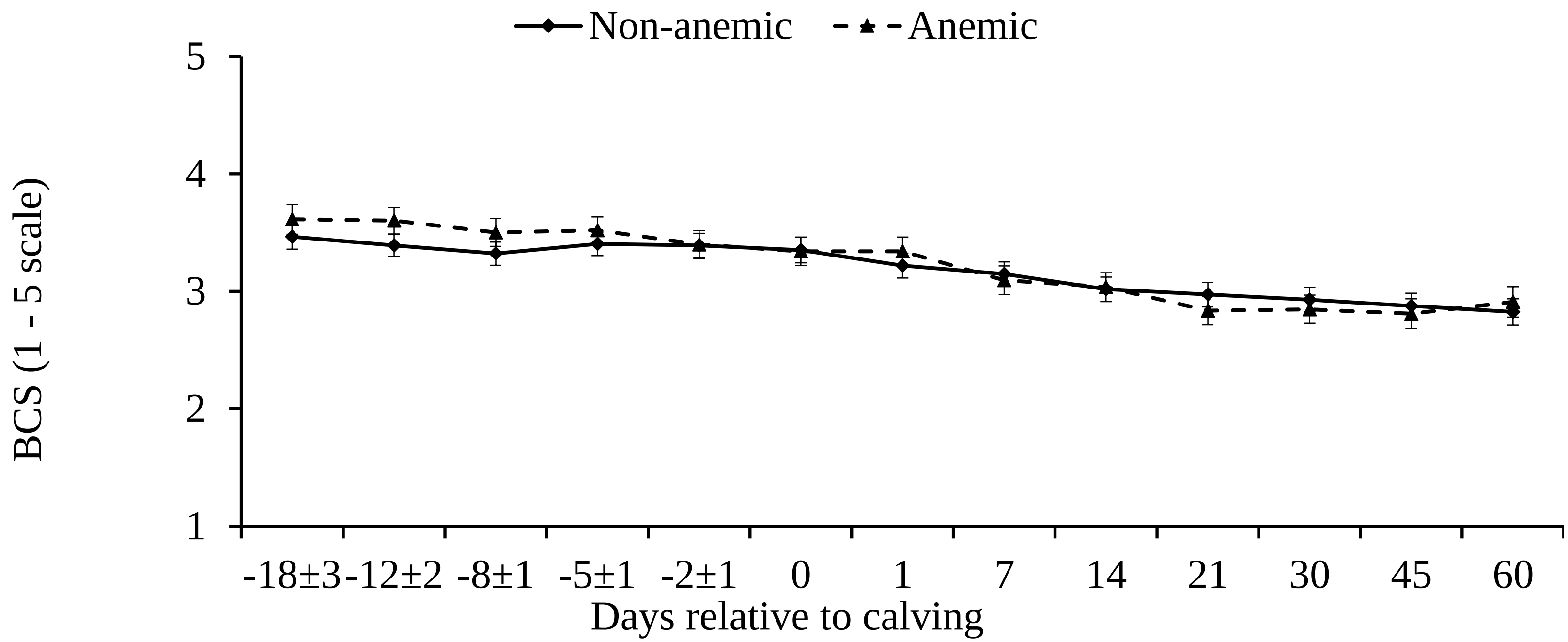


Figure 5 - Mean and standard error of mean obtained for milk yield in dairy cows with and without anemia

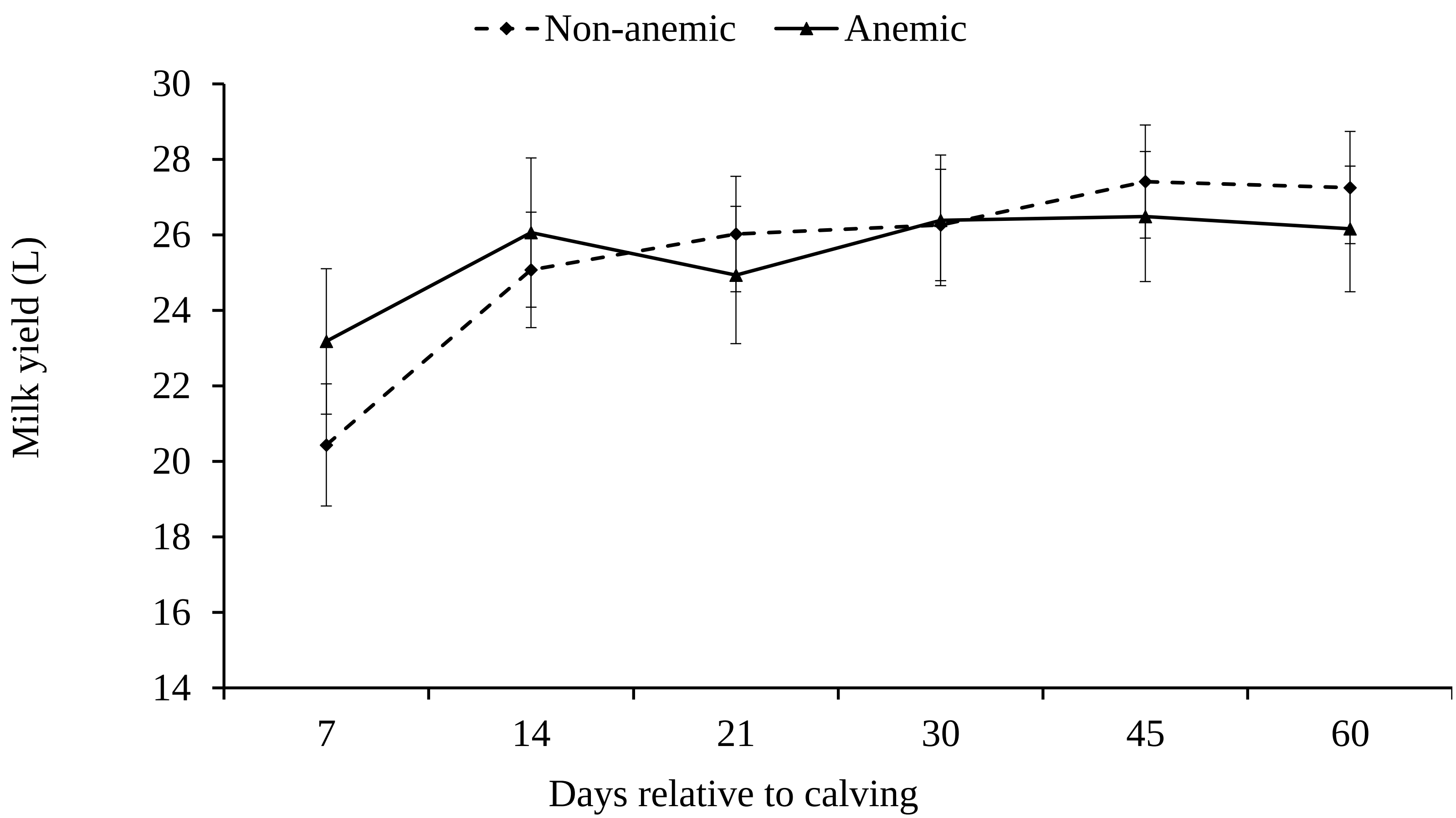


Table 5 – Body temperature parameters in dairy cows with and without anemia during 18 days before to calving up to 60 days postpartum[1]

	Temperature (°C)		
Period	Non-anemic	Anemic	*P* – value
-18±3	38.60±0.11	38.79±0.16	NS
-12±2	38.69±0.08	38.67±0.11	NS
-8±1	38.57±0.09	38.89±0.12	NS
-5±1	38.72±0.11	38.84±0.14	NS
-2±1	38.54±0.10	38.54±0.12	NS
Parturition	38.78±0.12	39.39±0.18	<0.01
1	38.98±0.11	38.78±0.14	NS
7	38.89±0.11	38.86±0.13	NS
14	38.75±0.12	38.48±0.14	NS
21	38.43±0.13	38.70±0.14	NS
30	38.36±0.11	38.60±0.12	NS
45	38.25±0.10	38.44±0.11	NS
60	38.44±0.10	38.17±0.12	NS

[1]Values are mean ±SEM.

Table 6 – Biochemical parameters in dairy cows with and without anemia during 18 days before to calving up to 60 days postpartum [1]

	Iron (mg/dL)			Phosphor (mg/dL)		
Period	Non-anemic	Anemic	*P* – value	Non-anemic	Anemic	*P* - value
-18±3	144.54±11.75	102.20±19.09	NS	5.97±0.31	5.01±0.38	NS
-12±2	108.26±15.96	88.64±27.80	NS	5.46±0.46	5.55±0.65	NS
-8±1	117.16±14.10	95.03±19.54	NS	5.64±0.40	5.09±0.42	NS
-5±1	125.25±11.81	109.50±15.19	NS	5.40±0.35	5.85±0.45	NS
-2±1	112.99±10.76	111.43±14.08	NS	5.59±0.42	5.39±0.47	NS
Parturition	101.68±10.43	93.80±12.55	NS	5.17±0.39	5.06±0.42	NS
1	93.34±11.36	100.32±12.97	NS	6.18±0.34	5.04±0.39	0.03
7	120.10±10.53	86.18±12.74	0.04	5.82±0.30	5.05±0.38	NS
14	105.90±10.99	82.01±13.05	NS	5.74±0.26	5.57±0.29	NS
21	102.44±12.86	108.09±14.03	NS	6.19±0.29	5.86±0.32	NS
30	119.97±11.39	123.71±13.05	NS	6.06±0.25	6.18±0.27	NS
45	117.76±12.17	111.84±15.51	NS	6.05±0.27	5.71±0.30	NS
60	138.17±12.89	113.01±15.25	NS	5.98±0.32	6.02±0.36	NS

[1]Values are mean ±SEM.

Table 7 – Total bilirubin during postpartum in dairy cows

	TBIL (mg/dL)		
Period	Non-anemic	Anemic	*P* - value
7	0.48±0.05	0.39±0.06	NS
14	0.40±0.05	0.24±0.04	0.02
21	0.35±0.03	0.24±0.06	NS
30	0.38±0.06	0.24±0.03	0.03
45	0.27±0.03	0.22±0.04	NS
60	0.15±0.03	0.19±0.04	NS

[1]Values are mean ±SEM.

Table 8 – Hematological parameters according to prevalence of acute puerperal metritis in dairy cows[1]

	Red blood cell (x10^6/uL)			Packed cell volume (%)			Hemoglobin (g/dL)		
Period	Without APM	APM	*P*-value	Without APM	APM	*P*-value	Without APM	APM	*P*-value
-18±3	5.56±0.13	5.80±0.14	NS	30.14±0.74	31.38±0.78	NS	9.51±0.22	9.84±0.23	NS
-12±2	5.58±0.14	5.95±0.15	NS	29.91±0.68	31.64±0.75	NS	9.37±0.20	9.91±0.23	NS
-8±1	5.67±0.19	5.59±0.20	NS	30.45±0.99	30.32±1.06	NS	9.59±0.31	9.60±0.33	NS
-5±1	5.58±0.13	5.58±0.16	NS	30.46±0.73	30.05±0.89	NS	9.61±0.22	9.53±0.27	NS
-2±1	5.91±0.14	5.87±0.16	NS	32.22±0.77	32.09±0.88	NS	10.04±0.24	9.94±0.27	NS
Parturition	5.87±0.14	6.20±0.15	NS	32.13±0.71	33.85±0.77	NS	9.99±0.23	10.54±0.24	NS
1	5.66±0.13	5.73±0.15	NS	30.94±0.73	31.67±0.83	NS	9.67±0.23	9.83±0.26	NS
7	5.18±0.12	5.32±0.13	NS	28.26±0.68	28.40±0.74	NS	8.81±0.21	8.85±0.23	NS
14	5.07±0.11	5.30±0.13	NS	27.58±0.55	28.33±0.65	NS	8.57±0.18	8.94±0.20	NS
21	4.90±0.11	5.20±0.13	NS	26.11±0.58	27.64±0.61	NS	8.34±0.18	8.77±0.19	NS
30	5.02±0.12	5.07±0.13	NS	25.99±0.52	26.12±0.59	NS	8.23±0.15	8.32±0.18	NS
45	5.13±0.12	5.32±0.13	NS	26.54±0.55	27.28±0.61	NS	8.50±0.17	8.73±0.19	NS
60	5.25±0.12	5.22±0.13	NS	26.46±0.59	26.52±0.63	NS	8.42±0.19	8.30±0.21	NS

[1]Values are mean ±SEM.

Table 9 – Hematological parameters according to prevalence of lameness in dairy cows[1]

	Red blood cell (x10^6/uL)			Packed cell volume (%)			Hemoglobin (g/dL)		
Period	Without Lameness	Lameness	*P*-value	Without Lameness	Lameness	*P*-value	Without Lameness	Lameness	*P*-value
-18±3	5.65±0.10	5.73±0.20	NS	30.43±0.59	31.58±1.10	NS	9.66±0.18	9.63±0.33	NS
-12±2	5.56±0.12	6.19±0.20	<0.01	29.82±0.55	33.00±0.92	<0.01	9.37±0.16	10.24±0.28	<0.01
-8±1	5.56±0.15	5.92±0.29	NS	30.06±0.79	31.54±1.46	NS	9.58±0.24	9.70±0.45	NS
-5±1	5.58±0.12	5.56±0.23	NS	30.23±0.64	30.51±1.25	NS	9.66±0.19	9.29±0.38	NS
-2±1	5.82±0.12	6.18±0.23	NS	31.79±0.66	33.67±1.30	NS	9.94±0.20	10.26±0.39	NS
Parturition	6.09±0.11	5.81±0.21	NS	33.35±0.61	31.56±1.10	NS	10.44±0.19	9.63±0.34	0.04
1	5.70±0.12	5.68±0.21	NS	31.41±0.63	30.89±1.13	NS	9.83±0.19	9.46±0.35	NS
7	5.20±0.10	5.36±0.20	NS	28.19±0.57	28.80±1.06	NS	8.85±0.18	8.77±0.34	NS
14	5.20±0.10	5.09±0.17	NS	28.15±0.47	27.26±0.81	NS	8.81±0.15	8.47±0.26	NS
21	5.15±0.10	4.70±0.17	0.02	27.55±0.46	24.58±0.83	<0.01	8.78±0.15	7.81±0.26	<0.01
30	5.07±0.10	4.96±0.18	NS	26.33±0.45	25.24±0.79	NS	8.35±0.13	8.03±0.24	NS
45	5.24±0.11	5.14±0.19	NS	26.98±0.47	26.59±0.83	NS	8.65±0.15	8.49±0.26	NS
60	5.28±0.11	5.10±0.19	NS	26.48±0.50	26.46±0.92	NS	8.41±0.15	8.19±0.31	NS

[1]Values are mean ±SEM.

Table 10 – Effect of BHBA concentration on hematological parameters in dairy cows[1]

	Red blood cell (x10^6/uL)			Packed cell volume (%)			Hemoglobin (g/dL)		
Period	Normal BHBA	Acetonemia[2]	*P*-value	Normal BHBA	Acetonemia[2]	*P*-value	Normal BHBA	Acetonemia[2]	*P*-value
-18±3	5.73±0.12	5.67±0.16	NS	30.98±0.70	30.43±0.96	NS	9.73±0.20	9.67±0.29	NS
-12±2	5.74±0.11	5.62±0.18	NS	30.80±0.63	30.57±1.03	NS	9.66±0.19	9.51±0.31	NS
-8±1	5.58±0.12	5.56±0.25	NS	30.03±0.71	30.45±1.42	NS	9.60±0.21	9.59±0.43	NS
-5±1	5.69±0.12	5.50±0.22	NS	30.88±0.72	30.43±1.31	NS	9.83±0.22	9.55±0.40	NS
-2±1	5.83±0.12	5.89±0.21	NS	31.76±0.70	32.45±1.15	NS	9.98±0.21	10.04±0.35	NS
Parturition	6.09±0.12	5.91±0.20	NS	33.41±0.65	32.93±1.07	NS	10.41±0.20	10.30±0.33	NS
1	5.52±0.11	5.99±0.19	0.03	30.37±0.58	33.15±0.98	0.02	9.48±0.18	10.22±0.31	0.03
7	5.14±0.11	5.57±0.18	NS	27.72±0.58	29.83±0.96	NS	8.63±0.18	9.24±0.30	NS
14	5.04±0.12	5.45±0.19	0.03	27.16±0.55	29.44±0.95	0.04	8.50±0.17	9.27±0.30	0.03
21	5.00±0.11	5.01±0.18	NS	26.77±0.50	26.39±0.81	NS	8.52±0.16	8.47±0.26	NS
30	4.92±0.10	5.32±0.16	NS	25.60±0.45	27.20±0.77	NS	8.14±0.14	8.59±0.25	NS
45	5.16±0.10	5.34±0.16	NS	26.61±0.45	27.48±0.77	NS	8.57±0.14	8.69±0.25	NS
60	5.38±0.10	4.82±0.18	<0.01	27.12±0.45	24.10±0.84	<0.01	8.59±0.14	7.68±0.29	<0.01

[1]Values are mean ±SEM.

[2]Acetonemia = serum BHBA values > than 1.2 mmol/L in the postpartum samples.

Table 11 – Effect of NEFA concentration on hematological parameters in dairy cows[1]

	Red blood cell (x10^6/uL)		Packed cell volume (%)		Hemoglobin (g/dL)	
Period	Normal NEFA	Elevated NEFA concentration	Normal NEFA	Elevated NEFA concentration	Normal NEFA	Elevated NEFA concentration
-18±3	5.94±0.27	5.64±0.10	31.08±1.50	30.66±0.51	9.92±0.44	9.64±0.15
-12±2	5.97±0.29	5.68±0.11	30.26±1.36	30.62±0.53	9.31±0.41	9.62±0.16
-8±1	5.53±0.27	5.64±0.10	29.35±1.34	30.66±0.51	8.99±0.41	9.72±0.15
-5±1	5.56±0.28	5.56±0.11	29.13±1.38	30.29±0.57	9.15±0.43	9.59±0.17
-2±1	5.60±0.31	5.84±0.13	29.71±1.69	31.89±0.69	9.32±0.53	9.93±0.21
Parturition	5.95±0.30	6.11±0.12	31.93±1.57	33.37±0.61	9.93±0.50	10.41±0.19
1	5.49±0.31	5.69±0.12	29.27±1.64	31.40±0.65	9.06±0.51	9.80±0.20
7	4.96±0.28	5.30±0.11	26.57±1.52	28.73±0.58	8.40±0.48	8.94±0.19
14	5.01±0.27	5.20±0.11	27.51±1.34	28.02±0.52	8.30±0.42	8.81±0.16
21	5.09±0.24	5.02±0.10	26.63±1.23	26.82±0.49	8.43±0.39	8.56±0.15
30	4.89±0.26	5.06±0.10	25.70±1.16	26.10±0.46	8.14±0.36	8.30±0.14
45	5.44±0.27	5.19±0.10	27.18±1.21	26.82±0.48	8.80±0.39	8.58±0.15
60	5.35±0.29	5.22±0.10	26.54±1.39	26.56±0.51	8.49±0.45	8.41±0.16

[1]Elevated NEFA concentration = serum NEFA concentration higher than 0.3 mmol/L during the last 7 days prior to gestation, or > than 0.7 mmol/L during the first 7 DIM.

Table 12 – Effect of body condition score at prepartum on hematological parameters in dairy cows[1]

	Red blood cell ($x10^6$/uL)			Packed cell volume (%)			Hemoglobin (g/dL)		
Period	BCS ≤ 3	BCS 3.0-3.5	BCS ≥ 3 .75	BCS ≤ 3	BCS 3.0-3.5	BCS ≥ 3 .75	BCS ≤ 3	BCS 3.0-3.5	BCS ≥ 3 .75
-18±3	5.88±0.16	5.47±0.13	5.75±0.15	31.27±0.85	29.60±0.72	31.54±0.77	9.91±0.26	9.31±0.22	9.86±0.23
-12±2	5.65±0.21	5.68±0.17	5.86±0.20	30.11±0.93	30.24±0.77	31.54±0.89	9.55±0.30	9.42±0.25	9.85±0.28
-8±1	5.57±0.14	5.52±0.12	5.84±0.13	29.53±0.68	30.36±0.61	31.71±0.65	9.56±0.23	9.56±0.20	9.85±0.22
-5±1	5.53±0.18	5.55±0.16	5.69±0.20	29.34±1.05	30.54±0.92	31.12±1.20	9.47±0.32	9.60±0.28	9.81±0.36
-2±1	5.75±0.22	5.73±0.20	6.28±0.23	31.01±1.38	31.74±1.22	34.84±1.43	9.85±0.43	9.92±0.38	10.69±0.45
Parturition	6.16±0.20	6.05±0.18	6.13±0.22	32.91±1.11	33.66±0.96	33.80±1.22	10.43±0.36	10.53±0.31	10.44±0.39
1	5.59±0.33	6.09±0.29	5.64±0.33	30.18±1.84	34.09±1.62	30.89±1.84	9.61±0.59	10.62±0.52	9.39±0.59
7	5.18±0.16	5.30±0.14	5.33±0.16	27.84±0.79	28.75±0.68	28.76±0.79	8.84±0.23	8.93±0.20	8.86±0.23
14	5.09±0.15	5.14±0.13	5.23±0.16	27.16±0.73	27.98±0.63	27.91±0.75	8.50±0.24	8.88±0.21	8.73±0.25
21	4.87±0.19	5.10±0.16	5.12±0.19	25.57±1.01	27.52±0.83	27.06±0.99	8.18±0.32	8.80±0.26	8.61±0.31
30	4.96±0.16	5.12±0.14	5.06±0.17	25.78±0.62	26.53±0.54	25.97±0.64	8.29±0.20	8.40±0.18	8.17±0.20
45	5.16±0.16	5.14±0.14	5.40±0.16	26.29±0.76	26.99±0.64	27.26±0.76	8.59±0.26	8.53±0.22	8.86±0.26
60	5.40±0.18	5.15±0.15	5.22±0.18	27.02±0.90	26.14±0.78	26.10±0.93	8.72±0.28	8.27±0.24	8.20±0.29

[1]According to the value in first evaluating.

Table 13 – Effect of body condition score change at prepartum on hematological parameters in dairy cows[1]

	Red blood cell ($x10^6$/uL)			Packed cell volume (%)			Hemoglobin (g/dL)		
Period	Without BCS loss	Low BCS loss	High BCS loss	Without BCS loss	Low BCS loss	High BCS loss	Without BCS loss	Low BCS loss	High BCS loss
-18±3	5.61±0.14	5.68±0.16	5.83±0.24	30.43±0.75	30.31±0.81	31.89±1.23	9.5±0.23	9.76±0.25	9.79±0.38
-12±2	5.50±0.16^{b}	5.64±0.19^{b}	6.51±0.28^{a}	29.56±0.70^{b}	29.98±0.82^{b}	34.28±1.22^{a}	9.25±0.21^{b}	9.42±0.25^{b}	10.71±0.37^{a}
-8±1	5.56±0.0.12	5.58±0.14	5.96±0.21	30.21±0.57^{b}	29.93±0.70^{b}	32.69±1.01^{a}	9.50±0.19	9.67±0.23	10.11±0.34
-5±1	5.52±0.0.16	5.53±0.19	5.75±0.36	29.90±0.91	29.84±1.11	31.85±2.18	9.56±0.27	9.48±0.33	9.69±0.65
-2±1	5.94±0.19	5.59±0.24	6.48±0.36	32.76±1.20	30.40±1.50	36.14±0.2.27	10.25±0.37	9.69±0.47	11.00±0.71
Parturition	6.12±0.16	6.19±0.21	5.96±0.29	33.50±0.88	33.74±1.11	32.85±1.57	10.54±0.28	10.61±0.36	10.12±0.51
1	5.47±0.17	5.94±0.21	5.60±0.30	30.00±0.96	32.66±1.22	31.01±1.77	9.32±0.30	10.35±0.38	9.34±0.55
7	5.12±0.14	5.37±0.17	5.38±0.24	27.76±0.0.66	28.95±0.82	28.97±1.17	8.70±0.19	9.05±0.24	8.84±0.34
14	5.00±0.12	5.43±0.16	5.04±0.22	27.18±0.60	28.92±0.75	26.72±1.07	8.55±0.20	9.10±0.26	8.41±0.37
21	4.91±0.15	5.21±0.20	4.92±0.27	26.58±0.80	27.17±1.06	25.85±1.42	8.43±0.26	8.81±0.34	8.20±0.46
30	5.05±0.14	5.10±0.18	4.96±0.25	26.03±0.52	26.47±0.66	25.37±0.93	8.25±0.17	8.49±0.21	8.01±0.30
45	5.33±0.13	5.05±0.17	5.47±0.23	27.55±0.59	26.01±0.76	27.47±1.05	8.87±0.20	8.40±0.26	8.81±0.36
60	5.26±0.15	5.36±0.18	5.00±0.28	26.29±0.79	27.17±0.99	25.05±1.41	8.44±0.24	8.66±0.30	7.71±0.45

[a–b] Means within a row with different superscript letters differ ($P < 0.05$).
[1] Change from prepartum until parturition.

4.5 DISCUSSION

This study characterized the increase in the prevalence of anemia during the postpartum period in dairy cows, with a reduction in hemoglobin and packed cell volume, mainly after 21 days postpartum. Marked for being an anemia of mild degree, classified as normocytic, normochromic and regenerative, because the values of VCM, CHCM, and RDW are in the range described as physiological that is 40-60 pg for VCM, 14.4-18.6 g / dL for CHCM and 16-24% for RDW according to described according to Jain, (1996).

Anemic animals did not develop fever and the vital parameters represented by heart rate, respiratory rate, ruminal movement and body temperature were within the physiological limits. The main causes of anemia in cattle are diseases that destroy the red blood cells. However, other causes such as reduced production of red cells, inflammatory causes, and mineral deficiencies can also cause anemia in ruminants (THRALL, 2004).

Anemia caused by hemolysis may occur due to gastrointestinal endoparasites and hematophagous ectoparasites, (THRALL, 2004). Fecal Egg Counting and blood smear tests were performed to rule out the presence of endoparasites and hemoparasites, it was not observed the presence of parasites that justified the hemolytic crisis, associated with these findings the concentration of TBIL in the anemic animals was within the biological values, which is 0.01-0.47 mg/dL according to Kaneko, (2008), indicate that there was no hemolytic anemia, that is, the changes observed in the erythrogram were not due to the existence of a specific disease of as the anaplasmosis and babesiosis.

In addition, through biochemical examination, it was ruled out that anemia originated from iron and phosphorus deficiency, mainly because the values are within normal range, which is 57-162 µg/dL for iron (KANEKO, 2008) and 4.0-8.5 mg/dL for phosphorus (GOFF, 2000). In high-producing cows, the occurrence of hemoglobinuria during postpartum, characterized by intravascular hemolysis, anemia and hemoglobinuria is described (THRALL, 2004). This is related to the decrease in phosphorus concentration in the blood circulation (THRALL, 2004). Hypophosphatemia can cause oxidative damage in the red blood cells, due to the reduction of ATP and glutathione (THRALL, 2004). The results did not indicate the possibility of a hemolytic crisis that justified the anemia observed in the animals. There was no repercussion of anemia on milk yield and BCS.

In the erythrogram of animals with APM and lameness, it was not evidenced that inflammation from the uterus and the locomotor system caused profound modifications in the performed tests, and the observed values are within the physiological limits. Excluding with this the occurrence of inflammatory anemia, which can happen mainly in chronic processes, in this condition anemia occurs due to side effects caused by cytokines that act suppressing the production of erythropoietin, proliferation and differentiation of progenitor cell lines of erythrocytes, decrease the availability and alteration in iron metabolism, among other effects, leading to a decrease in the production of erythrocytes by the bone marrow (NEMETH; GANZ, 2014). The results obtained in the present research could not prove the observations of Heidarpour et al., (2014) who reported lower values of RBC and PCV in cows with uterine inflammation when compared with healthy animals, being this finding related to the production of inflammatory cytokines.

Regarding the BHBA metabolite, it was observed that cows with acetonemia presented erythrogram values below the physiological limits at 60 days postpartum. In a study conducted by Marutsova et al., (2015) to evaluate the effect of ketosis on the erythrogram, the authors observed higher values of RBC, hemoglobin and PCV, in cows with ketosis when compared to the group of healthy cows, while Sahinduran et al., (2010) did not notice difference between the red blood cell values between ketosis cows and healthy cows. In addition, Bélici et al., (2010) observed a reduction in hemoglobin concentration and RBC count in cows with ketosis, which is related to the state of oxidative stress and altered liver function.

According to the BCS in the prepartum and changes in the BCS until the calving it was not observed that fat or thin cows, and cows that lost body condition showed a greater reduction of the erythrogram during postpartum. Despite the high prevalence of anemic animals during 21 days postpartum, the results of the present study did not show a relationship between the loss of body score in the last 21 days of gestation and the occurrence of anemia during the puerperium. Cows with low BCS or that lose excess body condition may be suffering from nutritional deficiency, in which condition the animals may develop anemia due to the lack of some minerals such as iron and copper (JONES; ALLISON, 2007). Bélici et al., (2010) reported reduced hemoglobin content in obese cows. Rafia et al., (2012) evaluated the effect of the body condition score on the hemogram of dairy cows from 30 days before calving up to 30 days after calving, the animals were separated into three groups according to BCS, thin cows BCS ≤2.75, moderate BCS 3-3.75 and obese cows with BCS ≥ 4, blood samples were performed every ten days, PCV reduction, RBC count and hemoglobin after parturition were observed, however there was no difference between groups at periods 10, 20 and 30 days postpartum.

4.6 CONCLUSION

Although the increased prevalence of anemia during postpartum period in Holstein cows, it was not possible to characterize the main association of the reduction of erythrogram values between the categorizations and the parameters evaluated in this study.

5. CHAPTER 2 - PREVALENCE OF ANEMIA IN DAIRY COWS DURING THE PUERPERIUM

5.1 INTRODUCTION

The puerperium is defined as the period between the parturition until the first estrus (HORTA, 1995; RIBEIRO, 2016). The puerperium is characterized by physiological changes as the opening the cervix, and lochia discharge, these alterations enabling the entrance of the uterus by environmental bacteria. On the other hand, during this phase because of the severe negative energy balance the animal can suffer immunodepression. This deficiency can contribute to modify the defense mechanisms, allowing to the persistence of pathogenic bacteria and favor the establishment of diseases (KIM et al., 2005; SHELDON et al., 2006, 2008, 2009; HAMMON et al., 2011; GUMEN et al., 2011).

Consequently, the animal is more susceptible to suffer several diseases, as puerperal (retained fetal membranes (RFM), acute puerperal metritis (APM) and endometritis), and metabolic (ketosis, hypocalcemia and fatty liver) (BUTLER, 2003; LEBLANC, 2010). Heidarpour et al., (2014) observed decreased erythrogram values in cows with endometritis.

Until the present moment, no study has reported the prevalence of anemia during puerperium dairy cows. Therefore, the objectives of this study were to characterize the prevalence of puerperium anemia during 21 to 30 DIM and their relationship with several disorders.

5.2 MATERIALS AND METHODS

5.2.1 Animals

336 female Holstein animals (252 multiparous and 84 heifers), in seven dairy farms located in southeast Brazil were used in this study.

5.2.2 Characterization and diagnosis of health problems

Cows were considered anemic if the hemoglobin and hematocrit values were lower than 8.0 g/dL and 24% respectively (JAIN, 1986). Clinical signs of disease (dystocia, RFM, APM, mastitis, digestive problems and lameness were recorded.

The classification of dystocia was an animal that needed at least two human to intervention at the parturition (NOAKES et al., 2001). RFM was characterized as a cow that shown failed to eliminate the placenta during the first 24 hours after calving (SHELDON, 2004). APM was classified as an animal that suffer inflammation on uterus epithelium, an enlarged and flaccid uterus, with a discharge purulent and fetid, in firstly 21 days postpartum, associated with fever (>39,5 ºC) (SHELDON et al., 2006). Mastitis was diagnosed when the California Mastitis Test (CMT) was positive (> 3 score). Digestive problems was diagnosed when the animal suffer any disease associated with tract gastrointestinal (diarrhea, displacement of the abomasum, bloat). Lameness was diagnosed when the animal suffer any disease located in claws and limbs. Cows that suffer acetonemia had β-hydroxybutyrate value > than 1.2 mmol/L (OETZEL, 2004).

5.2.3 Blood samples

Blood samples were obtained from each animal, once between 21 to 30 DIM. Blood was collected by puncture of the coccygeal vein or artery into Vacutainer ® systems.

5.2.4 Hematological and biochemical analyses

Two blood samples were from each cow, with EDTA for hematological analysis, and other tube without anticoagulant to determine biochemical profile. The analysis were be realized at Multiuser Laboratory in Clinical Veterinary Analysis of University of São Paulo. The erythrogram analyses were performed using BC-2800 Vet Mindray ® to measure a count of red blood cell (RBC), hemoglobin concentration (HB), hematocrit (HT) percentage, and mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW). This equipment uses the impedance method to determine erythrogram parameters.

Serum urea, creatinine, aspartate transaminase (AST), gamma glutamyl transpeptidase (GGT), albumin, triglyceride, total cholesterol, very-low density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), BHBA, NEFA and total calcium were determined by commercial kits of the Randox Laboratories brand, Crumlin, UK, in an automatic biochemistry system (RX Daytona® - Randox Laboratories, UK). Total protein, plasmatic protein and fibrinogen were measured by heat precipitation and refractometry methods according to Kaneko (2008).

5.2.5 BCS and milk yield

Cows were scored for body condition in a 1 to 5 scale (Ferguson et al., 1994) during the collect. The BCS was also categorized as ≤2.75 and ≥3.0 to be used as explanatory variable. Milk yield were measured when the sample were obtained by software Alpro®.

5.2.6 Statistical analysis

The effects of parity, milk yield, BCS, dystocia, RFM, APM, mastitis, problem digestive, lameness, acetonemia, presence of single clinical disease or multiple clinical disease on the prevalence of anemia were analyzed by logistic regression using PROC GLIMMIX of SAS (version 9.3 SAS/STAT; SAS Institute Inc., Cary, NC) fitting binary distribution.

The response of anemia was calculated for all farm as the proportion of cows recruited in the farm that developed anemia. The distribution of anemia was compared between farms by means of the xsquared test of association. Descriptive statistics for qualitative and quantitative variables under study were computed and estimated using logistic regression. The model-building strategy all variables were screened using univariable and multivariable conditional logistic regression with the data grouped by herd using the PROC GLIMMIX of SAS (version 9.3 SAS/STAT; SAS Institute Inc., Cary, NC).

The hematological and biochemical analyses, milk yield and BCS, were analyzed by The GLIMMIX procedure of SAS for animals with and without anemia. Models also included the effects of cow, parity and farm. Differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were considered tendencies.

5.3 RESULTS

The most prevalence disease was APM affecting 33.9% of the cows. The prevalence of dystocia, RFM, mastitis, digestive problems, lameness and acetonemia were 11.2%, 9.6%, 9.9%, 2.2%, and 10.6% and 4.3% respectively (Table 1). Additionally, the prevalence of single clinical disease and multiple clinical disease were 54.0% and 35.5% respectively.

The prevalence of anemia was 16.3% (55/336), being 12.7% (7/55) presented the HT value ≤ 19.9%, 30.9% (17/55) between 20-21.9% and 17 % (31/55) between 22-23.9%. The prevalence of anemia in animals with dystocia, RFM, APM, mastitis, digestive problems, lameness and acetonemia were 16.7%, 12.9%, 13.8%, 12.5%, 28.6%, 23.5% and 11.1% (Table2).

The prevalence of anemia in animals with single clinical disease and multiple clinical disease were 13.2% and 12.2%. The prevalence of anemia did not differ ($P < 0.05$) between cows diagnosed any disorders. The prevalence of anemia in multiparous and primiparous were 15.9% and 16.7% respectively (Table 3). There were association with anemia by interaction of parity with metritis ($P<0.05$).

The prevalence of anemia in BCS ≤2.75 and BCS ≥3.0 were 16.2% and 15.7% respectively (Table 3). The RBC, HB, and HT were less ($P < 0.01$) in cows with anemia (Table 3). Additionally, the MCH and RDW was greater ($P < 0.01$) for cows with anemia. Cows with anemia tended ($P = 0.05$) to have lower serum concentrations of fibrinogen than cow without anemia (Table 5). Concentrations of cholesterol were lower ($P < 0.04$) in cows with anemia than cows without anemia.

The concentration of LDL tend ($P = 0.08$) to be less for anemic cows than cows without anemia. Anemic cows had higher ($P <0.02$) serum concentration of NEFA, and had lower ($P <0.02$) serum concentration of calcium. The milk yield and the BCS did not differ among the groups (Table 6).

Table 14 - Prevalence of diseases in late puerperium dairy cows[1]

Disorder	Prevalence, %
Clinical diseases (n = 322)	-
Single clinical disease	54.0
Multiple clinical disease	35.7
Type of disease	-
Dystocia	11.2
RFM	09.6
APM	33.9
Mastitis	09.9
Digestive problems	02.2
Lameness	10.60
Acetonemia (n = 210)	04.31

[1]A total of 322 cows were enrolled in the study and evaluated for prevalence of clinical diseases and prevalence of anemia, and 210 cows had blood sampled and analyzed for concentrations of BHBA, for prevalence of acetonemia and prevalence of anemia.

Table 15 - Prevalence of anemia according with disorders in late puerperium dairy cows

	Anemic with disorder		Anemic without disorder		
Disorder	%	N	%	N	*P*-value
Clinical diseases	-	-	-	-	-
Single clinical disease	13.2	(23/174)	19.6	(29/148)	0.12
Multiple clinical disease	12.2	(14/115)	19.6	(38/207)	0.11
Type of diseases [1]	-	-	-	-	-
Dystocia	16.7	(6/36)	16.1	(46/286)	0.93
RFM	12.9	(4/31)	16.5	(48/291)	0.60
APM	13.8	(15/109)	17.4	(37/290)	0.41
Mastitis	12.5	(4/32)	16.6	(48/290)	0.60
Digestive problems	28.6	(2/7)	15.5	(49/315)	0.38
Lameness	23.5	(8/34)	15.3	(44/288)	0.22
Acetonemia	11.1	(1/9)	15.5	(31/201)	0.72

[1]A total of 322 cows were enrolled in the study and evaluated for prevalence of clinical diseases and prevalence of anemia, and 210 cows had blood sampled and analyzed for concentrations of BHBA, for prevalence of acetonemia and prevalence of anemia.
[2]Number of Animal with Anemia / Number of Animal with Disorder.
[3]Number of Animal with Anemia / Number of Animal without Disorder.

Table 16 - Prevalence of anemia in late puerperium dairy cows

Variable	Prevalence, %
Parity[1]	-
Multiparous (n=251)	15.9
Primiparous (n=84)	16.7
BCS [2]	-
BCS $\leq$2.75 (n=212)	16.2
BCS $\geq$3.0 (n=115)	15.7

[1]Durring the current lactation.
[2] Scale from 0 = thin to 5 = fat, recorded during the first sample.

Table 17 – Erythrogram parameters according to anemia in late puerperium [1]

	Anemic		
Parameters	No[2]	Yes[3]	*P*-value
RBC ($x10^6$/uL)	5.73±0.10	4.31±0.15	<0.01
HB (g/d)	9.00±0.13	6.89±0.20	<0.01
HT (%)	28.75±0.42	21.20±0.63	<0.01
MCV (fL)	50.60±0.43	49.94±0.65	0.29
MCH (pg)	15.65±0.23	16.67±0.35	<0.01
MCHC (g/dL)	31.24±0.23	31.81±0.34	0.08
RDW (%)	16.54±0.14	16.99±0.21	0.03

[1]Values are mean ±SEM.
[2]No = cow was not diagnosed with anemia.
[3]Yes = cow was diagnosed with anemia.

Table 18 - Biochemical parameters according to anemia in late puerperium[1]

	Anemic		
Parameters	No[2]	Yes[3]	*P*-value
Urea (mg/dL)	24.62±0.77	27.24±1.71	NS
Creatinine (mg/dL)	1.00±0.03	1.08±0.07	NS
AST (U/L)	67.97±1.95	65.20±4.12	NS
GGT (U/L)	18.42±0.87	15.93±1.80	NS
Albumin (g/dL)	3.17±0.04	3.01±0.08	0.06
Total protein (g/dL)	7.72±0.05	7.59±0.11	NS
Plasmatic protein (g/dL)	7.52±0.06	7.59±0.11	NS
Fibrinogen (g/dL)	0.25±0.04	0.44±0.10	0.05
Triglyceride (mg/dL)	12.93±0.43	12.00±0.89	NS
Cholesterol (mg/dL)	94.92±2.93	81.39±6.26	0.04
VLDL (mg/dL)	3.07±0.13	2.62±0.24	NS
HDL (mg/dL)	74.31±3.37	62.08±7.41	NS
LDL (mg/dL)	24.31±2.11	17.29±3.38	0.09
BHBA (mmol/L)	0.58±0.03	0.48±0.07	NS
NEFA (mmol/L)	0.41±0.10	0.94±0.21	0.02
Calcium (mg/dL)	9.17±0.67	8.92±1.38	0.03

[1]Values are mean ±SEM.
[2]No = cow was not diagnosed with anemia.
[3]Yes = cow was diagnosed with anemia.

Table 19 - Milk yield and BCS according to anemia in late puerperium [1]

	Anemic		
Item	No[2]	Yes[3]	*P*-value
Milk yield (L)	31.60±1.23	31.51±2.08	NS
BCS (points)	2.84±0.03	2.74±0.06	NS

[1]Values are mean ±SEM.
[2]No = cow was not diagnosed with anemia.
[3]Yes = cow was diagnosed with anemia.

5.4 DISCUSSION

This study characterizes the prevalence of anemia on postpartum dairy cows, and their effects on hematological and biochemical parameters.

The prevalence of dystocia (11.2%), was considerably higher in the study than in previous studies reporting 8.5% (RIBEIRO et al., 2013), 6.9% (GAAFAR et al., 2011), and was considerably lower than 20.5 by study of Jeong et al., (2015). The prevalence of RFM (9.6%) was considerably higher than 12.3% of Vergara et al., (2014), and lower than 30.4% of Jeong et al., (2015). The prevalence of APM (33.9%) in the present study, was very similar by 33.3% by study of Jeong et al., (2015), and were higher than 5.3%, 9.6%, 17.1 founded by several authors (RIBEIRO et al., 2013; SUTHAR et al., 2013, VERGARA et al., 2014).

The prevalence of mastitis (9.9%) was similar than 9.4% related by Jeong et al., (2015), and was lower than 15.3% observed by Ribeiro et al., (2013), and was higher than 6.1% reported by Suthar et al., (2013). The prevalence of digestive problems (2.2 %) was lower than results of 5.4% and 4.0% founded by Jeong et al., (2015) and Ribeiro et al., (2013). The prevalence of lameness (10.6%) and acetonemia (4.3%) were higher than related in a previous study 3.2%, and were lower than 35.4% by lameness and acetonemia respectively (RIBEIRO et al., 2013). The prevalence of single clinical disease (54.0%) and multiple clinical disease (35.7%) were higher than related in a previous study 26.7%, 10.8% respectively (RIBEIRO et al., 2013).

The anemia is the main disease involving red blood cells in ruminants, defined as reducing the amount of red blood cells in the body (THRALL, 2004). Hemocyte mass can be measured by packed cell volume, hemoglobin content, as well as by red blood cell count (THRALL, 2004). Anemia is characterized in three etiological forms: hemorrhagic, hemolytic, and aplastic (KERR, 2004). The main causes related to anemia are diseases that destroy erythrocytes or hemolysis, blood loss and reduced production of red blood cells (THRALL, 2004), as well as inflammatory causes, mineral deficiencies, being the iron element most common in causing anemia (THRALL, 2004).

The animals evaluated in this study none presented history of high fever and none of the blood smears were observed hemoparasites, demonstrating that the cows did not suffer by babesiosis or anaplasmosis, or infestation by ecto - and endoparasites.

The higher prevalence of anemia for dairy cows with lameness can be occurred by inflammatory response that higher the cytokines produced, causing reduction on hematological value. Cytokines contribute to modulate the inflammatory responses (HENDERSON; WILSON, 1996). Inflammatory cytokines act as inhibitors of erythropoiesis via direct toxic effects on erythroid precursors, decreased expression of hematopoietic factors including erythropoietin and stem cell factor, and decreased expression of erythropoietin receptors (FRY, 2010).

Cows that suffered anemia presented lower values of RBC, HB and HT than the biological range, which is 5-10 $x10^6$/ µL for RBC, 8-15 g/dL for HB and 24-46% for HT (RADOSTITS, 2007; JAIN, 1986). The pattern of anemia was characterized as regenerative, normocytic, normochromic. Variations in erythrogram have been reported in cows with milk fever (STARIC; ZADNIK, 2010), left displacement of the abomasum (MOKHBER et al., 2011), RFM (SAUT; BIRGEL JUNIOR, 2008), and metabolic problems (SAKHARE et al., 2011). However, not yet related by cows that suffer with anemia during late puerperium.

Hemoglobin and packed cell volume values can decreased in dairy cows during the second and third lactation months associated with increased milk production (SEKER; UNSUREN, 1989). The packed cell volume value can decrease due to protein catabolism that occurs during the transition period to provide amino acids for milk production and gluconeogenesis (KIDA, 2002). In our study, this hypothesis was not confirmed, because the milk yield did not differ among anemic and not anemic cows.

Saut and Birgel Junior, (2008) evaluated the erythrogram constituents in postpartum dairy cows and observed reduction of packed cell volume value at times between 30 until 90 days postpartum.

The AST value was above that described as physiological (78-132 UI/L) according to Radostits, (2007) in both groups, whereas the values for GGT were above that recommended as physiological (6.1-17.4 UI/L) as described by Radostits, (2007) for animals without anemia, this increase in liver enzyme values may occur due to intense metabolism of fat in the hepatic tissue, for the production of energy at the early lactation (GRUMMER, 1993).

The serum fibrinogen concentrations in anemic cows were higher than cows without anemia and near the higher reference limits, which is 0.3-0.7 g/dL (KANEKO, 2008). Fibrinogen is an acute phase protein being used to evaluate the inflammatory process (BORGES et al., 2006). The exacerbated pro-inflammatory state may predispose the suppression of erythropoiesis by direct effects of cytokines on the marrow, resulting a limit the erythropoietic response to erythropoietin, which becomes insufficient to compensate the destruction of erythrocytes causing the reduction of hematological values (NEMETH; GANZ, 2014).

Additionally, NEFA values was higher that related as normal (<0.7 mmol/L) by González, (2000) in the anemic animals, this finding represents an excess of lipid mobilization, the excess of NEFA is related to the increased risk of developing fatty liver syndrome (DRACKLEY, 1999; ADEWUYI et al., 2005).

5.5 CONCLUSION

In conclusion, anemia presented a prevalence of 16.3% during the puerperium in dairy cows and no association with several diseases in Holsteins cows such as dystocia, retention of fetal membranes, acute puerperal metritis, mastitis, digestive problems, lameness and acetonemia. In addition, the biochemical profile in cows with anemia did not show no associations of anemia during late puerperium in dairy cows. Overall, anemia in dairy cows that did not suffer from endo or ectoparasites demonstrated no losses in the health of the animals and their production.

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