FERNANDA CAROLINA RAMOS DOS SANTOS

Effect of maternal late-gestation status on metabolic profile, health and immunity of dairy calves

São Paulo
2020

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Dissertation submitted to the Postgraduate Program in Veterinary Clinical Science of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Master's degree in Sciences.

Department:

Internal Medicine

Area:

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Advisor:

Prof. Viviani Gomes, Ph.D.

In agreement:_____

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CERTIFICADO

Certificamos que a proposta intitulada "Efeito do perfil materno durante o período final de gestação sobre o perfil metabólico, saúde e imunidade de bezerras leiteiras", protocolada sob o CEUA nº 6740260218 (ID 004857), sob a responsabilidade de **Viviani Gomes** *e equipe; Fernanda Carolina Ramos dos Santos* - 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 09/05/2018.

We certify that the proposal "Effect of maternal late-gestation status on metabolic profile, health and immunity of dairy calves", utilizing 180 Bovines (180 females), protocol number CEUA 6740260218 (ID 004857), under the responsibility of **Viviani Gomes** *and team; Fernanda Carolina Ramos dos Santos* - 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 05/09/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 07/2018 a 09/2018 Área: Clínica Médica Veterinária

Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	2 a 10 anos	N:	90
Linhagem:	Holandesa			Peso:	400 a 700 kg		
Origem:	Animais de proprietários						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	0 a 30 dias	N:	90
Linhagem:	Holandesa			Peso:	30 a 50 kg		

Local do experimento: A parte de campo será realizada na Fazenda Agrindus situada no município de Descalvado localizada no estado de São Paulo. As análises laboratoriais serão realizadas no laboratório do departamento de Clínica Médica Veterinária (VCM), da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (USP). As análises hormonais e químico-bromatológicas serão realizadas em local a definir.

São Paulo, 16 de fevereiro de 2020

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenador
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

EVALUATION FORM

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Date: _____/_____/_____

Committee Members

Prof. _____

Institution:_____ Decision: _____

Prof. _____

Institution:_____ Decision: _____

Prof. _____

Institution:_____ Decision: _____

DEDICATION

For everyone that I love…

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“Eu seguro minha mão à sua mão
E uno o meu coração ao seu,
Para que juntos possamos fazer
Aquilo que sozinho eu não consigo.”
Unknown author

ABSTRACT

SANTOS, F. C. R. **Effect of maternal late-gestation status on metabolic profile, health and immunity of dairy calves.** 2020. 131 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

Maternal status during the transition period can have carryover effects on several health and performance variables of Holstein dairy calves. However, the effects of maternal late gestation status on profile presented by dairy calves during the neonatal period are not well established. The general objective of this study was to evaluate the impact of maternal variables at the calving on the metabolism and immunity of dairy calves during the first month of life. Holstein cows (n=28) were blood sampled at calving. The mean of the results for the maternal variables [non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), glucose, total protein, albumin, triglycerides, cholesterol, haptoglobin (Hp), iron, thoracic perimeter and body condition score (BCS)] was calculated and used as maternal factors. The 28 calves from these cows were subsequently divided into two groups according to their dams' high or low degree of each factor. The responses of calves in each of these groups were compared throughout their first month of life. Calves were blood sampled following birth and at ~ 2, 7, 14 and 28 days of age by assessing of IgG levels, biochemical parameters, inflammatory status, and innate immunity response. At the same timepoints, the calves were evaluated clinically for diarrhea and other clinical indices. When compared when grouped as maternal groups of low or high glucose, a statistical difference was found only on D14 with calves from cows with higher blood glucose had a lower cholesterol concentration when compared with calves from dams with lower blood glucose. Inflammatory status also varied with calves born from dams with low NEFA had lower levels of haptoglobin (Hp) when compared to calves born to dams with high NEFA on D28. Calves from dams with low maternal albumin, low maternal cholesterol, and low maternal BCS had high levels of basal reactive oxygen species on D7 and D28. Calves from dams with low BCS also had neutrophils more reactive for *S. aureus, S. hyicus*, and *E. coli*, considering the AFU. The response ratio against *S. aureus* and *S. hyicus* was higher in the calves was born from the low maternal BCS. Collectively, these data suggest that prenatal exposure to different maternal factors may adversely affect

metabolic, inflammatory and immune responses of the calves during the first month of life that could influence disease susceptibility and performance.

Keywords: transition period. inflammation. innate immune response. maternal factors.

RESUMO

SANTOS, F. C. R. **Efeito do perfil materno durante o período final de gestação sobre o perfil metabólico, saúde e imunidade de bezerras leiteiras**. 2020. 131 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

O status materno durante o período de transição pode ter efeitos na saúde e desempenho de bezerras da raça Holandesa. No entanto, o efeito do perfil materno ao final da gestação sobre o status apresentado pelas bezerras durante o período neonatal não estão bem estabelecidos. O objetivo geral deste estudo foi avaliar o impacto de variáveis maternas no momento do parto sobre o metabolismo, a saúde e a imunidade de bezerras leiteiras durante o período neonatal. Vacas da raça Holandesa (n = 28) tiveram amostras de sangue coletadas no parto. A média dos resultados para as variáveis maternas [ácidos graxos não esterificados (AGNE), β-hidroxibutirato (BHB), glicose, proteína total, albumina, triglicerídeos, colesterol, haptoglobina (Hp), ferro, peso e escore de condição corporal (ECC)] foram calculados e utilizados como fatores maternos. As bezerras foram posteriormente divididas em grupos de acordo com o alto ou baixo grau de cada fator materno. As respostas das bezerras em cada um desses grupos foram comparadas ao longo do primeiro mês de vida. As bezerras tiveram amostras de sangue coletadas após o nascimento e em torno de 2, 7, 14 e 28 dias de vida, avaliando os níveis de IgG, parâmetros, estado e resposta imune inata. Nos mesmos momentos, as bezerras foram avaliadas por parâmetros vitais, frequência de diarreia e doenças respiratórias e desempenho. As bezerras nascidas de mães mais pesadas, com maior escore de condição corporal e nascidos na primavera, apresentaram maior frequência de diarreia. Vacas que pariram mais pesadas e com maior escore de condição corporal geraram bezerras com melhor desempenho desde a primeira semana de vida em relação à altura da cernelha e largura da garupa. Bezerras de mães com baixas concentrações de proteína apresentaram alta concentração de AGNE no D1. Vacas com alto nível de proteína também apresentaram alto nível de Hp e baixos níveis de albumina. Os filhos nascidos de vacas com alta concentração de ferro apresentaram altos valores de glicose. Em relação à resposta imune, as bezerras filhas de vacas com baixa albumina, baixo colesterol e baixo BCS apresentaram altos níveis basais de espécies reativas de

oxigênio expressos em taxa média de fluorescência em D7 e D28. Os filhos nascidos de vacas com baixo BCS também apresentaram neutrófilos mais reativos para *S. aureus, S. hyicus* e *E. coli*, considerando a taxa média de fluorescência. A taxa de resposta contra *S. aureus* e *S. hyicus* foi maior nas bezerras nascidas da baixa BCS materna. Em suma, esses dados sugerem que a exposição pré-natal a diferentes fatores maternos pode afetar adversamente algumas respostas metabólicas, inflamatórias e imunológicas das bezerras durante o primeiro mês de vida que podem influenciar a suscetibilidade à doenças e o desempenho das bezerras.

Palavras-chave: período de transição. inflamação. resposta imune inata. fatores maternos.

SUMMARY

1. INTRODUCTION

The transition period from late gestation to early lactation is critical for the next production cycle and full of challenges for the dairy cow. It can be characterized by metabolic stress due to excessive lipid mobilization, oxidative stress, and inflammatory dysfunction (ABUELO et al., 2015), when the cow fails to physiologically adapt to the profound increase in nutrient requirements associated with fetal growth, colostrogenesis and lactogenesis (SORDILLO and MAVANGIRA, 2014). In addition to substantial influence on the dam, maternal stress during late gestation also affects the fetus and probably prenatal stress also exerts carryover effects on the offspring in postnatal life (MCMILLEN and ROBINSON, 2005; MERLOT et al., 2008).

The first month of life is a time when the greatest neonatal morbidity and mortality rates are observed on dairy farms (WINDEYER et al., 2014); hence, maternal carryover effects can have a significant effect on neonatal health. The USDA reported preweaning heifer morbidity and mortality were 33.8% and 5%, respectively in 2014 (URIE et al., 2018). In a data survey carried out in Brazil in 2018, the morbidity rate during the preweaning phase was 34% for diarrhea, 17% for pneumonia and 14% for bovine babesiosis and anaplasmosis, while the mortality rate was 10% (AZEVEDO et al., 2018). Understanding the factors associated with morbidity, mortality and growth is an essential part of improving calf health and performance (WINDEYER et al., 2014).

Studies in humans and mice demonstrated that intrauterine conditions during development lead to changes in tissue structure and function (FOWDEN et al., 2009). This suboptimal condition which may have long-term consequences on offspring physiology and cause greater chances of developing diseases (McMillen and Robinson, 2005; Merlot et al., 2008).

In cattle, the type of stress that consistently affects pregnant dairy cows is heat stress, where cows that suffer heat stress give birth to calves 5 kg lighter than calves born to dams who did not suffer heat stress (Tao et al., 2012). Maternal metabolic stress during the final stage of pregnancy alters immune response and metabolic profile of calves in the first month of life, with calves born from cows with high concentrations of NEFA having lower thoracic perimeter at birth (LING et al., 2018). In addition, a pro-inflammatory profile was found in calves exposed to high concentrations of maternal NEFA.

Late gestation heat stress results in calves with lower thoracic perimeter at birth, shorter stature at weaning, and failure to achieve the same weight or height at 12 months of age observed in calves from dams that are cooled when dry (Dahl et al 2016). Furthermore, the effects of in utero exposure to heat stress lowers milk yield and reproductive performance (MONTEIRO et al. 2016). These effects extend to at least the first lactation of offspring. In this way, prenatal conditions have the potential of affecting the productivity and health status of replacement heifers.

It remains unknown whether others maternal factor during late gestation has a similar detrimental effect on health, performance and immunity of neonatal calves. Thus, the hypothesis of this research is that maternal factors during late gestation influence the health, metabolism and immunity of calves during the neonatal period.

2. OBJECTIVES

The hypothesis of this research is that maternal factors influence the health, metabolism and immunity of their offspring during the neonatal period. The general objective of this research was to evaluate the impact of maternal variables in the transition period on the profile presented by Holstein calves during the neonatal period. The specific objectives included the evaluation of maternal factors on:

a) vital parameters, diarrhea and Bovine Respiratory Disease occurrence of calves;
b) performance of neonates by measuring thoracic perimeter, withers height and rump width of calves;
c) innate immune response and inflammatory profile by measuring haptoglobin, iron, total protein, White Blood Cells (WBC) parameters and the production of reactive oxygen species of calves.

3. LITERATURE REVIEW

This literature review will present articles about the metabolism profile of cows in the transition period, as well as the metabolism and immunity of calves in the neonatal period. The relationship between dams and calves, and fetal programming was also addressed to understand how maternal variables can influence the calf's life in the first month after birth.

3.1. MATERNAL METABOLISM DURING THE TRANSITION PERIOD

At the end of the dry period, the cow is in the period understood as transition period (GRUMMER, 1995), between 21 days pre-partum to 21 days post-partum (VAN KAMPEN; MALLARD, 1997). This period is critical for the health, production and profitability of dairy herds (MULLIGAN and DOHERTY, 2008; INGVARTSEN and MOYES, 2013).

During the dry period, many adaptations necessary for the maintenance of fetal nutrition occur and as the dam prepares for the beginning of lactation. These adaptations to the physiological state and nutritional conditions occur through homeostatic and homeorhetic mechanisms. Homeostatic control involves the maintenance of physiological balance, while the homeorhexia can be defined as the orchestrated control of the metabolism of body tissues necessary to support a new physiological state (BAUMAN and CURRIE, 1980).

The major regulator of energy metabolism in mammals is blood glucose (LEHNINGER *et al*., 1995). In ruminants, only 5% of the ingested glucose is directly absorbed (HERDT, 1988). Much of the dietary glucose (starch and fiber) undergoes rumen fermentation and is converted to volatile fatty acids, and thus a constant state of gluconeogenesis is required to meet glucose demand (HERDT, 1988).

Cellulose and other structural carbohydrates in plant cell walls (polysaccharides) are fermented by microorganisms from the rumen environment in short chain volatile fatty acids, especially acetic, propionic and butyric acid. Anaerobic fermentation products are absorbed directly into the rumen and, to a lesser extent in the reticulum and the large intestine and transported to the liver (REYNOLDS et al., 1988; CORREA; GONZÁLEZ; SILVA, 2010).

In the ruminal epithelium, the short chain volatile fatty acids are absorbed and metabolized: 80% of butyrate is converted to acetoacetate and ketone bodies; and 50% of propionate can be metabolized to lactate or pyruvate (GONZÁLEZ; SILVA, 2006). The remaining absorbed propionic acid is transformed into glucose in the liver through gluconeogenesis (REYNOLDS et al., 1988; CORREA; GONZÁLEZ; SILVA, 2010).

Hepatic gluconeogenesis may be performed by various biochemical pathways, depending on glucose precursors. In ruminants, the primary source of glucose is propionate, but there are other precursors of gluconeogenesis, such as glycerol obtained from lipolysis; lactate from anaerobic glycolysis in skeletal muscle; and glycogenic amino acids originated from proteolysis (GONZÁLEZ; SILVA, 2006).

Elevated plasma glucose concentrations result in glycogenesis, under the influence of hormones as insulin and glucagon. This process occurs in the cytosol of cells in all tissues, especially in the liver and skeletal muscles, that are glycogen storage sites. On the contrary, increased energy demand stimulates glycogenolysis, in which hepatic glycogen is degraded into glucose, a source for oxidative process and energy formation. The glycogenolysis process is regulated by catecholamines and glucagon that act directly on the liver and muscles (GONZÁLEZ; SILVA, 2006).

During the transition period, there is an intense decrease in dry matter intake (DMI), especially in the last week before birth (HAYIRLI et al., 2002), associated with the increase in energy demand. This condition usually results in energy deficiency, i.e., the animal enters a state of negative energy balance (NEB). In the demand for energy, the glucagon is released, stimulating lipolysis, so that there is hydrolysis of triglycerides and release of non-esterified fatty acids (NEFA) (DRACKLEY, 1999; BRUSS, 2008).

Non-esterified fatty acids present in the bloodstream can be used by body tissues for energy generation and glucose utilization savings (PULLEN et al., 1989). Therefore, the NEFA serum concentrations reflect the magnitude of adipose tissue mobilization and normally these compounds are incorporated into milk fat in early lactation. In the hepatic parenchyma, most NEFA are completely or partially oxidized, esterified or exported as very low-density lipoprotein (VLDL) (GRUMMER, 1995). The liver apparently metabolizes NEFA according to the proportion of its supply via the bloodstream (EMERY et al., 1992; GRUMMER, 1995; DRACKLEY, 1999; HERDT, 2000; BUTLER, 2004; HOEDEMAKER et al., 2004).

However, hepatic fatty acid oxidation and VLDL export capacity is relatively low in ruminants (GRUMMER, 1995). Thus, excess NEFA in the liver during the transition period and early lactation may be partially oxidized to produce ketone bodies, like as β-hydroxybutyrate (BHB), that are released in the blood and serve as energy sources for other tissues or reconverted to triacylglycerol (BERTICS et al., 1992; GRUM et al., 1994, NRC, 2001).

Accumulation of triacylglycerol in the hepatic parenchyma reduces the liver's ability to detoxify ammonia in urea (STRANG et al., 1998), resulting in decreased hepatic gluconeogenesis capacity. Thus, there is a decrease in synthesis from propionate, which is the main precursor of glucose in ruminants (CADORNIGA-VALINO et al., 1997; OVERTON et al., 1999).

In the peripartum, the serum levels of NEFA and BHB are indicators of the ability of cows to deal with metabolic challenges in the transition period, because it measures the mobilization and oxidation of fat, respectively, reflecting the success of the cow in adapting to NEB (HERDT, 2000). These adaptations that culminate in NEB can be noted by the abrupt decrease in the body condition score (BCS) (HAYIRLI et al., 2002).

Lago et al. (2001) showed more intense weight loss and higher BHB levels in overweight Holstein cows (BCS≥4,0) when compared to cows with lower BCS (BCS≤3,5). In addition, the authors concluded that obese animals (BCS≥4,0) presented more intense NEB considering that the glucose levels were lower.

Hayirli et al. (2002) studied Holstein cows during the final three weeks of gestation, which were classified as lean (BCS from 1 to 3), medium (BCS from 3.01 to 4) and obese (BCS 4.01 to 5). The authors found a 40% decrease in DMI in obese animals compared to a decrease of 28% and 29% in lean and medium animals, respectively, from the third pre-partum week onwards.

Chebel et al. (2018) found that loss of BCS during the dry period was associated with negative health, reproductive, and productive performances, but this change during the dry period was not associated with the likelihood of metabolic diseases on the cow.

Metabolic status and organ functioning disorders, as well as body adaptation to nutritional, physiological challenges and metabolic imbalances, can be monitored by biochemical, hematological and hormonal parameter. The monitoring of these variables, combined with clinical examination, are very useful in the diagnosis of clinical cases that are not well evidenced, but that may cause losses in the productive

and reproductive performance of dairy herds (GONZÁLES, 1997; GONZÁLES et al., 2009).

LeBlanc et al. (2005) found that high NEFA concentrations (≥ 0.4 mmol/L) for pre-partum cows prior is associated with increased risk displaced abomasum, retained placental and decreased milk production. Duffield et al. (2009) reported that higher BHB values (> 1.2 mmol/L) in the first or second week postpartum is associated with increased risk of abomasum displacement and metritis.

Endocrine changes interfere with energy metabolism. The stress of cows in the peripartum triggers an important endocrine mechanism that controls glucose levels, which are mediated by activation of the somatotropic axis that results in the release of catecholamines (epinephrine and norepinephrine) and cortisol, which stimulate the sympathetic nervous system, resulting in lipolysis: increases NEFA mobilization from adipose tissue and stimulates gluconeogenesis (GRUMMER, 1995; DRACKLEY, 1999)

Maternal and fetal cortisol is produced by activation of the somatotropic axis. The endocrine mechanisms involved are controlled by the increased production of corticotropin-releasing hormone (CRH) by the hypothalamus, which in turn acts on the adenohypophysis gland by increasing the adrenocorticotropic hormone (ACTH), which ultimately acts on the adrenal gland producing the cortisol (JACKSON, 2006).

Glucocorticoid concentrations begin to increase three days before calving, with peak at calving and return to reference intervals on the third postpartum day. Corticosteroids have a hyperglycemic effect by stimulating lipolysis and proteolysis. Cortisol promotes liver enzyme expression for gluconeogenesis from precursors such as amino acids and glycerol (GOFF; KEHRLI; HORST, 1989; PATEL et al., 1996).

During the transition period, cows also have unregulated immune responses and this can be attributed to the metabolic and hormonal variations that occurred in this period in response to increased energy demand (MALLARD et al., 1998; INGVARTSEN et al., 2003; MEGLIA et al. al., 2005; LOISELLE et al., 2009). In addition, high NEFA and BHB, and low blood glucose are important contributors to the dam's immune dysregulation in pre-partum, since glucose is the major metabolic fuel for many of the cellular functions. Moreover, the inflammatory response of the cow in the transition period is marked by a high production of acute phase inflammatory proteins. In response, there is the production of proinflammatory cytokines (ABUELO et al., 2019; LING et al., 2018).

In inflammatory processes there is intense production of reactive oxygen species (ROS) by cells of the immune system. This combination of proinflammatory state in the immediate postpartum also stimulates fat mobilization (ABUELO et al., 2019; LING et al., 2018). Ling and colleagues (2018) point out that cows that undergo severe degradation in response to an imbalance caused by excessive fat mobilization, oxidative stress and immune dysfunction with exacerbated inflammatory response, go through a state called metabolic stress (LING et al., 2018).

3.2. NEONATAL METABOLISM

At the end of pregnancy, fetal maturation results in increased gluconeogenesis and hepatic glycogen stores, caused by increased glucocorticoids, catecholamines and thyroid hormones. Glucose during the fetal period depends almost exclusively on placental glucose supply, and there is a linear relationship between maternal and fetal glucose concentrations (FOWDEN et al., 2009).

After birth, the source of glucose will no longer be passive, i. e. via the placenta, and the newborn must meet the demand for glucose by ingesting lactose (via ingestion of colostrum, milk and replacer), or by endogenous production of glucose (via gluconeogenesis or glycogenolysis). Lactose intake alone is not enough to meet neonatal glucose demands (GIRARD et al., 1992; GIRARD et al., 1985; MELLOR; COCKBURN, 1985), so liver glycogen, stored in late pregnancy, will be the first source of energy to ensure blood glucose after birth (LIGGINS, 1994; STEINHOFF-WAGNER et al., 2011).

After ingestion of colostrum, considering the first 24 hours of calf life, plasma glucose concentrations will remain low, so endogenous glucose production in calves should avoid a marked decrease in plasma glucose concentration (STEINHOFF-WAGNER et al., 2011; HADORN et al., 1997). However, in preterm calves, severe hypoglycemia and insufficient endogenous glucose production are observed due to low hepatic glycogen stores (BITTRICH et al., 2002).

Glucose concentrations after birth are lower compared to the second day of life, even when lactose is absorbed from ingestion of colostrum and milk, indicating maturation of the gluconeogenic pathway after birth. During the first days of life, endogenous glucose production guarantees 25 to 30 µmol of glucose, with 60%

derived from gluconeogenesis (STEINHOFF-WAGNER et al., 2011; SCHEUER et al., 2006).

Lactose, amino acids, especially alanine, and glycerol are substrates for gluconeogenesis and used by the newborn within the first eight hours after birth. Calves can use lactose from birth instead of amino acids in gluconeogenesis, while having reduced ability to use glycerol (GIRARD et al., 1992).

Calves also use propionate as a substrate for the gluconeogenic pathway, being measurable in hepatocytes from 14 days of age, indicating that from ruminal development, i. e. when it becomes functional, the production of short chain fatty acids from increases and propionate becomes the main precursor of glucose via endogenous glucose production (DONKIN; HAMMON, 2005; DONKIN; ARMENTANO, 1994).

Calves also have energy production via renal gluconeogenesis, contributing 10-15% of the total glucose produced by gluconeogenesis, and their functionality is independent of age, since calves have a rate like dry cows when it comes to kidney gluconeogenesis (KREBS and YOSHIDA, 1963).

Pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PPECK) and glucose 6-phosphatase (G6Pase) enzymes are the limiting enzymes of gluconeogenesis. Pyruvate Carboxylase and PPECK enzymes have been present in the liver since the fetal period; however, only PEPCK concentrations increase after birth and are related to increased endogenous glucose production and hepatic glycogen storage (KALHAN and YOSHIDA, 1963).

The decline in hepatic glycogen stores immediately after birth coincides with the increased concentration of G6Pase as it ensures the release of glucose into the bloodstream. Glucose 6-phosphatase activities remain low during the fetal period, increase at birth, peak during the neonatal phase, and remain constant until adulthood (BOHME et al., 1983).

In relation to fat metabolism, represented by fatty acid oxidation, it increases rapidly after birth to supply the energy demand of the newborn calf (GIRARD et al., 1992; GIRARD et al., 1985). Fatty acid oxidation capacity is low in the fetal liver and increases markedly during the first 24 hours after birth (ODEN; TREEN, 2003).

Fatty acids are available due to the oxidation of fat from milk intake after birth, however, the production capacity of ketone bodies is low, and plasma concentrations of β-hydroxybutyrate and acetoacetate after birth are minimal (GIRARD et al., 1992).

Calves, even if born prematurely, have fat stores and can mobilize them and provide non-esterified fatty acids for energy supply when milk intake is insufficient (STEINHOFF-WAGNER et al., 2011; HADORN et al., 1997).

Brown adipose tissue, equivalent to 2% of thoracic perimeter, is the largest fat reserve in a newborn calf and ensures heat production and body temperature regulation as its adipocytes have many mitochondria. When stimulated by cold, sympathetic nervous system signals stimulate adipocytes present in brown adipose tissue, so that triglycerides break down, generating glycerol and free fatty acids, which are used as a substrate by mitochondria for heat production. Approaching one month of age, brown adipose tissue is converted to white adipose tissue due to less sympathetic stimulation (SMITH et al., 2004).

There are several hormones that regulate endocrine metabolism of glucose and fatty acids in calves. The main mediators are insulin, glucagon, growth factor (GH), the IGF system (Insulin Growth Factor 1 - IGF-1 and Insulin Growth Factor 2 - IGF-2) and cortisol (HAMMON et al., 2012).

Plasma insulin concentration is reduced at birth and increases with ingestion of colostrum, milk or replacers, indicating that nutrient intake, especially glucose, stimulates insulin secretion (GIRARD et al., 1992). There is no evidence that colostrum insulin is absorbed in significant quantities from the gastrointestinal tract in calves, reaching systemic concentrations. Thus, plasma insulin concentration originates from pancreatic secretion. The amount, timing and frequency of ingestion influence the concentration and duration of plasma insulin effect (FOWDEN et al., 2009).

Regarding plasma glucagon, its concentration also increases after ingestion of colostrum. Glucagon is an insulin antagonist, so it is necessary to maintain glucose homeostasis (KRAUS-FRIEDMANN, 1984). During the calf's first week of life, the plasma glucagon concentration is higher in animals fed on replacement and formulas than in animals receiving colostrum, probably due to insufficient glucose intake when compared to colostrum (FOWDEN et al., 2009).

After birth, plasma GH concentrations do not respond to feeding and plasma IGF-I concentrations are low. Due to the low number of hepatic GH receptors in the newborn, GH may not be relevant for gluconeogenesis stimulation in neonates. In contrast to GH, IGF-I plasma concentrations are affected by the amount and timing of colostrum ingestion, and the frequency of feeding, as colostrum ingestion stimulates

glucose absorption, resulting in elevated plasma insulin concentrations. Glucose and insulin are known to stimulate IGF-I synthesis (HAMMON et al., 2012).

Plasma cortisol, which is increased at birth, decreases after colostrum ingestion and decays in the first weeks of life. However, plasma cortisol concentrations may remain during food deprivation or after reduced colostrum ingestion as glucocorticoids are able to stimulate activity of hepatic gluconeogenic enzymes (HADORN et al., 1997; HAMMON; BLUM, 1998, LEE et al., 1985).

Colostrum provides newborns with large amounts of nutrients, vitamins, and non-nutrient biologically active substances such as immunoglobulins, hormones, growth factors, cytokines, and other biologically active peptides. In addition to the great importance of immunoglobulins from colostrum in the passive immunity of the neonate, colostrum is of great importance for intestinal development (BLUM, 2006; BLUM; BAUMRUCKER, 2008; BLUM; HAMMON, 2000). This profile is not induced by an isolated growth factor, but by the interaction of many growth promoting substances present in colostrum, such as insulin-like growth factors (IGF-1 and IGF-2) and transforming factors. Transforming growth factor beta 1 - TGF-β1 and Transforming growth factor beta 2 - TGF-β2 and GH do not directly regulate newborn metabolism, but stimulate gastrointestinal tract development to improve nutrient absorption and thus indirectly affect calf metabolism, probably by their binding to receptors present in the intestinal mucosa of newborns (BURRIN et al., 1996; ODLE et al., 1996).

Colostrum ingestion stimulates small bowel cell proliferation, increases villus size, and increases absorptive capacity while reducing apoptosis. In short, it stimulates lactose digestion and increases glucose absorption (BLUM, 2006; BLUM; BAUMRUCKER, 2008; ODLE et al., 1996; BLÄTTLER et al., 2001; BÜHLER et al., 1998).

3.3. MATERNAL-CALF RELATIONSHIP AND FETAL PROGRAMMING

Fetal programming are the events that happen during pregnancy that can affect long term effects on the newborn. The term “fetal programming” resulted from studies by Dr. David Barker and colleagues. They studied birth records in the United Kingdom and Europe, and related different maternal stresses to infant weight and physical characteristics at birth and subsequent health status in later life. They determined that maternal undernutrition in the first half of gestation, followed by adequate nutrition from mid-gestation to term, resulted in infants of normal birth weight, which were proportionally longer and thinner than normal. This early fetal undernutrition resulted in an increased incidence of health problems experienced by these individuals as adults, including obesity, diabetes, and cardiovascular disease (BARKER et al., 1993; GODFREY and BARKER, 2000).

Applying the concept of fetal programming on dairy production, it is any event that occurred with the dam during gestation that affects calf health, production and performance (NATHANIELSZ et al., 2007). Calf development and growth depend on maturation events occurring in the fetal period, allowing the development of multiple organs, metabolic pathways, immune system, endocrine system, and thermoregulatory pathways (VONNAHME, 2007).

The bovine embryo enters the uterus 4 days after ovulation. The critical period of maternal recognition of gestation occurs between 15-18 days after ovulation, followed by the initial stages of early placentation. In the cow, the placenta attaches to discrete sites on the uterine wall called caruncles. These caruncles are aglandular proliferations of connective tissue which appear as knobs along the uterine luminal surface. These caruncles are arranged in two dorsal and two ventral rows throughout the length of the uterine horns. The placental membranes attach at these sites via chorionic villi in areas called cotyledons (ROSENFELD et al., 1974; REYNOLDS et al., 1988; REYNOLDS; REDMER, 1995).

By day 120 of gestation, the placental vasculature can be seen radiating out from the umbilicus to the individual cotyledons. The caruncular-cotyledonary unit is called a placentome and is the functional area of physiological exchanges between cow and calf. In association with the formation of the placentome, the caruncular area is progressively vascularized to meet the increasing demands of the conceptus. Approximately day 120 of gestation is a transitional period in caruncular

vascularization, which sets the stage for subsequent increases in nutrient transfer required to support the rapidly growing fetus (FORD, 1995; REYNOLDS; REDMER, 1995). It is clear that the placenta plays a fundamental role in providing for the metabolic demands of the fetus; thus, although placental growth slows during the last half of gestation, placental function increases dramatically to support the exponential rate of fetal growth (METCALFE et al., 1988; REYNOLDS; REDMER, 1995). The bovine fetus grows at the fastest rate and accumulates approximately 60% of its birth weight during the last two months of gestation (BAUMAN and CURRIE, 1980).

Establishment of a functional fetal/placental vascular system is one of the earliest requirements during conceptus development (REYNOLDS; REDMER, 1995). However, for the conceptus to effectively draw nutrients from the maternal system, the uterine vasculature must be properly developed. In cows, preferential vascularity of the caruncles begins around day 90 of gestation, with a marked increase in both blood flow and vascular density by day 120 of gestation (FORD, 1995).

The establishment of the vascular architecture is essential if the maternal side is to support the exponentially growing fetus during the last trimester of gestation (REYNOLDS; REDMER, 2001). Any detrimental effects of maternal nutrition during this critical establishment of the maternal-fetal vascular systems would impact the ability of the fetus to acquire the proper amount of nutrients and oxygen. All the respiratory gases, nutrients, and wastes that are exchanged between the maternal and fetal systems are transported via uteroplacental (REYNOLDS; REDMER, 1995; REYNOLDS; REDMER, 2001).

Establishment of functional fetal and uteroplacental circulations is one of the earliest events during embryonic/placental development (PATTEN, 1964; RAMSEY, 1982). It has been shown that the large increase in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation (ELEY et al., 1978; PRIOR.; LASTER, 1979), depends primarily on the dramatic growth of the uteroplacental vascular beds during the first half of pregnancy (REYNOLDS; REDMER, 1995).

Fetal organogenesis is occurring simultaneously as placental development. In the beef cow fetus, as early as 21-22 days post-ovulation, the heartbeat is apparent. Limb development occurs as early as day 25 of pregnancy followed by a sequential development of other organs, including the pancreas, liver, adrenals, lungs, thyroid, spleen, brain, thymus, and kidneys (HUBBERT et al., 1972). As the growth trajectories

for these tissues vary, each tissue is susceptible to suboptimal conditions (i.e. maternal undernutrition) at different time periods.

Fetal growth and development depend upon the acquisition of maternal digested or metabolic products, which can promote fetal growth directly by providing essential chemical elements for fetal tissue growth (GAO et al., 2009). Inadequate maternal nutrition or low energy during pregnancy influences the fetal growth trajectory (ROBINSON et al., 1999; MCMILLEN et al., 2001) because partitioning of fetal substrates is disturbed or destroyed by changes of maternal metabolic status, which would compromise postnatal growth, metabolism, and health (GAO et al., 2009; MICKE et al., 2010).

Undernutrition of the pregnant cow during the initial stages of fetal development may appear to be unimportant because of the limited energy requirements of the fetus for growth and development during the first half of gestation. This is accentuated by the fact that 75% of the growth of the ruminant fetus, for example, occurs during the last two months of gestation (ROBINSON et al., 1977). However, it is during this early phase of fetal development that maximal placental growth, differentiation and vascularization occurs, as well as fetal organogenesis, all of which are critical events for normal conceptus development (VONNHAME, 2007).

Restrictive nutrition during gestation culminates in the development of fetal hypoglycemia (BELL, 2002), because fetal glycemia depends almost exclusively on placental supply of glucose (FOWDEN et al., 2009). As an attempt to maintain the maternal-fetal gradient of glucose, the transfer of glucose to the placenta is restricted and the consumption of placental glucose is reduced (HAY, 1995), so that conditions of maternal malnutrition induce fetal gluconeogenesis (HAY, 2006).

The maturation of the fetus near birth results in increased gluconeogenesis and hepatic glycogen stock. These changes are caused by increased glucocorticoids, catecholamines and thyroid hormones during late pregnancy (BATTAGLIA, 1978; FORHEAD et al., 2009). Glucocorticoids, such as cortisol, increase the gluconeogenic capacity before birth and their action on glucose metabolism after birth is prolonged when the supply of nutrients is insufficient (HAMMON, 1998; STEINHOFF-WAGNER, 2011).

Studies have concluded that maternal nutritional status affects the quality of colostrum in ruminants (BANCHERO et al., 2006, PHOMVISITH et al., 2016). Colostrum is fundamental for the growth of neonates (BLUM; BAUMRUCKER, 2008),

thus increasing maternal influence on offspring after birth. It is known that colostrum is responsible for the transfer of passive immunity (URUAKPA et al., 2002) and stimulates the development of the gastrointestinal tract (HAMMON et al., 2013). Studies with piglets demonstrated that colostrum could influence body temperature and heat production by the neonate, because digestion and nutrient absorption metabolism produce heat (HERPIN et al., 2005).

Ling et al. (2018) found that offspring born from cows that underwent excessive fat metabolism (high NEFA and BHB) or oxidative stress during pre-partum had lower thoracic perimeter at birth, higher circulating concentrations of haptoglobin (inflammation marker protein) and lower immune response than calves that had lower concentrations of these parameters. These data suggest that prenatal exposure to maternal metabolic stress can affect calf metabolic and inflammatory responses, which may influence disease susceptibility during the first month of life.

In addition to substantial influence on the dam, maternal heat stress during late gestation also affects the fetus. Further, prenatal stress exerts carryover effects on the offspring in postnatal life (TAO; DAHL, 2013). Heat stress in late gestation decreases birth weight of newborn farm animals, which reflects compromised fetal development in utero (TAO et al., 2011; TAO et al., 2012).

Evidence exists in other nonruminant species that maternal stress during gestation influences fetal development and the immune responses consequently. Ambient temperature during late gestation also affects the transfer of passive immunity. During late gestation, piglets from heat-stressed sows have lower circulating IgG compared with those from sows under thermoneutral conditions (MACHADO-NETO et al., 1987). In contrast, cold stress in late pregnancy increases the IgG absorption of piglets relative to their counterparts from sows in thermoneutrality (BATE; HACKER, 1985). The same was observed in dairy calves: heat stress during the dry period of dairy cows compromised the passive IgG transfer from colostrum and cell-mediated immune function of the calves during the preweaning period (TAO et al., 2012).

Low maternal energy density in the last 21 days of pregnancy resulted in decreased calf birth thoracic perimeter, body height, body length, thoracic girth, and umbilical girth, as well as decreased expression of CD4 and CD4:CD8 (GAO et al., 2012).

4. MATERIAL AND METHODS

The Animal Research Ethics Committee of the School of Veterinary Medicine and Animal Science at the University of São Paulo approved all procedures involving animals in this study (Protocol number 6740260218). This research was performed between July and November 2018 in a commercial dairy farm located in Descalvado, São Paulo, Brazil, latitude 21°57'44.9"S and longitude 47°41'44.8"W.

During the experiment, weather data (minimum, maximum and mean temperatures) from the region of Descalvado were collected from the AccuWeather database for the region of Descalvado/São Paulo, Brazil. During July and August, the average maximum temperature was 28,2◦C; average minimum temperature was 9,2◦C and average thermal amplitude was 21,2◦C; while during the months of September and October the average maximum temperature, average minimum temperature and average thermal amplitude were 29.3◦C; 15.9◦C and 13.4◦C, respectively.

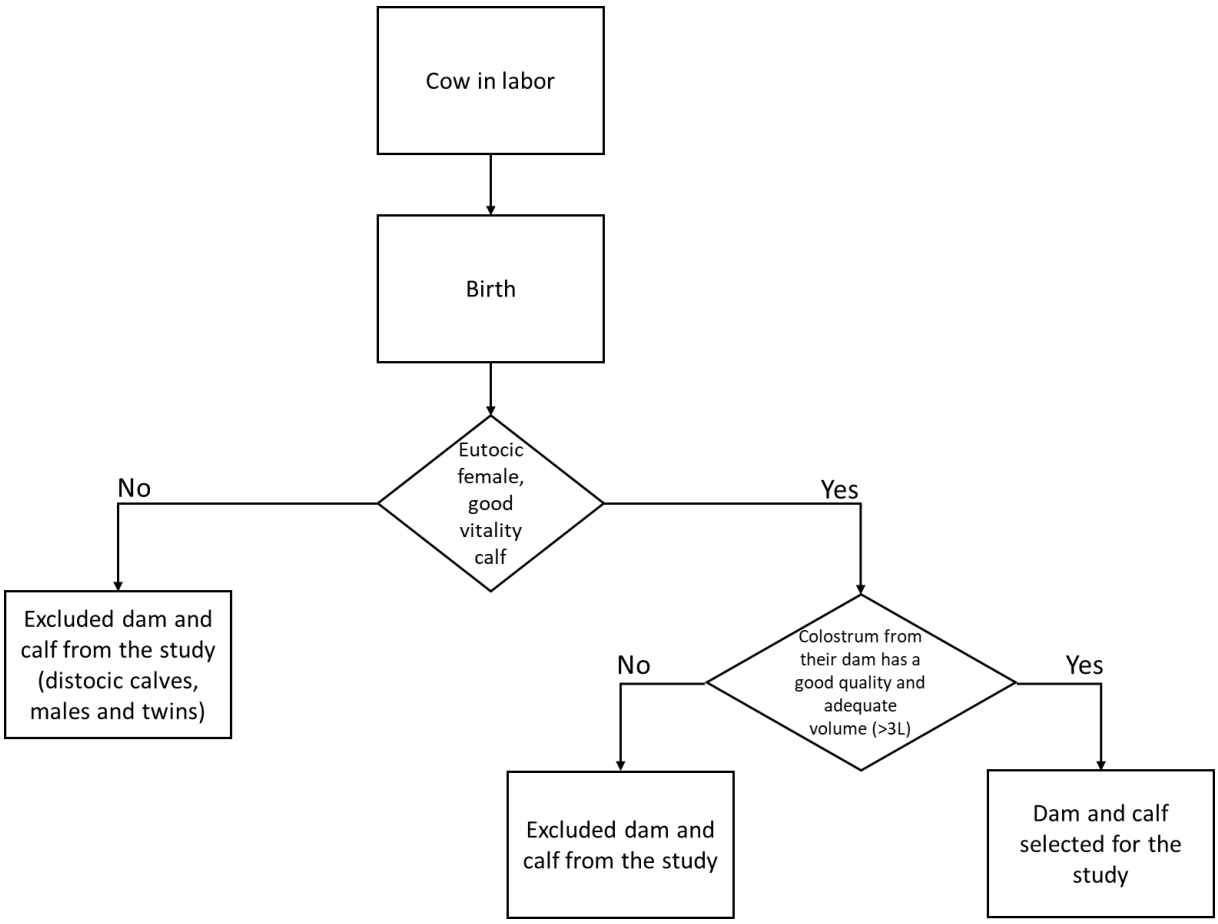
4.1. EXPERIMENTAL ANIMALS, FACILITIES AND MANAGEMENT

The management procedures involving cows and calves using normal husbandry procedures and done under as identical conditions as possible.

4.1.1. Dams management and housing

Twenty-eight multiparous Holstein dairy cows between 2nd to 5th lactation were screened for this research based on the proximity of their calving dates and offspring profile, excluding dams that calved males, stillbirths, twins, and neonates with low vitality immediately after birth. An additional criterion was that the dams produced a minimum volume of 3 liters with good quality colostrum to prevent passive immune failure in the offspring (Figure 1). Aiming at this last inclusion criterion, nulliparous cows were not included to reach the minimum volume suitable for the process of supplying colostrum to calves.

Figure 1 – Flow table of calves and dam's selection process for the present study



Source: SANTOS (2020).

Cows were dried off at approximately 220 d of gestation (dry period of 73 ± 29.9 d). At dry off, cows received an intramammary treatment with a long-acting antimicrobial (Cepravin® Dry Cow, MSD Animal Health) for the prevention of mastitis and an internal teat sealant (Teatseal®, Zoetis). To improve protection against neonatal diarrhea, cows were vaccinated with a single dose of an inactivated combination of Bovine Rotavirus, Bovine Coronavirus and *E. coli* F5 (K99) (Rotavec®, MSD Animal Health), besides of one dose of vaccine against mastitis composed by *E. coli* J5 bacterin and toxoid (J-VAC®, Boehringer-Ingelheim). Dams also received two doses of commercial vaccines against Bovine Respiratory Disease (BRD) in the dry period (Cattle Master Gold FP5/L5®, Zoetis).

The dams were transferred to the indoor compost-barn, collective maternity housing with cross-ventilation (Figure 1a, b and c), 30 days before expected calving date.

Between 60 and 30 days from the expected date of calving, the animals received diet A (Table 1) and Concentrate A (Table 2), while between 30 days from the expected date of calving until the real time of calving the cows receive diet B (Table 1, Figure 2d) and the Anionic Concentrate (Table 2). The TMR diets were offered twice a day, considering the intake of 13-14 kg of dry matter (DM) per animal. Minerals and vitamins were added in the manufacture of concentrate and mineral salt and water were provided *ad libitum*. The diet met or exceed nutrients and energy requirements for pre-calving dairy cows (NRC, 2001).

Table 1. – Diet A provided to dams between 60 and 30 days and diet B provided to dams between 30d to calving time during the experiment.

Diet A (60-30d from expected calving)		**Diet B (30d to calving)**	
Item	**% Dry Matter**	**Item**	**% Dry Matter**
Concentrate A	5.92	Anionic concentrate	9.06
Soybean meal	8.33	Wheat straw	12.41
Wheat straw	7.80	Soybean meal	19.82
Tifton grass	13.25	Corn silage	58.71
Corn silage	64.70	-	-
Total	100 %	Total	100%

Source: SANTOS (2020).

Table 2. – Concentrate A provided to dams between 60 and 30 days (recipe to 2000 kg) and Anionic concentrate provided to dams between 30d to calving time during the experiment (recipe to 1000.8 kg).

Concentrate A		**Anionic concentrate**	
Item	**Quantities (kg)**	**Item**	**Quantities (kg)**
Vitamin E	2,0	NutriCAB® (Kemin)	25
Corn grain	1086,0	Colina	15
570 ca (Nutron) *	510,0	Vitamin E	1
Urea	200,0	Salus NA **	440
OmniGen® (Phibro)	100,0	Corn grain	425
Yeast	50,0	Vitamin AD3	0,25
Magnesium oxide	25,0	OmniGen® (Phibro)	40
Mycofix® (Biomin)	20,0	Yeast	25
Sel-plex® (Alltech)	2,0	Urea	15
Difly® (Champion)	1,5	Mycofix® (Biomin)	7
Biotin	1,0	Nexulin® (Pancosma)	4
Probios® (Ourofino Saúde Animal)	2,5	Biotin	1
-	-	Sel-plex® (Alltech)	1
-	-	Difly® (Champion)	1
-	-	Probios® (Ourofino Saúde Animal)	0,5

570 ca (Nutron®):** Guarantee levels of the vitamin and minerals: Calcium (min) 180 g/kg; Calcium (max.): 200 g/kg; Phosphor (min.): 20 g/kg; Vitamin A (min.): 450000 UI/kg; Vitamin D(min.): 300000 UI/kg; Vitamin E (min.): 7000 UI/kg; Magnesium (min.): 145 g/kg; Sulfur (min.): 45 g/kg; Manganese (min.): 3000 mg/kg; Zinc (min.): 3000 mg/kg; Cobalt(min.): 63 mg/kg; Copper (min.): 800 mg/kg; Iodine (min.): 35 mg/kg; Selenium (min.): 22 mg/kg; Fluorine (max): 200 mg/kg; Chrome (min.): 60 mg/kg; Sodic monensin (min.): 1500 mg/kg. * Salus NA**: Guarantee levels of the vitamin and minerals: non-protein nitrogen (max): 280 g/kg; Calcium (min) 100 g/kg; Calcium (max.): 150 g/kg; Phosphor (min.): 4500 mg/kg; Magnesium (min.): 60 g/kg; Sodium (min): 20 g/kg; Sulfur (min.): 50 g/kg; Zinc (min.): 1350

mg/kg; Cobalt(min.): 25 mg/kg; Copper (min.): 315 mg/kg; Iodine (min.): 35 mg/kg; Chrome (min.): 25 mg/kg; Selenium (min.): 15 mg/kg; Manganese (min.): 1260 mg/kg; Vitamin A (min.): 225000 UI/kg; Vitamin D(min.): 70000 UI/kg; Vitamin E (min.): 3600 UI/kg; Sodic monensin (min.): 600 mg/kg.
Source: SANTOS (2020).

Figure 2 – Collective maternity housing with maximum capacity for 50 animals with 30 days before expected calving date (a and b); diet compost for corn silage and concentrate provided for the dams during the period of study (c and d).

Source: SANTOS (2020).

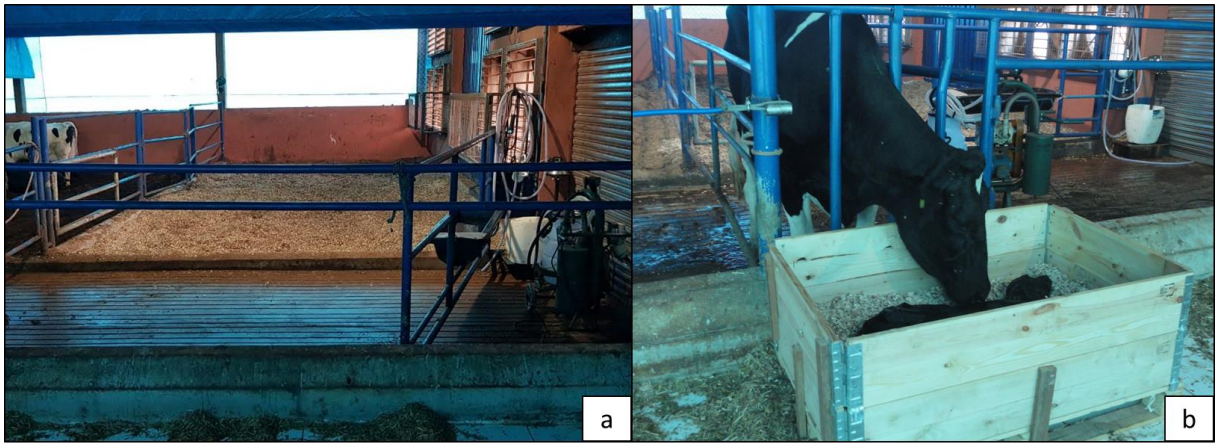
4.1.2. Calving and birth management

During the experimental period, the research team monitored the animals in the maternity housing, monitoring pregnant group in the compost-barn every 2 hours for 24 hours to guarantee that all deliveries would be monitored. Cows that were near parturition were moved to clean maternity pens (Figure 3a). The signs of calving that were monitored was discomfort (swishing tail, arched back, restless, peeing, kicking and nosing at her side and tail raising), amniotic sac protruding, and swollen vulva.

Immediately after birth, calves were separated from their dams and immediately placed in a “cuddle box” (Figure 2b) to receive maternal initial contact, including sniff and lick the newborn calf. In this moment, the calf vitality score was evaluated to

include in this research only neonates from eutocic (spontaneous) births and vitality score greater than 7 (VANNUCCHI, 2014). Males and twins were all excluded from this investigation.

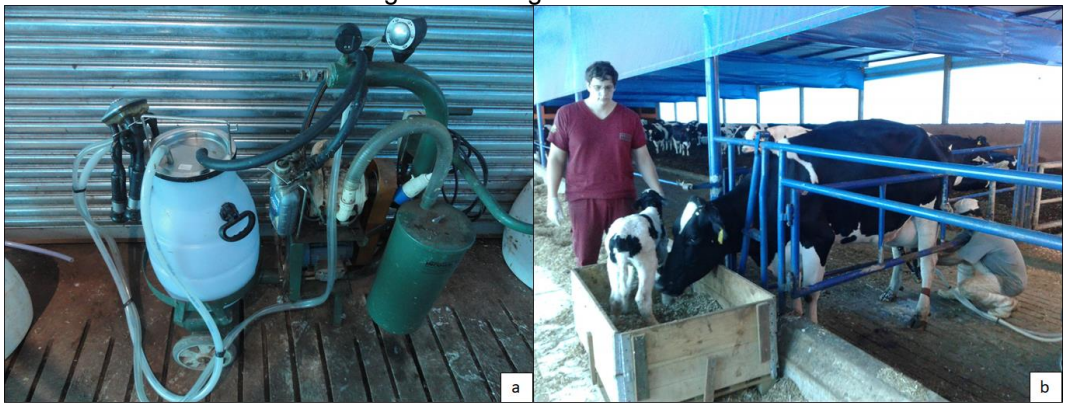
Figure 3 – Maternity pen with shaving bed and portable milking machine (a); cow licking their offspring after birth.



Source: SANTOS (2020).

Dams were milked immediately after calving using a portable milking machine at the maternity pen (Figure 4). Fresh colostrum was collected after cleaning teats with soap and water, dipping in a 1% chlorine solution, and drying with fresh paper towels. Initially, colostrum was measured and screened according to the quality using a digital Brix handheld refractometer (MISCO DD-2 Refractometer, Misco®) (Figure 5a) and colostro *balls* (Ms colostro balls, Nutri Support©, 3705010) (Figure 5b). Only dams that produced colostrum with ≥21% were included in the study (Quigley et al., 2013). Aliquots of colostrum pool (50 mL) were taken after complete milking from the bucket to evaluate the concentration of IgG.

Figure 4 – Portable milking machine at the maternity pen (a); cow being milked immediately after birth while licking and sniffing calf in a “cuddle box”.

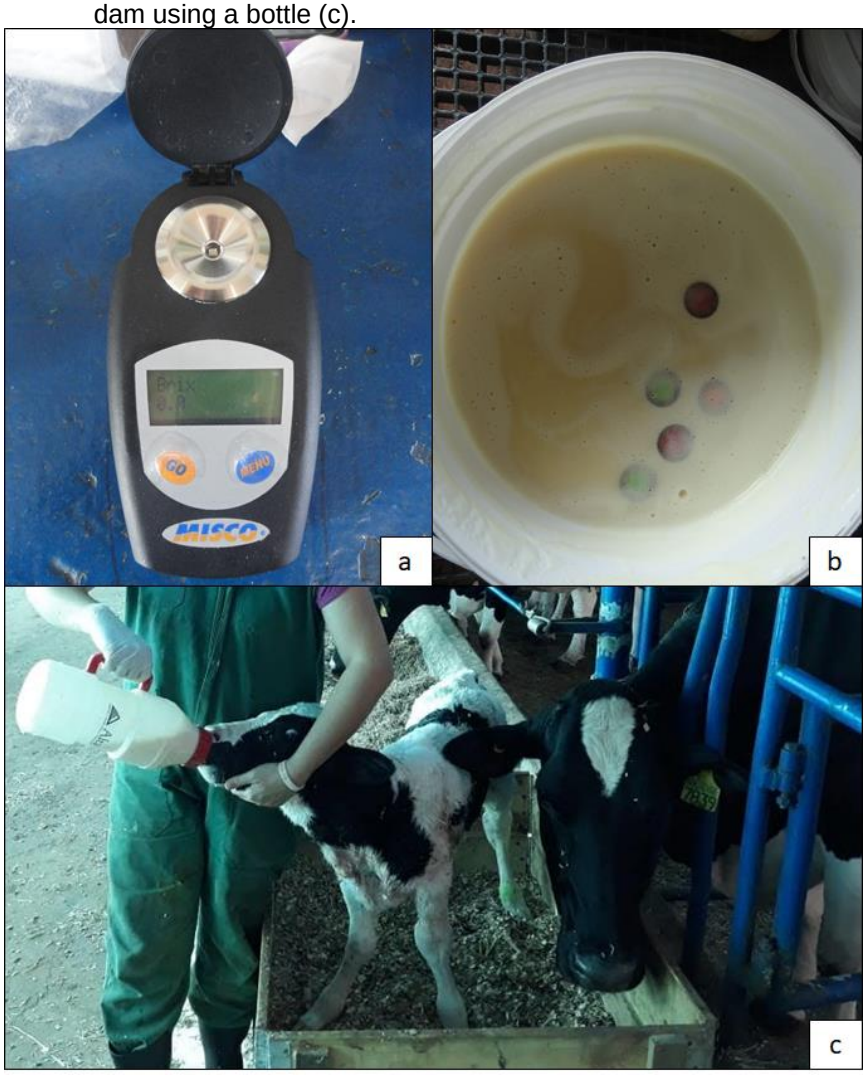


Source: SANTOS (2020).

The first colostrum feeding was provided by using a 3-liter bottle (Agrozootec®, model 04.02.0207) (Figure 5c) offered no later than 1 hour after birth. Multiple attempts have been made to supply all colostrum produced by the cow to calves in the first 18 hours after birth. Among the attempts, the colostrum was kept under refrigeration at 4ºC, being previously heated to 37ºC. After colostrum sampled, the cows finished their participation in the experiment and were transferred to the postpartum farm facilities of the farm.

The mean of colostrum ingested by the calves was 3.71 ± 1.05 L during the first 18 hours, with a maximum and a minimum volume ingested was 6.5 L and 2.0 L, respectively.

Figure 5 – Evaluation of colostrum by electronic Brix refractometer (a); by "*colostro* balls" (b); and calve receiving fresh colostrum from their dam using a bottle (c).



Source: SANTOS (2020).

4.1.3. Calf's management and housing

After the birth until the 14th day, calves were housed in closed-sided individual housing with bed of hay (Figure 6a), distributed in covered barn. Initially, cleaning was performed with removal of organic matter, washing with water and soap. Subsequently, disinfection fire equipment was used. A disinfectant solution based on chlorine dioxide 7% (Dioxiplus®, Dioxide, Brazil) was applied on the housing with a pump. At each animal exchange, the pens were cleaned in the same way.

During this period, each calf was fed with 6 L of non-medicated milk replacer (Nattimilk E Max®, Auster Nutrição Animal) divided into two feedings (7 AM and 3 PM). The dilution used to prepare the milk replacer was 1 kg of the product for every 7 liters of water, totaling 10% of total solids content. The milk was offered in bottles during the first 2 or 3 days of life and, after this period, in buckets fixed in each pen restrict to each individual calf (Figure 6b). Water and starter (Table 3 and 4) produced on farm ad libitum without probiotics were introduced from the 2nd and 3rd day of life, respectively. The starter was supplied from the 3rd day until the end of the experiment, being that the average of starter intake was 100 g per day per animal during the first 14 days.

Table 3.– Concentrate provided to calves during the experimental phase (recipe for 2000 kg).

Concentrate A	
Item	**Quantities (kg)**
Corn grain	870
Soybean meal	605
Wheat bran	400
Minerals and vitamins	60
Milk replacer **	50
Yeast	10
Mycofix® (Biomin)	5

Source: SANTOS (2020).

Table 4. – Guarantee levels of the vitamins and minerals provided to the animals during the experiment

Item	**Quantities**
Calcium (min.)	150 g/kg
Calcium (max.)	165 g/kg
Phosphor (min.)	20 g/kg
Vitamin A (min.)	180000 UI/kg
Vitamin D(min.)	50000 UI/kg
Vitamin E (min.)	3000 UI/kg
Sodium (min.)	42 g/kg
Fluorine (max.)	200 mg/kg
Magnesium (min.)	5000 mg/kg
Sulfur (min.)	5000 mg/kg
Manganese (min.)	1500 mg/kg
Zinc (min.)	1500 mg/kg
Cobalt (min.)	10 mg/kg
Copper (min.)	300 mg/kg
Iodine (min.)	15 mg/kg
Selenium (min.)	20 mg/kg
Chrome (min.)	20 mg/kg
Monensin (min.)	386 mg/kg
Sodic monensin (min.)	900 mg/kg

Source: SANTOS (2020).

The bottles and buckets used for providing colostrum, milk replacer and water were washed before and immediately after use with common detergent and chlorine solution, followed by rinsing with plenty of hot water. When there is an exchange between animals' blocks, the containers are disinfected with a disinfectant solution based on chlorine dioxide 7% (Dioxiplus®, Dioxide, Brazil), followed by immersion in boiling water for 10 minutes.

To prevent umbilical infection between birth and 10 days of life approximately, the external components of the umbilical stump of the animals were dipped in an antiseptic commercial based on dichlorvos, picric acid and iodoform (Umbicura®, Umbicura, Brazil).

On D3, the calves received an intranasal vaccine against Infectious Bovine Rhinotracheitis, Parainfluenza 3 and Bovine Respiratory Syncytial Virus (Inforce 3®, Zoetis). The volume of 2mL was administered to each nostril of the animal, using the cannula provided by the vaccine manufacturer.

On D7, the animals were disbudding according to farm management, using a caustic paste (Bovicor®, Bovitec, Brazil) with an anesthetic ointment. Hair was clipped around each horn bud, a thin film of caustic paste was rubbed into the scalp until each horn bud is evenly coated, and a ring of zinc oxide and permethrin spray (Unguento Plus, Pearson, Brazil) was applied around the paste to prevent spreading.

On D+15, the animals were transferred to another unit of calf rearing (phase 2) inside the same commercial farm (Figure 6c). Between the 15th and 28th day of life, the calves were housed in closed-sided individual housing with bed of hay and received eight liters of transitional milk pool supplemented with corrected dry matter, divided into two feedings a day. In this phase, the animals received a concentrate produced on farm with an average consumption of 200 g (Tables 3 and 4). During all the experiment, the diet met or exceeded nutrient requirements for pre-weaned Holstein calves to achieve max-growth rate (NRC,2001). After D28, at the end of the study, the calves remained inserted in the rearing calves on the farm where the study was conducted.

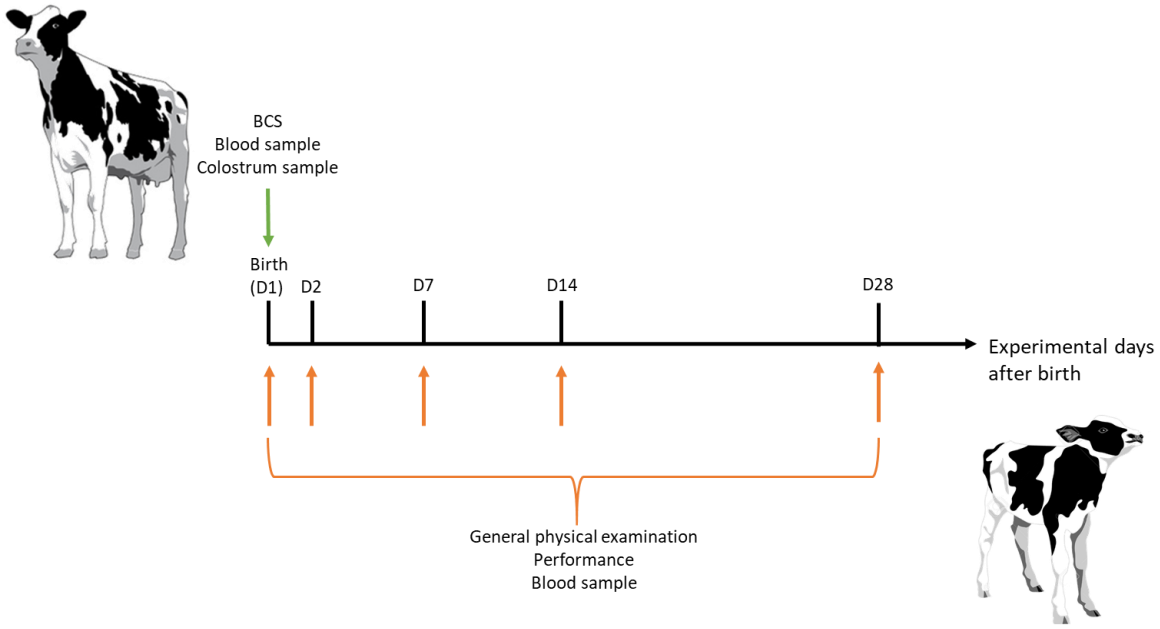
Figure 6 – Suspended closed-sided individual housing in a covered shed (a); individual pen with buckets fixed for water/milk and starter (b).

Source: SANTOS (2020).

4.2. SAMPLING

The general schedule used to collect samples and perform the clinical examination of dams and offspring is shown in Figure 7.

Figure 7 – Experimental schedule to sampling and performing clinical evaluation, according to the animal category and moments of evaluation



BCS = body condition score.
Source: SANTOS (2020).

4.2.1. Sampling and data collections from the dams

At calving, the BCS of each dam were performed by the same evaluator, using the BCS composed of a scale from 1 to 5 with intervals of 0.25, according to the criteria established by Edmonson et al. (1989) and adapted by Rosenberger et al. (2008). After milking, blood samples were collected via coccygeal venipuncture by using two plain vacuum tubes with fluoride sodium and without anticoagulant to obtain aliquots of plasma and serum, respectively. Blood samples were maintained in the room temperature to allow clot formation. Serum/plasma were subsequently obtained after centrifugation at 2,000 × g for 15 min, aliquoted, and stored at −20°C pending analysis within three months of collection.

Additional information from the dams, such as parity, conception date, drying date and age were obtained from the farm management software (Ideagri® software, Ideagri, Brazil).

4.2.2. Sample and data collections from the calves

Calves were blood sampled via jugular venipuncture for the first month of life, being immediately after birth (first day of life – D1) and D2 (1.89 ± 0.74 d), D7 (6.11 ± 1.37 d), D14 (13 ± 1.47) and D28 (28.11 ± 1.29 d) days of life. Sampling time was kept consistent throughout the study, with calves being sampled 2 h before the morning milk feeding. The blood was also collected from the calves into two plain vacuum tubes containing fluoride sodium and with no anticoagulant to obtain plasma and serum, respectively. Blood was allowed to clot at room temperature and serum/plasma were subsequently obtained after centrifugation at 2000 × g for 15 min, aliquoted, and stored at −20°C pending analysis within three months of collection.

In the sampling time, calves were given a general physical examination that included: vital signs, hydration status, ocular mucous, capillary refill time and palpation of lymph nodes (Dirksen et al., 2008). Furthermore, fecal (Table 5) and bovine respiratory disease (BRD) scores (Table 6) were assessed in accordance with the Calf Heath Scoring Criteria previously published by The University of Wisconsin (Madison) by McGuirk (2008). Calves were assessed as having diarrhea when the scores were 2 or 3. BRD was scored using a combination of the following parameters: rectal temperature, cough, nasal and ocular secretion and ear position with a score of 0–3 for each based on severity of each. The scores were added to obtain a final value. Calves whose score was ≥ 4 were screened for the diagnosis of BRD (Table 6).

Table 5.- Fecal scores adopted for detecting diarrhea in calves in the first month of life.

Parameter	Score
Normal consistency: firm, brownish color, perineum and tail clean and dry	0
Semi formed or pasty	1
Loose but enough consistency to remain on bedding: fecal content adhered to the perineum and tail	2
Watery feces that sift through bedding material, fecal content adhered to the perineum and tail	3

Source: (McGUIRK, 2008)

Table 6.- Bovine Respiratory Disease scores adopted to detect disease in calves in the first month of life. Calves are assessed based on four categories: rectal temperature, nasal discharge, cough, and eye or ear. Scores from each category are combined to come up with a total respiratory score.

Score	Rectal temperature (ºC)	Cough	Nasal discharge	Eye discharge	Ear positioning
0	<38.3	None	Normal serous discharge	Serous	Normal
1	38.3 – 38.8	Induce single cough	Small amount of unilateral cloudy discharge	Small amount of ocular discharge	Ear flick or head shake
2	38.9 – 39.3	Induced repeated cough or occasional spontaneous cough	Bilateral, cloudy or excessive mucus discharge	Moderate amount of bilateral discharge	Slight unilateral droop
3	≥ 39.4	Repeated spontaneous cough	Copious bilateral mucopurulent discharge	Slight unilateral droop	Head tilt or bilateral droop

Source: POULSEN; McGUIRK (2009).

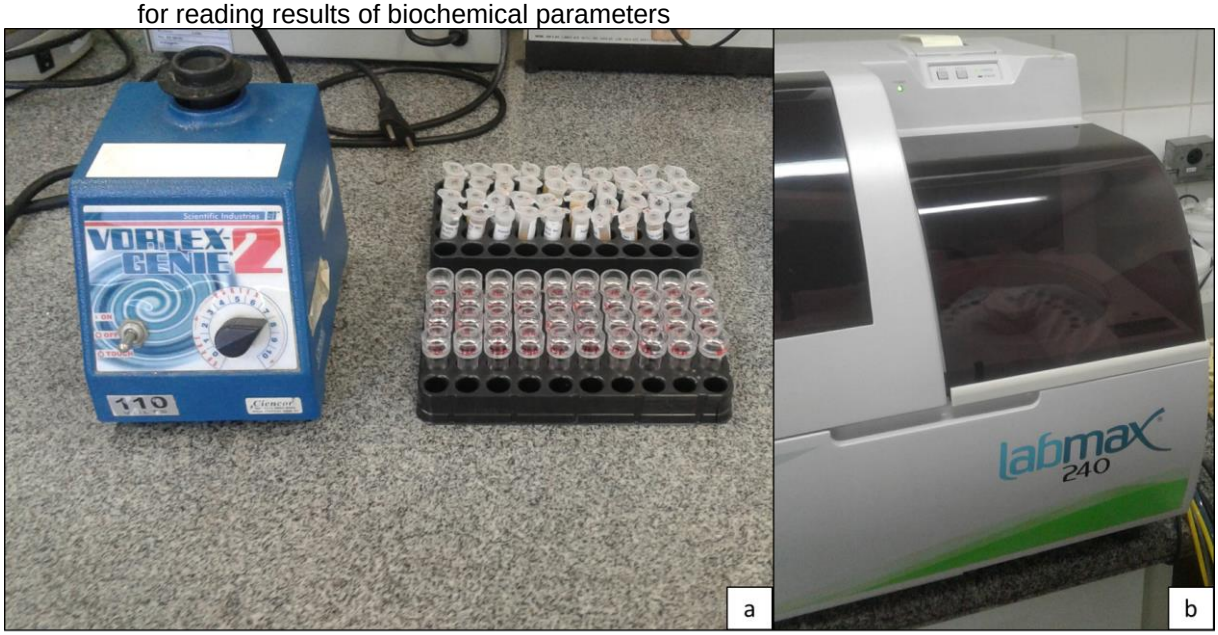
Calf thoracis perimeters were measured with a tape placed vertically at the point of the elbow (Heinrichs et al., 2007). Body measurements including rump width (distance between the points of hook bones) and height at the withers (distance from base of the front feet to the withers) of the calves were performed during the neonatal period, according to the method described in Khan et al. (2007).

4.3. ANALYTICAL DETERMINATIONS

4.3.1. Biochemical parameters

For samples obtained from dams and calves (Figure 7), the measurements of biochemical biomarkers were performed with specific commercial kits for each parameter, using the methodology provided by the manufacturer. Quantification of cholesterol (Labtest®, 76-2/100, Brazil) and triglycerides (Labtest®, 87-2/250, Brazil) were performed from serum samples, and the quantification of glucose (Labtest®, 133-1/500, Brazil), NEFA (Randox®, FA115, United Kingdom) and BHB (Randox®, RB1007, United Kingdom) were obtained from fluoride plasma samples. The aliquots were thawed in the refrigerator 4◦C overnight. When the samples were fully thawed, it was homogenized by a vortex and biochemical tests were performed on an automated biochemical analyzer (Labmax 240, Labtest®, Japan).

Figure 8 – Aliquots of samples pipetted on buckets of the automated biochemical analyzer and vortex utilized during the homogenization of the samples (a); automated biochemical analyzer used for reading results of biochemical parameters



Source: SANTOS (2020).

4.3.2. IgG ELISA

Immunoglobulin G (IgG) levels in calf serum samples were quantified using an in-house sandwich ELISA. First, we standardize the technique with the most appropriate concentrations of capture and detection antibodies, concurrently with the optimization of the best dilution to obtain the bovine gamma globulin standard curve.

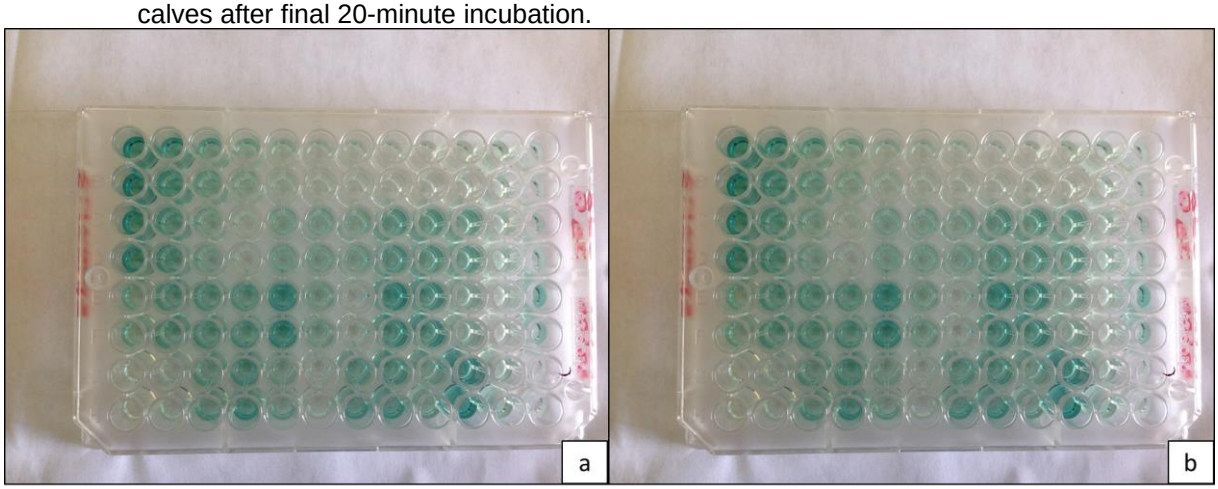
Rabbit anti-bovine IgG antibody (B5645; Sigma, St. Louis, MO) diluted 1: 400 in pH 9.7 sodium carbonate buffer was used to coat Immulon 4HBX plates (Thermo Corp., Milford, MA), which were incubated refrigerated (4-8°C) overnight. After overnight incubation, the plates were washed three times with phosphate buffered saline containing 0.5% Tween 20 (wash buffer). Serum samples were diluted 1:1,000,000 in wash buffer to determine IgG concentrations.

Samples of serum were placed in duplicate wells and incubated for 1 hour at room temperature. The plates were again washed three times with wash buffer, after which bound IgG was detected using a rabbit-anti-bovine IgG conjugated to horseradish peroxidase (A5295; Sigma, St. Louis, MO). The detection antibody was added to the plate diluted 1:1000 in wash buffer and incubated for 30 minutes. After incubation, the plates were washed three times with wash buffer and the 2,20-azino-

bis (3-ethylbenzthiazoline-6-sulfonic acid) substrate (A-9941; Sigma, St. Louis, MO) containing hydrogen peroxide 30% was added.

The plates were incubated for 20 min in the dark to allow color development. For measuring, a plate reader with a 405 nm filter was utilized. The concentration of IgG was determined relative to a nine-point serial dilution of the bovine gamma globulin standard (I5506; Sigma, St. Louis, MO).

Figure 9 – Plate of in-house sandwich ELISA assay for quantification of IgG in serum samples from calves after final 20-minute incubation.



Source: SANTOS (2020).

4.3.3. Inflammatory biomarkers

4.3.3.1. Iron, total protein and albumin levels

Serum aliquots were thawed in a refrigerator (4◦C) overnight, homogenized in an automatic vortex for later testing in the same automatic biochemical analyzer described previously (Labmax 240, Labtest®, Japan). Commercial kits were used to measure iron (Randox®, SI250, United Kingdon), total protein (Labtest®, MG3880, Brazil) and albumin (Labtest®, 19-1/250, Brazil) concentrations using the methodologies described by the manufacturer.

4.3.3.2. Leukogram

Total and differential WBC counts were also performed. In ADVIA 2120, the peroxidase method was used to evaluate WBC (WBCP). In the peroxidase method, the RBCs are lysed, and peroxidase reagents are used to distinguish between

peroxidase-positive cells, such as neutrophils, eosinophils, and monocytes, and peroxidase-negative cells, which include lymphocytes, basophils and “large unstained cells” (LUCs). This channel allowed also to determine the lobularity index (LI), which is the relationship between the number of cells producing high light scattering (PMN with higher lobularity) and cells with lower light scattering (mononuclear, immature granulocytes and blasts) (BRAGA, 2014).

4.3.3.3. Haptoglobin determination

To determine the haptoglobin (Hp) levels, a method was developed that was modification of several methods (JONES; MOLD, 1984; BASTOS et al., 2013). Hemolysate solution was prepared by adding 2 ml of 0.9% saline solution to 10 ml of healthy bovine blood, preserved in EDTA, and centrifuged (1000 x G / 5 minutes) three times to remove plasma, platelets, leukocytes and concentrate red blood cells. The erythrocytes were lysed with distilled water (600 µL) and the solution was frozen. After 24 hours, the solution with the lysed erythrocytes was extracted with chloroform in a 1:3 ratio, centrifugated at 1000 x G for 5 minutes, forming the concentrated hemoglobin solution. This solution when added to the potassium ferricyanide solution (2%) and with an adjustment calculation, allowed to obtain the 0.3 g / L methemoglobin solution, a necessary molecule for binding with Hp and subsequent formation of the composite.

Serial dilution with Hp standard (human serum as measured by the Beckman Coulter, Brea, CA, USA commercial kit) yielded a linear Hp concentration from 0 to 0.59 g/L. All standard and test serial samples had a control and test wells. To the test wells, 50 µL of methemoglobin solution was added and in the control wells of each sample, 50 µL of physiological solution (0.9%). Complex formation occurred after the addition of 150 µL of Guayaquil substrate and 50 µL of hydrogen peroxide (0.02 mol/L) in all test and control wells of each sample. After 10 minutes, a spectrophotometric reading with a wavelength of 490 nanometers was taken.

4.3.3.4. Assessment of radical oxygen species (ROS)

Production of ROS was assessed by using the fluorescent dye dihydrorhodamine 123 (batch 1861629, Life Technologies do Brasil), adapting the methodology describe by Donovan et al. (2007) and Nace et al. (2004).

First, 3 mL of blood were added in silicon tubes of 15 mL sterile. Initially the blood was lysed by the addition of 6 mL of Tris-chloride ammonia lysis solution, incubated at 37 °C for 30 minutes. Posteriorly, the tubes were centrifuged in 290xg for 10 minutes, afterwards, the cells were washed in 5 mL of salt buffered solution twice at 290xg for 5 minutes. After the last washing, the supernatant was discarded and the leucocytes were diluted in 1 mL of RPMI1640 cell culture medium without red phenol (Cat no.: 7509, Sigma-Aldreich, St Louis, MO) supplemented with 10% of fetal serum inactivated by heat and 2 mM L-glutamine (Cat no.:21051-024, Gibco®, Brazil).

The concentration and viability of leucocytes was determined by Trypan Blue exclusion. For this, 50 µL of cell suspension was diluted in 450 µL of Trypan Blue 0.04%. From this mixture, 10 µL was pipetted in Newbauer chamber, counting the number of living and dead cells in a quadrant of the chamber specific for counting leucocytes. Finally, the cell suspension was adjusted to $3x10^6$ cells/mL.

The leucocytes suspension (100 µL) was plated in flat bottom cell cultivation plates, being afterwards diluted in 100 µL of supplemented cell cultivation medium (none stimulated leucocytes) or in a cell cultivation medium containing specific treatment (stimulated leucocytes). The cells were stimulated with inactivated antigens like *Staphylococcus aureus* (pure)*; Escherichia coli* (pure)*; S. hyicus* (pure) and Phorbol Myristate Acetate at 10^{-6}M (PMA - P8139, Merck Milipore, Darmsdtadt, Germany, Sigma). The bacteria dilutions used were determined in previous pilots in which was obtained the maximum production of ERO's after the incubation of leucocytes with the stimulus. Finally, 10 µL of subtract DHR-123 (dihydrorhodamine 123) with final concentration of 10 µM was added in each well, except for the negative control containing only the medium (medium background).

The plates were incubated for two hours in CO_2 incubator at 37°C. The fluorescent reading was performed in Fluoroskan Ascent FL (Thermo Scientific), excitation 485 nm and emission 538 nm. The fluorescence was obtained with the average rate of fluorescence unit (AFU). In each test, the PMA was used as a

maximum positive control and RPMI-1640 medium was used as negative control. The production of EROs in the concentration of each stimulus is presented as a response ratio, calculated as follows: AFU value for stimulated cells/ AFU cell value not stimulated.

4.4. MATERNAL FACTORS AND GROUPS CLASSIFICATION AND STATISTICAL ANALYSIS

The maternal parameters BCS, NEFA, BHB, glucose, total protein, albumin, triglycerides, cholesterol, Hp, iron and thoracic perimeter were considered as quantitative independent variables. The experimental groups were established according to the method used by Ling and collaborators, so it was calculated the medians for each maternal metabolic biomarker to set offspring´s data in low and high groups. In cases where the maternal result was equal to the median, the offspring was allocated to the low group (Table 7).

Table 7. - Median (cut-off values) of maternal biomarkers used to set the offspring in experimental groups.

Maternal factors	Median	Groups
NEFA	0.53 mmol/L	High n= 14 Low n= 14
BHB	0.42 mmol/L	High n= 13 Low n= 15
Glucose	89.5 mg/dL	High n= 14 Low n= 14
Total protein	23.3 µmol/L	High n= 14 Low n= 14
Albumin	6.2 g/dL	High n= 14 Low n= 14
Triglycerides	2.75 g/dL	High n= 14 Low n= 14
Cholesterol	30.70 mg/dL	High n= 13 Low n= 15
Hp	68.5 mg/dL	High n= 13 Low n= 15
Iron	23.11 mg/dL	High n= 14 Low n= 14
Thoracic perimeter	222.5 cm	High n= 14 Low n= 14
BCS	3.0	High n= 15 Low n=13

Source: SANTOS (2020).

The statistical analysis was performed using the SAS (SAS System for Windows, Institute Inc., Cary, NC, USA, version 9.4).

The quantitative variables that presented a non-parametric distribution were subjected to numerical transformations (inverse, log or quadratic root).

Within each maternal group formed in relation to the quantitative independent variables (BCS, NEFA, BHB, glucose, total protein, albumin, triglycerides, cholesterol, Hp, iron and thoracic perimeter), a comparison was made between the dams to determine the maternal profile within each situation (low and high). To compare adult cow data between groups, t test was used. A correlation analyses among dam variables were performed with the Pearson's test. To interpret the values resulting from the Pearson correlation test, the correlation coefficient (r) was used, with 0.9 (positive or negative) indicating a very strong correlation, 0.7 to 0.9 positive or negative indicates a strong correlation and 0.5 to 0.7 positive or negative indicates a moderate correlation, 0.3 to 0.5 indicates a low correlation (MUKAKA, 2012).

To evaluate the effects of maternal factor (independent variables), time and group-time interaction, the mixed linear model was used. Data distribution was checked using the Kolmogorov Smirnov test with level significance of 5%. The group and time factors were inserted into the linear model as fixed effects, and the subject (animal) factor as a random effect. The autoregressive (AR), symmetric component (SC) and unstructured (UN) covariance structures were tested based on the Akaike Information Criteria, and therefore the matrices with the lowest values were chosen. The EMEANS command with the Bonferroni test allowed comparisons of the main effects to be obtained. The ordinal qualitative clinical parameters were transformed into qualitative response variables (yes and no), with results expressed in positive and negative frequencies, the differences between groups being determined by the chi-square test. For all tests performed statistical differences were considered when the P-value was ≤ 0.05.

5. RESULTS

5.1. DAMS STATUS AT CALVING TIME

The comparison of metabolic biomarkers from dam´s according to the low and high experimental groups is shown on Tables 8 and 9. It was also detecting significant correlations among maternal variables (Table 10). The classifying variables differed significantly between all the low and high groups for each maternal variable (P<0.01), except for the albumin, which did not (P> 0.05) generate groups (low and high) with different results for albumin.

Regarding the group categorized in relation to the NEFA (Table 8), it is noted that dams with high NEFA had higher (P <0.05) levels of BHB and higher thoracic perimeter (P <0.01) when compared with the low NEFA group animals. The same result was defined by the correlation between the same variables: NEFA levels are negatively correlated with glucose levels (low correlation) and positively with thoracic perimeter (moderate correlation), in other words, higher NEFA results are observed in animals with higher thoracic perimeter and lower glucose availability (Table 10, Figure 10).

In relation to the group divided by BHB cut-off values (Table 8), dams in high level group had higher (P<0.01) NEFA concentrations, higher thoracic perimeter and lower concentrations of glucose and total protein (P<0.05) than animals on low-BHB group. The maternal BHB concentration was moderate correlated positively with NEFA and negatively correlated with glucose concentrations (Table 10, Figure 11).

The dams in the low glucose group (Table 8) had higher (P <0.05) concentrations of NEFA and BHB when compared with high-glucose group dams. Significant negative correlation was identified between the glucose levels and maternal NEFA (low correlation) and BHB (moderate correlation) (Table 10), as we can observe at Figures 10 and 11.

Dams in the high triglyceride group also had higher cholesterol (P <0.01) and albumin (P <0.05) levels when compared to dams in the low triglyceride group (Table 8). The triglycerides parameter has a moderate positively correlation with albumin and a high correlation with cholesterol levels (Table 10, Figure 11).

Regarding the group divided by the cholesterol variable, it is observed that the cows in the high group had higher (P <0.01) levels of albumin and triglycerides (Table 8). This variable was positively correlated with total protein (low correlation), albumin (moderate correlation) and triglycerides (high correlation) (Table 10, Figure 12).

The animals in the group with a high concentration of total protein when compared to the animals in the group with low protein showed lower (P <0.05)

concentrations of albumin (Table 2). The total protein levels were low correlated negatively with albumin and positively with cholesterol (Table 10, Figure 11).

As previously mentioned, the group categorized by albumin showed no statistical difference for the maternal parameters, so that animals with high and low albumin concentration did not differ (P> 0.05) from each other in relation to the evaluated parameters (Table 10), but it was negatively correlated (low correlation) with total protein and positively (low correlation) with triglycerides and cholesterol (Table 10).

Cows with high concentrations of Hp showed higher (P <0.05) iron levels (Table 9). Haptoglobin was positively correlated with iron levels (moderate correlation), while the iron levels were correlated positively with Hp (Table 10, Figure 12). However, animals in the high iron concentration group did not show (P> 0.05) any difference between the parameters evaluated (Table 9).

The dams with high thoracic perimeter showed differences (P<0.05) when compared with low thoracic perimeter cows regarding NEFA levels and BCS, which were higher in dams with high thoracic perimeter (Table 8). About damns with high BCS, these cows presented higher (P<0.05) BHB levels and thoracic perimeter when compared with cows with low BCS (Table 8). Body condition score was positively and moderately correlated with thoracic perimeter (Table 10, Figure 12).

Regarding the division of dams by thoracic perimeter (high and low), animals with high thoracic perimeter showed higher levels (P <0.05) of NEFA (Table 8) than the dams with low thoracic perimeter, so that a moderate positive correlation was found between these two variables (Table 10). There was also a positive correlation between thoracic perimeter and body condition score (Figure 12, Table 10).

Table 8. - Mean ± standard deviation of the maternal biomarkers categorized in groups according to the cut-off values established to distribute the dams in experimental groups

Maternal biomarkers	Groups													
	NEFA		BHB		Glucose		Thoracic Perimeter		BCS		Cholesterol		Triglycerides	
	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
NEFA (mmol/L)	0.35 ± 0.12	**0.79 ± 0.17 ****	0.41 ± 0.21	**0.72 ± 0.21 ****	0.68 ± 0.23	**0.47 ± 0.26 ***	0.45± 0.24	**0.70± 0.24***	0.51 ± 0.26	0.64 ± 0.27	0.60 ± 0.29	0.55± 0.25	0.65 ± 0.29	0.49 ± 0.22
BHB (mmol/L)	0.30 ± 0.12	**0.46 ± 0.17 ***	0.23 ± 0.09	**0.51 ± 0.08 ****	0.45 ± 0.14	**0.28 ± 0.15 ***	0.31± 0.13	0.43± 0.18	0.35± 0.14	**0.41 ± 0.20 ***	0.36 ± 0.19	0.39± 0.15	0.35 ± 0.16	0.39 ± 0.18
Glucose (mg/dL)	103.14± 30.06	88.29 ±24.92	108.15± 30.00	**84.93± 20.85 ***	71.93 ± 9.84	119.50 ±18.11******	101.07± 33.55	90.36± 21.35	100.13± 30.35	90.62 ± 25.56	93.15 ± 31.94	97.93± 25.30	94.86± 31.05	96.57 ± 26.03
Total protein (µmol/L)	6.36 ± 0.46	5.85 ± 1.74	6.63 ± 0.76	**5.64 ± 1.27 ***	6.01 ± 1.66	6.20 ± 0.77	6.31± 0.44	5.89± 1.76	5.76 ± 1.30	6.49 ± 1.18	25.38 ± 5.26	21.39± 6.17	5.97 ±1.48	6.23 ± 1.07
Albumin (g/dL)	2.71 ± 0.20	4.11 ± 5.74	2.73 ± 0.19	4.00 ± 0.41	4.08 ± 5.75	2.74 ± 0.17	2.72± 0.19	4.10± 5.74	4.08 ± 5.52	2.64 ± 0.32	2.49± 0.39	**4.21± 5.48****	2.57 ±0.39	**4.25 ± 5.69 ***
Triglycerides (g/dL)	33.14 ± 12.14	32.14± 21.33	29.83 ± 11.34	35.07± 16.50	32.53± 20.27	32.74 ±13.85	30.93± 14.76	34.34± 19.46	33.93 ± 19.79	31.14 ± 13.84	20.05± 8.74	**43.54± 14.83****	19.86 ±7.62	**45.41 ±13.91****
Cholesterol (mg/dL)	74.24 ± 13.68	68.06± 22.78	70.18 ± 10.96	72.27± 23.71	68.97 ±20.50	73.40 ± 16.97	71.34± 14.97	71.19± 22.37	69.41 ± 17.80	73.26 ± 19.79	56.61± 11.05	84.88± 12.60	58.82 ±12.53	**84.67 ±14.06****
Hp (mg/dL)	24.96 ± 13.18	23.72 ± 4.95	24.48 ± 12.05	24.13 ± 5.99	26.03 ±11.11	22.27 ±7.02	25.36± 12.29	23.06± 4.68	25.84 ± 11.46	22.19 ± 5.62	25.78± 12.17	22.81± 5.81	26.28 ±11.74	21.98 ±5.45
Iron (mg/dL)	23.29 ± 5.42	23.19 ± 6.76	24.50 ± 4.90	22.15 ± 5.03	22.81 ±7.66	23.66 ± 4.01	24.67± 5.05	21.81± 6.72	23.87 ± 7.41	22.51 ± 4.02	5.86± 1.47	6.31± 1.08	25.13 ±5.23	21.35 ±6.33
Thoracic perimeter (cm)	687.64 ± 86.37	**794.93± 52.23 ****	698.15 ± 81.78	**778.67± 70.93 ***	743.07 ±80.66	739.50 ±99.36	664.86± 50.43	**817.71± 35.50****	700.87± 80.12	**787.92± 76.57***	719.85± 85.5	759.87± 90.28	752.14 ±88.60	730.43 ±91.00
BCS (1-5)	2.98 ± 0.51	3.25 ± 0.37	3.00 ± 0.51	3.22 ± 0.36	3.14 ± 0.49	3.09 ± 0.45	2.86± 0.45	**3.38± 0.31***	2.75± 0.30	3,54± 0.09	2.98± 0.53	3.23± 0.36	3.13 ±0.46	3.11 ± 0.48

Groups were compared statistically with the t test for parametric data and Mann-Whitney test for non-parametric data. NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.

* P<0.05; ** P<0,01.

Source: SANTOS (2020).

Table 9. - Mean ± standard deviation of the maternal variables categorized by groups (total protein, albumin, triglycerides and cholesterol).

Maternal variables	Groups							
	Total protein		Albumin		Iron		Haptoglobin	
	Low	High	Low	High	Low	High	Low	High
NEFA (mmol/L)	0.62 ± 0.31	0.53 ± 0.21	0.53 ±0.24	0.61 ± 0.29	0.56± 0.29	0.59± 0.25	0.55 ± 0.35	0.60± 0.18
BHB (mmol/L)	0.42 ± 0.13	0.41 ± 0.14	0.40 ±0.16	0.34 ± 0.17	0.42± 0.17	0.32± 0.15	0.37 ± 0.16	0.37± 0.18
Glucose (mg/dL)	100.93± 24.99	90.50 ± 30.00	99.14 ±28.51	92.29 ±28.37	92.21± 25.88	99.21± 30.78	99.00± 27.90	92.87± 28.98
Total protein (µmol/L)	5.24 ± 1.00	**6.96 ± 0.57****	6.38 ±1.12	5.83 ± 1.40	5.94± 1.56	6.26± 0.93	5.84± 1.34	6.33± 1.21
Albumin (g/dL)	4.02 ± 0.38	**2.81 ± 0.12 ***	4.37 ±5.65	2.46 ± 0.36	4.15± 5.73	2.67± 0.19	4.37± 5.91	**2.58± 0.30***
Triglycerides (g/dL)	30.56 ± 15.10	34.71 ± 12.28	41.27 ±17.11	24.00 ±12.22	35.52± 19.51	29.75± 14.29	37.17± 20.29	28.71± 13.09
Cholesterol (mg/dL)	66.12 ± 22.48	76.04 ± 11.39	81.92 ±13.09	61.38 ±17.58	75.61± 20.42	67.24± 16.27	74.39± 20.91	68.77± 16.69
Hp (mg/dL)	21.42 ± 6.45	27.17 ± 11.26	23.81 ±6.30	24.78 ±12.10	21.65± 4.53	26.94± 12.29	**18.27± 4.04**	**30.32± 9.57****
Iron (mg/dL)	22.36 ± 4.32	24.12 ± 5.59	22.04 ±6.11	24.44 ±5.89	18.80± 4.39	**27.68± 3.65****	**20.82± 6.47**	**25.33± 4.87 ***
Thoracic perimeter (cm)	742.93± 97.75	739.64 ± 80.96	743.00 ±76.56	739.57 ±102.55	754.64± 93.89	727.93± 84.76	724.46± 90.45	755.87± 87.81
BCS (1-5)	3.07 ± 0.50	3.16 ± 0.42	3.07 ±0.37	3.16 ± 0.54	3.27± 0.33	2.96± 0.53	3.17± 0.34	3.07± 0.55

Groups were compared statistically with the t test for parametric data and Mann-Whitney test for non-parametric data. NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.

* P<0.05; ** P<0,01.

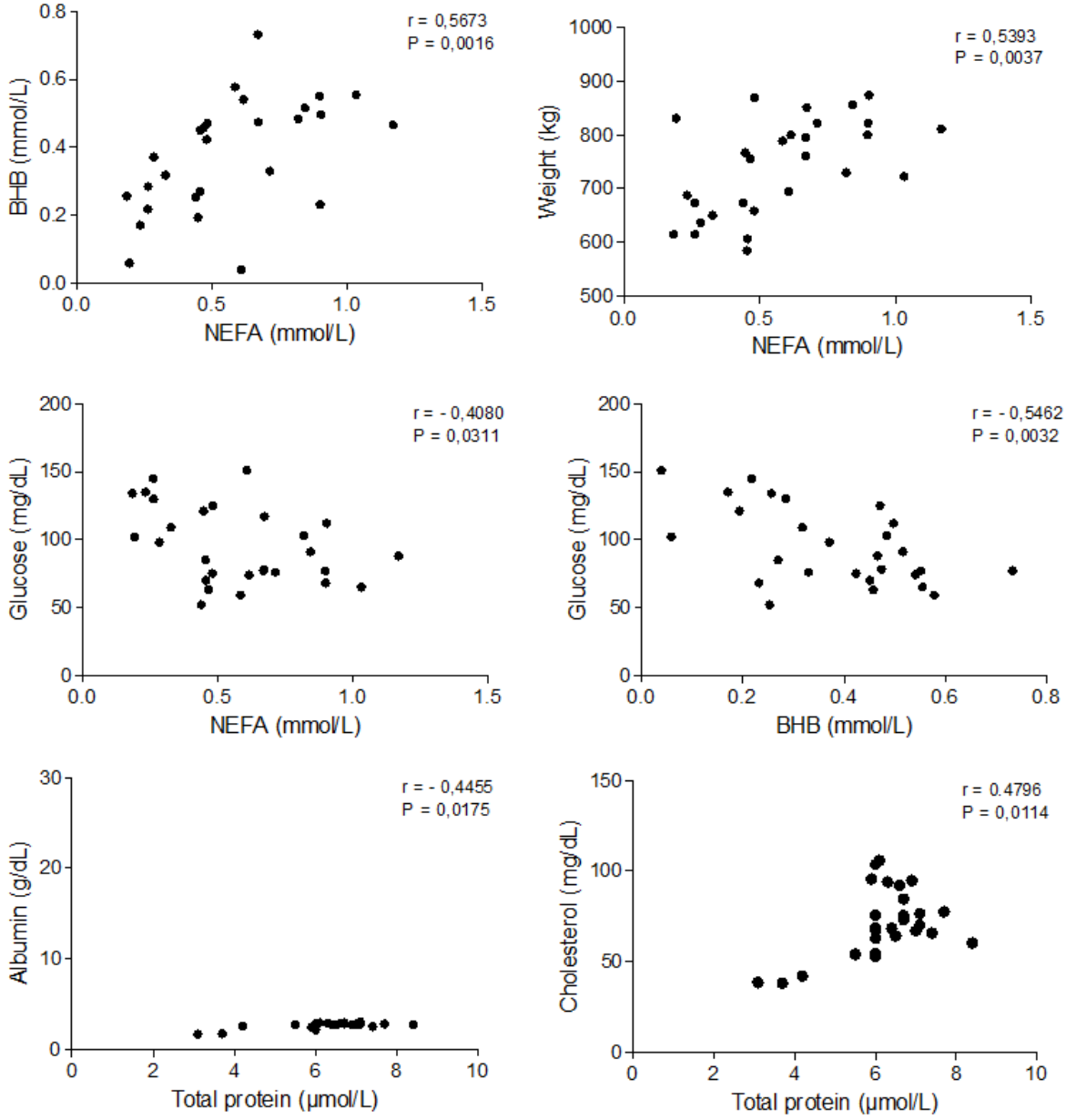
Source: SANTOS (2020).

Table 10.- Correlation analyses among maternal variables (shown as Pearson correlation coefficient).

Variables	NEFA (mmol/L)	BHB (mmol/L)	Glucose (mg/dL)	Total protein (µmol/L)	Albumin (g/dL)	Triglycerides (g/dL)	Cholesterol (mg/dL)	Hp (mg/dL)	Iron (mg/dL)	Thoracic perimeter (cm)	BCS (1-5)
NEFA (mmol/L)	---	**0.54****	**-0.41***	-0.15	0.01	-0.14	-0.16	0.00	0.06	**0.57****	0.31
BHB (mmol/L)	0.54	---	-0.55	-0.30	0.17	0.12	0.02	-0.07	-0.23	0.34	0.27
Glucose (mg/dL)	**-0.41***	**-0.55***	---	0.02	-0.14	-0.08	0.04	-0.35	-0.01	-0.14	-0.20
Total protein (µmol/L)	-0.15	-0.30	0.02	---	**-0.45***	-0.01	**0.48***	0.18	0.37	-0.13	0.18
Albumin (g/dL)	0.01	0.17	-0.14	**-0.45***	---	**0.60***	**0.60****	-0.10	**-0.56***	0.12	-0.05
Triglycerides (g/dL)	-0.14	0.12	-0.08	**-0.01***	**0.60****	---	**0.86****	-0.15	-0.34	0.04	0.06
Cholesterol (mg/dL)	-0.16	0.02	0.04	**0.48***	**0.60****	**0.86****	---	-0.08	-0.15	-0.01	0.19
Hp (mg/dL)	0.00	-0.07	-0.35	0.18	-0.10	-0.15	-0.08	---	**0.51***	0.04	-0.17
Iron (mg/dL)	0.06	-0.23	-0.01	---	-0.56	-0.34	-0.15	0.51	---	-0.14	-0.17
Thoracic perimeter (cm)	**0.57**	0.34	-0.14	-0.13	0.12	0.04	-0.01	0.04	-0.14	---	**0.59***
BCS (1-5)	0.31	0.27	-0.20	0.18	-0.05	0.06	0.19	-0.17	-0.17	**0.59****	---

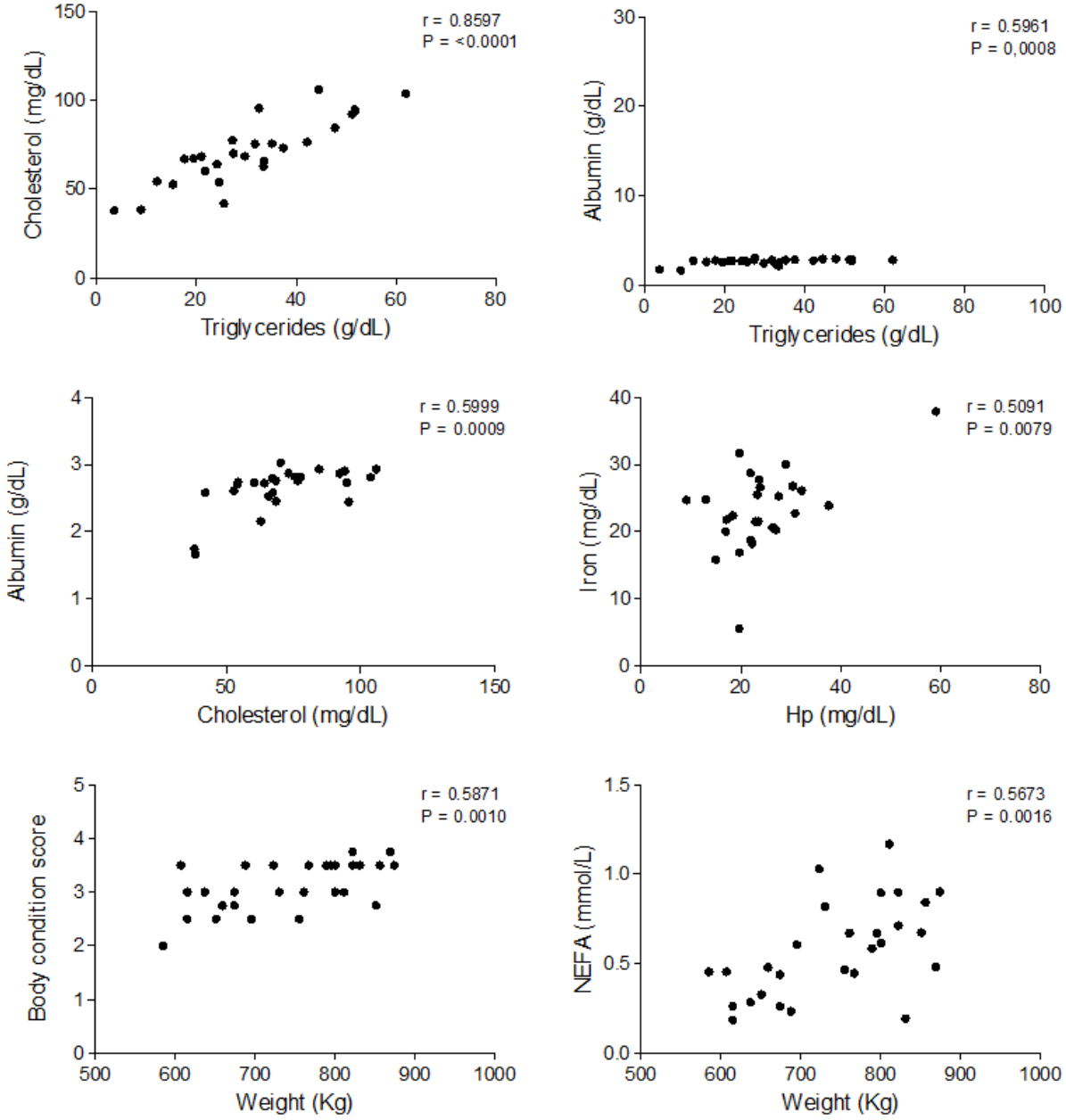
NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
* P<0.05; ** P<0,01.
Source: SANTOS (2020).

Figure 10 – Correlation between the maternal variables of dams.



Source: SANTOS (2020).

Figure 11 – Correlation between the maternal variables of dams.



Source: SANTOS (2020).

5.2. IMPACT OF MATERNAL FACTORS ON DAIRY CALVES HEALTH

5.2.1. Vital Parameters

The comparison between the maternal groups and calf vital parameters are shown in Table 11. Mean and standard deviation values found in each time of this study from D1 up to D28 are presented between Tables 21 and 31 in Appendix A.

The interaction between glucose maternal cut-off values and time (P=0.0534) was determined. Animals born to dams with low glucose had a higher respiratory rate than animals born to cows with high glucose at D28 (Figure 13). Maternal BCS (P=0.0082) and thoracic perimeter (P= 0.0288) had effect on heart rate (Table 11).

Animals born from cows with lower body condition (116.74 ± 2.54 bpm) had a lower heart rate than animals with higher body condition (129.50 ± 3.40 bpm), in the same way as animals born from dams with low thoracic perimeter (117.97 ± 3.40 bpm) had a lower heart rate than animals born from cows with high thoracic perimeter (127.36 ± 3.33 bpm).

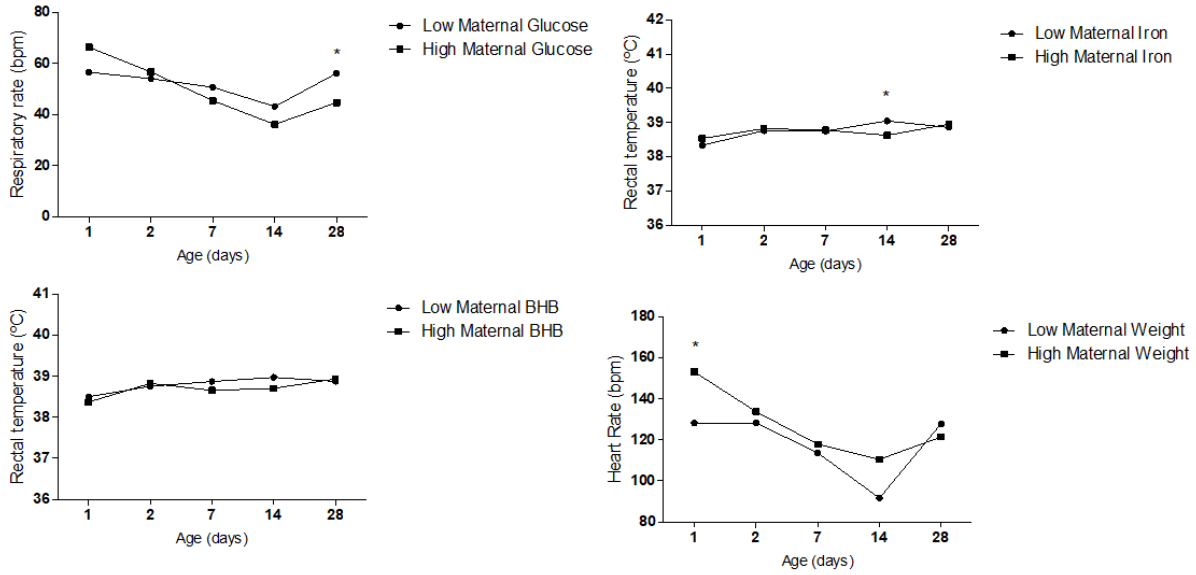
In addition, the interaction observed between time and maternal thoracic perimeter (P= 0.0344) for heart rate are observed (Table 11, Figure 13). Also, it detected interaction between time and the following maternal variables on rectal temperature of calves: BHB (P= 0.0530), albumin group (P=0.0070), iron (P=0.0078) and BCS (P=0.0402) (Table 11, Figure 13).

Table 11. – Relationship between the high and low maternal groups maternal groups and vital parameters, in the neonatal period (result expressed by P value from mixed linear model).

Maternal groups	Calf vital parameters								
	Respiratory rate			Heart Rate			Rectal Temperature		
	Group	Time	Group x time	Group	Time	Group x time	Group	Time	Group x time
NEFA	0.43	**<0.01**	0.74	0.11	**<0.01**	0.14	0.81	**<0.01**	0.30
BHB	0.47	**<0.01**	0.34	0.08	**<0.01**	0.08	0.64	**<0.01**	**0.05**
Glucose	0.53	**<0.01**	**0.05**	0.61	**<0.01**	0.97	0.98	**<0.01**	0.19
Total protein	0.11	**<0.01**	0.17	0.78	**<0.01**	0.39	0.20	**<0.01**	0.14
Albumin	0.12	**<0.01**	0.22	0.15	**<0.01**	0.46	0.58	**<0.01**	**0.01**
Triglycerides	0.24	**<0.01**	0.76	0.59	**<0.01**	0.91	0.34	**<0.01**	0.26
Cholesterol	0.09	**<0.01**	0.95	0.72	**<0.01**	0.90	0.93	**<0.01**	0.14
Hp	0.66	**<0.01**	0.50	0.91	**<0.01**	0.15	0.66	**<0.01**	0.40
Iron	0.19	**<0.01**	0.42	0.22	**<0.01**	0.42	0.91	**<0.01**	**0.01**
Thoracic perimeter	0.14	**<0.01**	0.53	**0.03**	**<0.01**	**0.03**	0.64	**<0.01**	0.29
BCS	0.29	**<0.01**	0.59	**0.01**	**<0.01**	**0.06**	0.99	**<0.01**	**0.04**

Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction.
P <0.05 was adopted for statistical differences.
Source: SANTOS (2020).

Figure 13 – Vital parameters of the calves according to different maternal groups at calving time during the neonatal period.



Groups were compared statistically with the t test.
* P<0.05
Source: SANTOS (2020).

5.2.2. Diarrhea

Diarrhea prevalence (%) along this research is shown on Table 12. It was found a difference in the frequency of the diarrhea rate between the high and low albumin maternal groups (P<0.05). Calves in the group of dams with low albumin concentration had a lower frequency of diarrhea in D14 (14.28%) than calves born to cows with a higher concentration of albumin (57.14%). In the following moment (D28), it was found higher frequencies of diarrhea (P<0.05) in the offspring born from dams with high thoracic perimeter and with a higher body condition score (Table 12).

Table 12. – Diarrhea frequency (%) (absolute number/total simple size) in calves divided by the different maternal groups (NEFA, BHB, glucose, total protein, albumin, triglycerides, cholesterol, Hp, iron, thoracic perimeter and BCS).

Maternal groups	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
NEFA	7.14% (1/14)	0.00% (0/14)	21.42% (3/14)	7.14% (1/14)	14.28% (2/14)	42.85% (6/14)	42.85% (6/14)	28.57% (4/14)	28.57% (4/14)	35.71% (5/14)
BHB	7.69% (1/13)	0.00% (0/15)	15.38% (2/13)	13.33% 2/15)	46.15% (6/13)	13.33% (2/15)	23.07% (3/13)	40.00% (6/15)	46.15% (6/13)	20.00% (3/15)
Glucose	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	14.28% (2/14)	35.71% (5/14)	21.42% (3/14)	21.42% (3/14)	50.00% (7/14)	42.85% (6/14)	21.42% (3/14)
Total protein	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	14.28% (2/14)	42.85% (6/14)	14.28% (2/14)	21.42% (3/14)	50.00% (7/14)	35.71% (5/14)	28.57% (4/14)
Albumin	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	14.28% (2/14)	35.71% (5/14)	21.42% (3/14)	**14.28%A (2/14)**	**57.14%B (8/14)**	28.57% (4/14)	35.71% (5/14)
Triglycerides	0.00% (0/14)	7.14% (1/14)	7.14% (1/14)	21.42% (3/14)	28.57% (4/14)	28.57% (4/14)	21.42% (3/14)	50.00% (7/14)	28.57 (4/14)	35.71% (5/14)
Cholesterol	0.00% (0/13)	6.67% (1/15)	7.69% (1/13)	20.00% (3/15)	30.76% (4/13)	26.66% (4/15)	23.07% (3/13)	46.66% (7/15)	23.07% (3/13)	40.00% (6/15)
Hp	0.00% (0/13)	6.67% (1/15)	23.07% (3/13)	6.66% (1/15)	30.76% (4/13)	26.66% (4/15)	46.15% (6/13)	26.66% (4/15)	38.46% (5/13)	26.66% (4/15)
Iron	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	14.28% (2/14)	28.57% (4/14)	28.57% (4/14)	28.57 (4/14)	42.85% (6/14)	42.85% (6/14)	21.42% (3/14)
Thoracic perimeter	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	14.28% (2/14)	14.28% (2/14)	42.85% (6/14)	42.85% (6/14)	28.57% (4/14)	**14.28%A (2/14)**	**50.00%B (7/14)**
BCS	0.00% (0/14)	7.14% (1/14)	7.14% (1/14)	21.42% (3/14)	21.42% (3/14)	35.71% (5/14)	42.85% (6/14)	28.57% (4/14)	**0.00%A (0/14)**	**64.28%B (9/14)**

A, B: different letters on the same line indicate statistical difference between groups in comparison with the chi-square test (P <0.05).
Source: SANTOS (2020).

5.2.3. Bovine Respiratory Disease

There was no statistical difference in prevalence (%) of the Bovine Respiratory Disease in relation to all the maternal groups evaluated (P> 0.05) (Tables 13).

Table 13. – Frequency of bovine respiratory score > 4 (%) for calves divided by the different maternal groups (NEFA, BHB, glucose, total protein, albumin, triglycerides, cholesterol, Hp, iron, thoracic perimeter and BCS).

Maternal groups	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
NEFA	7.14% (1/14)	7.14% (1/14)	0.00% (0/14)	7.14% (1/14)	0.00% (0/14)	21.42% (3/14)	14.28% (2/14)	28.57% (4/14)	28.57% (4/14)	28.57% (4/14)
BHB	0.00% (0/13)	6.67% (1/15)	7.69% (1/13)	0.00% (0/15)	0.00% (0/13)	20.00% (3/15)	30.76% (4/13)	13.33% (2/15)	30.76% (4/13)	26.66% (4/15)
Glucose	0.00% (0/14)	7.14% (1/14)	0.00% (0/14)	7.14% (1/14)	7.14% (1/14)	14.28% (2/14)	35.71% (5/14)	7.14% (1/14)	28.57% (4/14)	28.57% (4/14)
Total protein	0.00% (0/14)	7.14% (1/14)	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	7.14% (1/14)	21.42% (3/14)	21.42% (3/14)	35.71% (5/14)	21.42% (3/14)
Albumin	14.28% (2/14)	0.00% (0/14)	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	7.14% (1/14)	7.14% (1/14)	35.71% (5/14)	21.42% (3/14)	35.71% (5/14)
Triglycerides	14.28% (2/14)	0.00% (0/14)	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	7.14% (1/14)	7.14% (1/14)	35.71% (5/14)	21.42% (3/14)	35.71% (5/14)
Cholesterol	15.38% (2/13)	0.00% (0/15)	0.00% (0/13)	6.67% (1/15)	23.07% (3/13)	0.00% (0/15)	15.38% (2/13)	26.66% (4/15)	23.07% (3/13)	33.33% (5/15)
Hp	0.00% (0/13)	6.67% (1/15)	7.69% (1/13)	0.00% (0/15)	0.00% (0/13)	20.00% (3/15)	38.46% (5/13)	6.67% (1/15)	30.76% (4/13)	26.66% (4/15)
Iron	0.00% (0/14)	7.14% (1/14)	7.14% (1/14)	0.00% (0/14)	7.14% (1/14)	14.28% (2/14)	35.71 (5/14)	7.14% (1/14)	42.85% (6/14)	14.28% (2/14)
Thoracic perimeter	7.14% (1/14)	7.14% (1/14)	0.00% (0/14)	7.14% (1/14)	7.14% (1/14)	14.28% (2/14)	14.28% (2/14)	28.57% (4/14)	35.71% (5/14)	21.42% (3/14)
BCS	14.28% (2/14)	0.00% (0/14)	0.00% (0/14)	7.14% (1/14)	21.42% (3/14)	0.00% (0/14)	35.71% (5/14)	7.14% (1/14)	42.85 (6/14)	14.28% (2/14)

No differences founded between maternal groups in comparison with the chi-square test (P>0.05).
Source: SANTOS (2020).

5.3. IMPACT OF MATERNAL VARIABLES ON THE PERFORMANCE OF CALVES

The results between the maternal groups and calf performance are shown on Table 14. Means and standard deviations for each variable group in the individual times of this research are among Tables 21 to 31 in Appendix A.

Table 14. - Relationship between the high and low maternal groups and calf performance in the neonatal period (result expressed by P value from mixed linear model).

Maternal groups	Thoracic perimeter			Height at the withers			Rump width		
	Group	Time	Group x time	Group	Time	Group x time	Group	Time	Group x time
NEFA	0.91	**<0.01**	0.75	0.44	**<0.01**	0.86	0.13	**0.01**	0.41
BHB	0.95	**<0.01**	0.45	0.06	**<0.01**	0.31	0.10	**<0.01**	0.37
Glucose	0.60	**<0.01**	**0.02**	0.36	**<0.01**	0.83	0.90	**<0.01**	0.41
Total protein	0.74	**<0.01**	0.85	0.84	**<0.01**	0.84	0.96	**<0.01**	0.45
Albumin	0.41	**<0.01**	0.41	0.93	**<0.01**	0.22	0.34	**<0.01**	0.15
Triglycerides	0.86	**<0.01**	0.36	0.78	**<0.01**	**0.05**	0.29	**<0.01**	0.83
Cholesterol	0.28	**<0.01**	0.30	0.28	**<0.01**	0.15	0.08	**<0.01**	0.11
Hp	0.46	**<0.01**	0.89	0.50	**<0.01**	0.52	0.49	**<0.01**	0.95
Iron	0.20	**<0.01**	0.26	0.21	**<0.01**	0.20	**0.01**	**<0.01**	0.45
Thoracic perimeter	0.55	**<0.01**	0.33	0.07	**<0.01**	**0.01**	**0.02**	**<0.01**	**0.01**
BCS	0.59	**<0.01**	0.59	0.06	**<0.01**	0.08	**0.02**	**0.01**	0.19

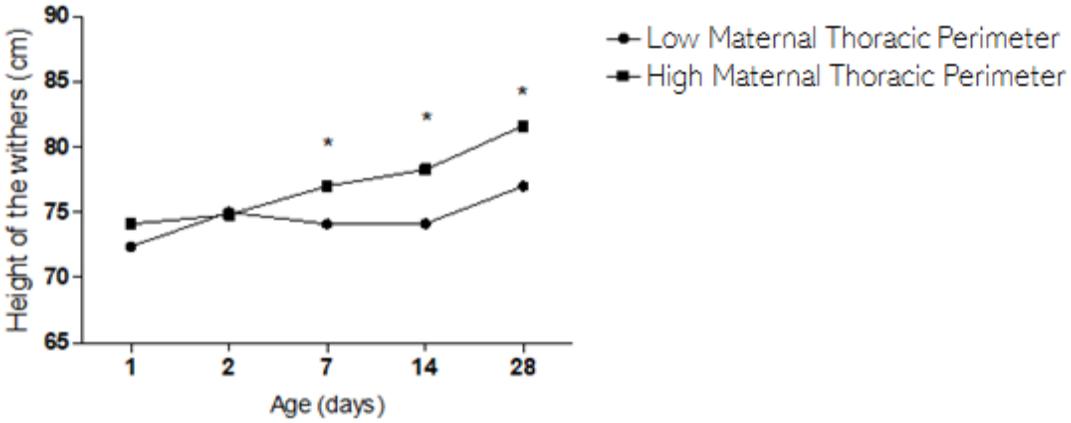
Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction.
P<0.05 was adopted for statistical differences.
Source: SANTOS (2020).

Calves' thoracic perimeter was affected by the interaction between maternal glucose levels and age (P = 0.0244), although the groups did not differ from each other within the sample at each time point (P > 0.05).

The interaction between maternal group and days of life for the variable wither height were observed for triglycerides (P= 0.0509) and thoracic perimeter (P=0.0018) (Figure 14).

Calves born from dams with low thoracic perimeter had lower (P<0.05) height at the withers in D7, D14 and D28 when compared to animals born from dams with high thoracic perimeters (Figure 14). At D28, calves born from animals with low thoracic perimeter had an average of 77.93 cm for withers height, while animals born to dams with high thoracic perimeter had 81.64 cm (Table 14, Figure 14).

Figure 14 – Height at the withers of the calves according to maternal thoracic perimeter group during the neonatal period.



Groups were compared statistically with the t test.
* P<0.05
Source: SANTOS (2020).

5.4. IMPACT OF MATERNAL VARIABLES ON THE BIOCHEMICAL PARAMETERS OF CALVES

The results about the relationship between the maternal groups and the biochemical parameters of calves are shown in Table 15. Means and standard deviations for each maternal group in the specific evaluated moments are presented among Tables 21 to 31 in Appendix A.

Several maternal parameters such as total protein (P=0.0200), albumin (P= 0.0258), triglycerides (P= 0.0401) and cholesterol (P= 0.0175) had effects on NEFA calf results. However, the comparison between the concentrations of these parameters in each time point did not presented statistical differences, except for total protein concentration in D1 (P<0.05), where the animals born from dams with low total protein showed low levels of NEFA (0.30 ± 0.05 mmol/L) when compared to the animals born from cows with a high concentration of total protein (0.56 ± 0.11 mmol/L).

Regarding to the calves BHB concentration, it was found only an effect of maternal triglycerides on this variable (P= 0.024). On D1, calves born from cows with low triglycerides were found to have higher values for BHB (0.06 ± 0.02 mmol/L) than calves born from cows with lower triglycerides (0.01 ± 0.01 mmol/L). Any other effect of the groups, time and interaction between groups and time were not detected in this research for calves BHB concentrations.

Calf blood glucose concentration had not any group maternal effect; however, it was possible to detect interactions between maternal groups and time for the maternal variables: iron (P= 0.0327) and BCS (P= 0.0327) (Figure 15).

The groups of calves were compared in relation to maternal iron levels (low and high), and it was found that calves born from dams with higher iron levels had higher (P <0.05) glucose levels soon after birth on D1 (83.84 ± 7.99 mg/dL) when compared with calves from cows with lower glucose concentration (57.07 ± 5.40 mg/dL). Another factor that influenced the blood glucose levels was the dam body condition score. Calves born from cows with greater BCS showed higher glucose levels on D2 (145.60 ± 9.42 mg/dL). Calf glucose concentration also had effect (P <0.05) of time during the realization of this study.

Triglycerides and cholesterol concentration in blood calves did not (P>0.05) had maternal group effect. However, it was possible to detect the interaction between calf triglycerides concentration with maternal iron (P= 0.0071). When comparing the groups (low and high) at each moment, we observe that animals born from low iron cows had higher concentrations of triglycerides in D2 (33.16 ± 4.14 mg/dL) when compared to calves born to dams with high iron (50.14 ± 4.85 mg/dL) (Figure 15). Also, it was observed interaction between calf cholesterol concentration with maternal NEFA (P= 0.0120), glucoses (P= 0.0451), iron (P= 0.0355) and thoracic perimeter (P= 0.0121).

Regarding the group categorized by the maternal NEFA, the profile of the calf in both experimental groups (low and high) did not differ at birth and on D2. However, animals born from cows with a lower concentration of NEFA showed higher concentrations of cholesterol in D7 (84.18 ± 4.94 mg/dL) when compared to calves born from cows with high NEFA (65.34 ±5.76 mg/dL). Arbitrarily, the profile changes in D14, so that the highest concentrations were found in calves born from dams with high NEFA levels (Figure 15).

In Figure 15, when comparing the groups of high and low concentration of maternal iron, statistical differences were found on D2, when calves born from dams with higher iron had a lower (P <0.05) concentration of cholesterol (37.42 ± 4.29 mg/dL) than animals born to dams with low iron (59.02 ± 8.31 mg/dL). In addition, on D1, calves from dams with high iron level showed greater glycemia when compared to calves born from cows with low iron concentration.

In relation to triglycerides level of calves, the neonates from cows with low maternal iron levels presented a greater level of triglycerides on D2 and D7 when compared to calves born from dams with high maternal iron (Figure 15).

Considering the maternal groups of low and high glucose, a statistical difference was found ($P < 0.05$) only in D14 with animals born from cows with higher blood glucose demonstrated to have a lower cholesterol concentration (99.06 ± 7.27 mg/dL) when compared with calves from dams with lower blood glucose results (78.57 ± 6.03 mg/dL) (Figure 15).

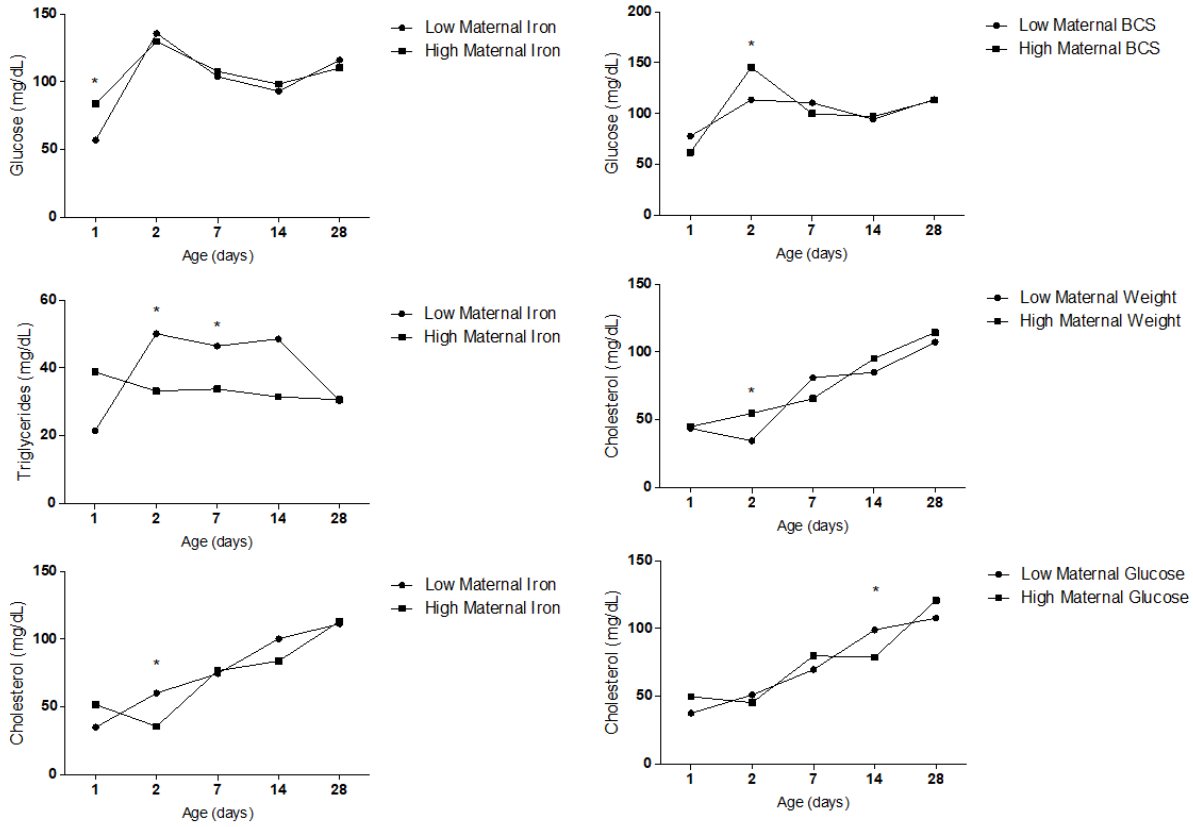
Regarding the influence of maternal thoracic perimeter on cholesterol levels, animals born from dams with low thoracic perimeter showed lower ($P < 0.05$) cholesterol concentration (33.25 ± 2.32 mg/dL) than calves born from cows with high thoracic perimeter on D2 (63.19 ± 8.10 mg/dL) (Figure 15).

Table 15. - Relationship between the high and low maternal cut-off values for different biomarkers (maternal groups) and the energetic metabolism in Holstein calves during the neonatal period.

Maternal groups	Calf biochemical parameters														
	NEFA			BHB			Glucose			Triglycerides			Cholesterol		
	G	T	G x T	G	T	G x T	G	T	G x T	G	T	G x T	G	T	G x T
NEFA	0.36	**0.01**	0.44	0.16	0.07	0.43	0.41	**<0.01**	0.44	0.55	**<0.01**	0.88	0.77	**<0.01**	**0.01**
BHB	0.67	0.52	0.41	0.48	0.19	0.22	0.83	**<0.01**	0.06	0.19	0.11	0.72	0.57	**<0.01**	0.11
Glucose	0.99	0.50	0.43	0.33	0.20	0.66	0.65	**<0.01**	0.58	0.57	0.09	0.12	0.71	**<0.01**	**0.05**
Total protein	**0.02**	0.52	0.27	0.57	0.21	0.88	0.44	**<0.01**	0.78	0.73	0.09	0.14	0.80	**<0.01**	0.29
Albumin	**0.03**	0.51	0.22	0.77	0.18	0.06	0.37	**<0.01**	0.24	0.84	0.10	0.38	0.24	**<0.01**	0.11
Triglycerides	**0.04**	0.51	0.33	**0.02**	0.19	0.14	0.72	**<0.01**	0.19	0.73	0.10	0.26	0.94	**<0.01**	0.17
Cholesterol	**0.02**	0.58	0.62	0.44	0.19	0.08	0.46	**<0.01**	0.25	0.70	0.11	0.82	0.22	**<0.01**	0.75
Hp	0.18	0.43	0.17	0.54	0.22	0.83	0.66	**<0.01**	0.44	0.75	0.09	0.36	0.43	**<0.01**	0.50
Iron	0.45	0.53	0.55	0.55	0.21	0.85	0.28	**<0.01**	**0.03**	0.20	0.07	**0.01**	0.45	**<0.01**	**0.04**
Thoracic Perimeter	0.89	0.50	0.57	0.54	0.19	0.18	0.15	**<0.01**	**0.01**	**0.10**	**0.10**	0.52	0.10	**<0.01**	**0.01**
BCS	0.22	0.90	0.69	0.59	0.65	0.90	0.87	**<0.01**	**0.03**	0.80	0.37	0.63	0.49	**<0.01**	0.61

Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction. G = group; T = time; G x T = group vs time
P <0.05 was adopted for statistical differences.
Source: SANTOS (2020)

Figure 15 – Biochemical parameters of the calves divided by different maternal groups at birth during the neonatal period.



Groups were compared statistically with the t test.
* P<0.05
Source: SANTOS (2020).

5.5. IMPACT OF MATERNAL VARIABLES ON THE INFLAMMATORY STATUS OF CALVES

The results about the relationship between the maternal groups and the inflammatory biomarkers are shown in Table 16. Means and standard deviations for each parameter in the time evaluated are presented among Tables 21 to 31 in Appendix A.

Calf haptoglobin serum concentration had effect of time for all experimental groups evaluated, except (P= 0.25) for the BCS groups. It is also noted the interaction between maternal groups and the calves' age considering the groups categorized according to maternal NEFA (P= 0.01) and maternal glucose concentrations (P= 0.02). The comparison between calves born from dams with high and low NEFA levels revealed that animals born to cows with low NEFA showed lower (P<0.05) levels of Hp (5.98 ± 1.62 mg/dL) when compared to calves born from dams with high NEFA (12.94 ± 3.42 mg/dL) on D28 (Figure 16).

Regarding the comparison between the high and low glucose groups, the blood glucose profile varied over time, with a statistical difference being observed only in D28, when the animals born from cows with low glucose had higher (P<0.05) levels of Hp (13.80 ± 3.38 mg/dL) (Figure 16).

Considering the iron levels of the calves, the only effect observed was detected in the comparison between the groups categorized in relation to the maternal level of total protein, and calves born from dams with low total protein had higher (P<0.05) of iron levels in D7 (26.87 ± 4.55 mg/dL). Although no other statistical differences were observed over time, the iron profile of animals born from cows with lower total protein was higher than that found for animals born from dams with low total protein concentration.

It was not detected effect of group or interaction between groups and time for calf total protein considering the maternal groups. However, the effect of age was detected only in comparisons of groups based on maternal levels of NEFA, cholesterol, iron and thoracic perimeter. For calf albumin concentration, it was observed the effect of maternal NEFA group (P= 0.0290). The interaction between group and time also was observed (P<0.01) for albumin considering the groups categorized by maternal thoracic perimeter: calves born from cows with high thoracic perimeter had higher

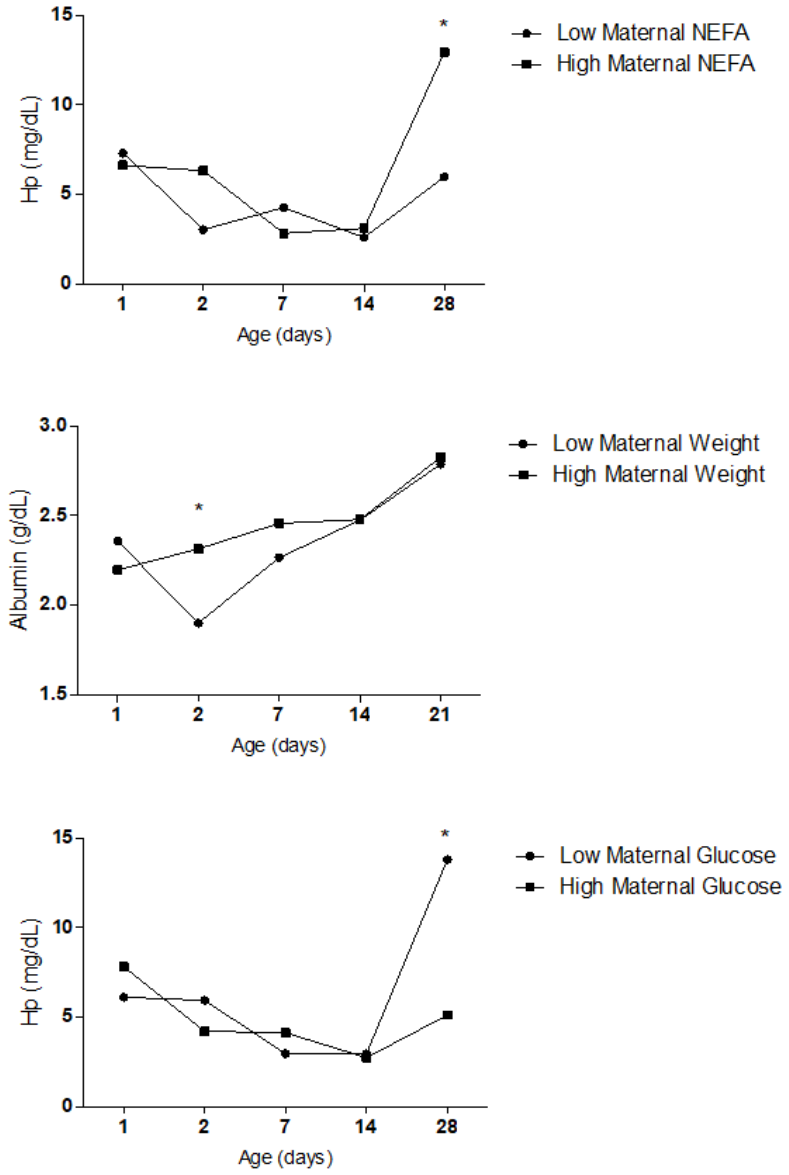
albumin levels (2.31±0.25 g/dL) than animals from cows with low thoracic perimeter (1.90±0.26 g/dL) on D2. We observed that for all evaluated experimental groups, the age effect was detected (P< 0.05).

Table 16. - Relationship between the high and low maternal groups maternal groups and the inflammatory biomarkers in the neonatal period (result expressed by P value from mixed linear model).

Maternal groups	Calf biochemical parameters											
	Haptoglobin			Iron			Total Protein			Albumin		
	G	T	G x T	G	T	G x T	G	T	G x T	G	T	G x T
NEFA	0.38	**0.01**	**0.01**	0.58	**0.04**	0.07	0.07	**<0.01**	0.85	**0.03**	**<0.01**	0.83
BHB	0.16	**<0.01**	0.44	0.94	0.05	0.64	0.49	0.23	0.36	0.28	**<0.01**	0.27
Glucose	0.21	**<0.01**	**0.02**	0.91	**0.04**	0.20	0.64	0.23	0.37	0.10	**<0.01**	0.94
Total protein	0.73	**<0.01**	0.49	**0.03**	**0.05**	0.39	0.25	0.24	0.47	0.57	**<0.01**	0.94
Albumin	0.37	**<0.01**	0.27	0.61	0.05	0.87	0.21	0.24	0.43	0.53	**<0.01**	0.85
Triglycerides	0.81	**<0.01**	0.61	0.71	**0.05**	0.38	0.14	0.23	0.33	0.19	**<0.01**	0.38
Cholesterol	0.96	**<0.01**	0.76	0.98	0.06	0.90	0.18	**<0.01**	0.89	0.60	**<0.01**	0.51
Hp	0.40	**<0.01**	0.75	0.81	0.06	1.00	0.59	0.29	0.17	0.37	**<0.01**	0.73
Iron	0.89	**<0.01**	0.24	0.53	0.05	0.78	0.58	**<0.01**	0.42	0.58	**<0.01**	0.42
Thoracic Perimeter	0.16	**<0.01**	0.23	0.07	**0.05**	0.56	0.13	**<0.01**	0.06	0.09	**<0.01**	**0.01**
BCS	0.58	0.25	0.15	0.66	0.19	0.85	0.88	0.50	0.93	0.32	**<0.01**	0.31

Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction. G = group; T = time; G x T = group vs time
P <0.05 was adopted for statistical differences.
Source: SANTOS (2020).

Figure 16 – Inflammatory biomarker of the calves divided by different maternal groups during the neonatal period.



Groups were compared statistically with the t test.
* P<0.05
Source: SANTOS (2020).

5.6. IMPACT OF MATERNAL VARIABLES ON THE INNATE IMMUNITY OF CALVES

5.6.1. Total leukocytes and neutrophils count

The results about the relationship between the maternal groups and neutrophils are shown in Table 17. Additional values (means and standard deviations) are described on Tables 43 to 53 in Appendix A.

Total leukocytes number and the number of neutrophils had not any effect of maternal groups or interaction between groups and time (P>0.05). However, it was possible to detect the effect of time for all maternal group's categorization used in this research for both leukocytes and neutrophils.

Table 17. - Relationship between the high and low maternal groups maternal groups and the leukogram parameters in the neonatal period (result expressed by P value from mixed linear model).

Maternal groups	Calf leukogram parameters					
	Leukocyte Total count			Neutrophils		
	G	T	G x T	G	T	G x T
NEFA	0.53	**0.01**	0.77	0.25	**0.01**	0.35
BHB	0.60	**0.02**	0.85	0.49	**0.01**	0.72
Glucose	0.49	**0.01**	0.71	0.34	**0.01**	0.84
Total protein	0.39	**0.01**	0.54	0.12	**<0.01**	0.25
Albumin	0.08	**0.04**	0.09	0.32	**0.04**	0.22
Triglycerides	0.51	**0.01**	0.74	0.39	**0.01**	0.78
Cholesterol	0.91	**0.01**	0.77	0.62	**<0.01**	0.27
Hp	0.43	**0.01**	0.61	0.19	**<0.01**	0.58
Iron	0.61	**0.02**	0.87	0.70	**0.01**	0.67
Thoracic perimeter	0.42	**0.01**	0.61	0.36	**0.01**	0.32
BCS	0.60	**0.02**	0.85	0.73	**0.01**	0.83

Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction. G = group; T = time; G x T = group vs time
P<0.05 was adopted for statistical differences.
Source: SANTOS (2020).

5.6.2. Impact of maternal variables on the innate immunity of calves

The ROS production expressed in AFU unit was very similar for the different dilution of inactivated bacterial antigen used in this research, so it was decided to present only the data from the tests performed with the pure stimulus.

The results regarding the comparison between groups, time and interaction between groups and time for ROS production variable (expressed in AFU) are shown in Table 18. Mean values and standard deviations for all stimulus used are presented among Tables 32 to 42 in Appendix A.

There was not effect of maternal group on basal ROS production, however it was found ($P<0.01$) interaction between groups and time for maternal levels of albumin, cholesterol and BCS (Figure 17). The basal ROS production also did not ($P>0.05$) show any age effect.

Calves born from dams with low albumin showed unstimulated cells with higher production of ROS in D7 (161.90 ± 132.98 AFU) when compared to calves born from cows with high albumin (90.13 ± 45.32 AFU). For groups divided in terms of maternal cholesterol content, in D2, calves born to mothers with a high concentration of cholesterol showed higher production of ROS by unstimulated cells (212.91 ± 168.70 AFU) when compared to calves born to mothers with a low amount of cholesterol (102.96 ± 46.77) (Figure 17).

For calves born to mothers with higher BCS, they presented a greater amount of ROS produced by cells not stimulated in D28 (203.60 ± 70.24 AFU) when compared to animals born to mothers with less body condition (109.23 ± 70.02 AFU) (Figure 17).

The effect of maternal BCS on ROS production after stimulation with pure *S. aureus* were also detected ($P<0.01$) (Table 18). Animals born to dams with low condition score presented a greater ROS production (574.04 ± 79.62 AFU) than calves from cows with a higher BCS (467.47 ±44.37 AFU).

The effect of maternal triglycerides concentration on EROS production against *E. coli* ($P= 0.04$) (Table 18) was observed. Calves from dams with low triglycerides presented lower ROS production (328.68 ± 25.68 AFU) when compared to animal born to cows with high triglycerides (475.87±63.16 AFU).

The effect of BCS on EROS production against *S. hyicus* ($P= 0.0071$) (Table 18) was founded, being that animals born to dams with higher BCS presented lower

ROS production (421.62 ± 25.76 AFU) when compared with animals from cows with lower BCS (614.91 ± 81.83 AFU).

Interaction between groups and time was detected (P<0.01) for maternal BSC and ROS produced against *S. aureus*, *E. coli* and *S. hyicus* (Figure 16). On D14, animals born to cows with low BCS showed higher ROS production when the neutrophils were stimulated with pure *S. aureus* (688.78 ± 1025.95 AFU) than calves born to dams with high BCS (441.66 ± 227.39 AFU). On D7, animals born to dams with low BCS presented higher ROS production when cells were stimulated by pure *S. hyicus* (622.80±330.06 AFU) than calves from cows with high BCS (408.89±172.88 AFU). Although the effect of BCS on ROS production was detected, no statistical difference was found in the *post-hoc* test for cells stimulated by pure *E. coli*.

The analysis of the response ration (RR = ROS produced by stimulated cells/basal ROS production) against different antigens is shown in Table 19. Mean values and standard deviations for all stimuli used are presented among Tables 29 to 40 in Appendix A. The effect of time was observed for almost all variables considering the different maternal groups established.

Interaction between group and time for maternal groups according to the albumin (P= 0.02), iron (P= 0.04) (Figure 18).

On D2, animals born to cows with low albumin demonstrated a higher response ratio of cells stimulated with PMA (7.24 ± 3.75 RR) than animals born to dams with high albumin (4.55 ± 2.62 RR). Although the interaction between the group based on maternal iron and age was found, no statistical difference was found in the post-hoc test.

For *S. aureus*, it was possible to detect effect of maternal groups according to iron concentration (P<0.01) and BCS (P= 0.02) (Table 19). Animals born to mothers with high iron showed a higher response rate of cells stimulated with S. aureus (6.04 ± 0.27 RR) when compared to calves born to mothers with low iron (4.18 ± 0.27 RR). Concerning the effect of the group categorized to maternal BCS, animals born to mothers with higher BCS showed a lower response rate to *S. aureus* (4.16 ± 0.29 RR) than calves born to cows with lower BCS (5.93 ± 0.38 RR).

Also, it was possible to detect interaction between group and time for RR of neutrophils against *S. aureus* when the data was categorized by maternal albumin (P= 0.03), cholesterol (P=0.01) and BCS (P<0.01) (Table 19). Although the interaction between maternal albumin levels and the calf's age was detected, no statistical

differences were found in the post-hoc test. When observing the groups categorized in relation to cholesterol, on D7, neutrophils from calves born to cows with high cholesterol showed a higher response ratio to the stimulus with pure *S. aureus* (6.37 ± 2.76 RR) than animals born of mothers with low cholesterol (4.14 ± 2.30 RR). Regarding the dam's body condition, calves born to mothers with low body condition had neutrophils with a higher rate of response to the stimulus with pure *S. aureus* on days D14 (8.29 ± 3.07 RR) and D28 (4.80 ± 2.27) than neutrophils born to mothers with high body condition (D14 = 5.63 ± 2.48 and D28 = 3.05 ± 1.13 RR). (Figure 18).

For *E. coli*, it was observed the effect of maternal iron concentration (P<0.01) (Table 19), as animals born to mothers with high iron had neutrophils more reactive to bacterial stimulus (4.44 ± 0.33 RR) than offspring of low iron cows (3.21 ± 0.20 RR). Effect of maternal BCS was also detected (P = 0.02) (Table 19), so that animals born from cows with low body condition had neutrophils more reactive to the stimulus with pure *E. coli* (4.46 ± 0.31 RR) than animals born to mothers with high body condition (3.08 ± 020 RR).

About response ratio for neutrophils stimulated by pure *E. coli, t*he interaction between groups and time was detected for maternal groups considering the cut-off values for cholesterol (P= 0.02) and iron (P= 0.04) (Table 19 and Figure 19). Calves born to cows with high cholesterol showed a high response rate (5.11±2.06 RR) when compared to the animals of cows with low cholesterol (3.29±1.77 RR) on D7. For the groups categorized by maternal iron, the offspring of the high group had a high response rate (6.48±3.22 RR) on D14 when compared to calves in the low group (3.76±1.58 RR).

For *S. hyicus*, it was detected effect of maternal group when dam values were considered to established groups according to iron concentration (P= 0.02) (Table 19). Concerning to maternal iron, calves born from cows with high iron showed greater reactivity to the stimulus with pure *S. hyicus* (5.93 ± 0.41 RR) than animals born from cows with lower iron concentration (4.44 ± 0.30 RR).

The interaction between maternal groups and time was also observed considering the cut- off maternal levels for albumin (P= 0.03), triglycerides (P<0.01), cholesterol (P= 0.03) and BCS (P<0.01) (Table 15). Although the effect of albumin on the response rate of neutrophils stimulated with *S. hyicus* was detected, no statistical differences were detected in the post-hoc tests.

Animals born to mothers with high triglycerides demonstrated to have neutrophils more reactive to *S. hyicus* on D7 (7.34 ± 3.00 RR) when compared to calves in the low group (3.72 ± 1.75 RR). Similarly, on D7, animals from dams with high cholesterol had a higher response rate (6.61 ± 3.03 RR) than animals born to cows with low cholesterol (4.28 ± 2.63 RR).

Concerning the interaction between maternal BCS and time, a statistical difference was detected in two moments, with animals born to cows with lower BCS showing cells with a higher rate of response to the stimulus with S*. hyicus* on D7 (6.59 ± 3.36 RR) and in D28 (4.71 ± 2.24 RR), when compared to animals born from cows with higher BCS (D7 = 4.31 ± 2.13 RR and D28 = 3.14 ± 1.13 RR).

Table 18. - Relationship between the high and low maternal groups maternal groups and the ROS production (average rate of fluorescence unit) in the neonatal period (result expressed by P value from mixed linear model).

Maternal groups	ROS production (average rate of fluorescence unit)														
	Unstimulated cells			Cell stimulated by PMA			*S. aureus* pure			*E. coli* pure			*S. hyicus* pure		
	G	T	G x T	G	T	G x T	G	T	G x T	G	T	G x T	G	T	G x T
NEFA	0.41	0.08	0.24	0.17	**<0.01**	0.19	0.35	0.87	0.13	0.53	0.84	0.14	0.25	0.96	0.13
BHB	0.63	0.09	0.76	0.09	**<0.01**	0.84	0.39	0.09	0.26	0.53	0.84	0.40	0.53	0.96	0.37
Glucose	0.07	0.09	0.92	0.06	**<0.01**	0.45	0.26	0.91	0.72	0.29	0.81	0.55	0.30	0.20	0.40
Total protein	0.75	0.08	0.29	0.90	**<0.01**	0.29	0.34	0.90	0.47	0.45	0.78	0.30	0.45	0.96	0.31
Albumin	0.19	0.05	**<0.01**	0.37	**<0.01**	0.27	0.14	0.87	0.31	0.07	0.80	0.33	0.10	0.96	0.49
Triglycerides	0.66	0.08	0.07	0.58	**<0.01**	0.57	0.07	0.88	0.44	**0.04**	0.83	0.62	**0.05**	0.96	0.54
Cholesterol	0.32	0.05	**0.01**	0.44	**<0.01**	0.42	0.21	0.94	0.47	0.15	0.84	0.52	0.13	0.99	0.57
Hp	0.97	0.06	0.15	0.27	**<0.01**	0.11	0.83	0.92	0.22	0.79	0.75	0.29	0.85	0.97	0.38
Iron	0.23	0.07	0.45	0.35	**<0.01**	0.96	0.29	0.91	0.49	0.60	0.79	0.43	0.63	0.99	0.39
Thoracic Perimeter	0.28	0.08	0.47	**0.01**	**<0.01**	0.94	0.58	0.91	0.38	0.68	0.88	0.50	0.76	0.98	0.43
BCS	0.08	0.44	**0.05**	0.17	**0.08**	0.21	**<0.01**	**<0.01**	**<0.01**	0.17	0.08	**0.01**	0.07	**<0.01**	**<0.01**

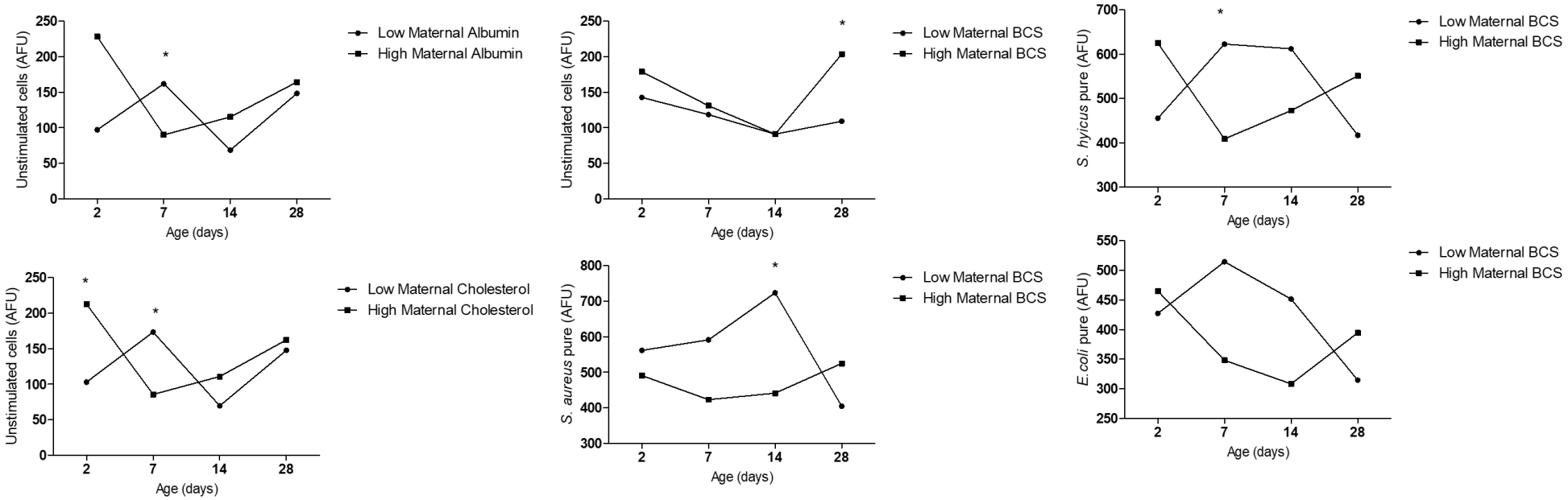
Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction. G = group; T = time; G x T = group vs time
P <0.05 was adopted for statistical differences.
Source: SANTOS (2020).

Table 19. - Relationship between the high and low maternal groups maternal groups and the ROS production (response ratio) in the neonatal period (result expressed by P value from mixed linear model).

Maternal groups	ROS production (response ratio)											
	Cell stimulated by PMA			*S. aureus* pure			*E. coli* pure			*S. hyicus* pure		
	G	T	G x T	G	T	G x T	G	T	G x T	G	T	G x T
NEFA	0.58	**<0.01**	0.85	0.34	**<0.01**	0.11	0.50	**<0.01**	0.07	0.06	**<0.01**	0.10
BHB	0.22	**<0.01**	0.57	0.09	**<0.01**	0.30	0.19	**<0.01**	0.40	0.10	**<0.01**	0.61
Glucose	0.19	**<0.01**	0.77	0.48	**<0.01**	0.60	0.24	**<0.01**	0.49	0.30	**<0.01**	0.83
Total protein	0.81	**<0.01**	0.46	0.40	**<0.01**	0.49	0.35	**<0.01**	0.32	0.41	**<0.01**	0.51
Albumin	0.64	**<0.01**	**0.02**	0.76	**<0.01**	**0.03**	0.55	**<0.01**	0.10	0.67	**<0.01**	**0.03**
Triglycerides	0.81	**<0.01**	0.31	0.55	**<0.01**	0.55	0.32	**<0.01**	0.32	0.29	**<0.01**	**<0.01**
Cholesterol	0.92	**<0.01**	0.15	0.85	**<0.01**	**0.01**	0.68	**<0.01**	**0.02**	0.45	**<0.01**	**0.03**
Hp	0.39	**<0.01**	0.91	0.49	**<0.01**	0.68	0.79	**<0.01**	0.56	0.80	**<0.01**	0.79
Iron	0.82	**<0.01**	**0.04**	**0.00**	**<0.01**	0.09	**0.02**	**<0.01**	**0.04**	**0.02**	**<0.01**	0.34
Thoracic perimeter	0.06	**<0.01**	0.16	0.20	**<0.01**	0.85	0.32	**<0.01**	0.76	0.16	**<0.01**	0.82
BCS	0.25	0.14	0.75	**0.02**	**<0.01**	**<0.01**	**0.02**	**<0.01**	0.12	0.12	**<0.01**	**<0.01**

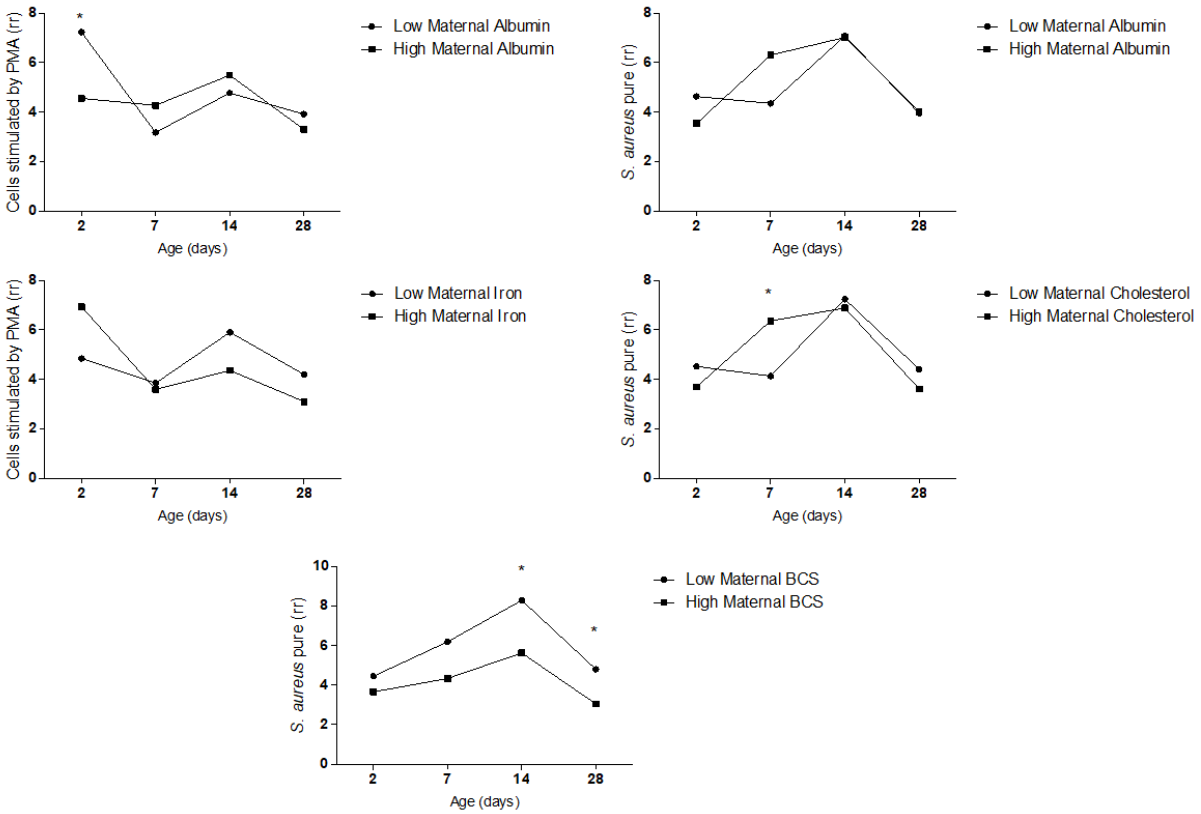
Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction. G = group; T = time; G x T = group vs time
P <0.05 was adopted for statistical differences.
Source: SANTOS (2020).

Figure 17 – ROS production (average rate of fluorescence unit) by neutrophils from calves divided by different maternal groups during the neonatal period.



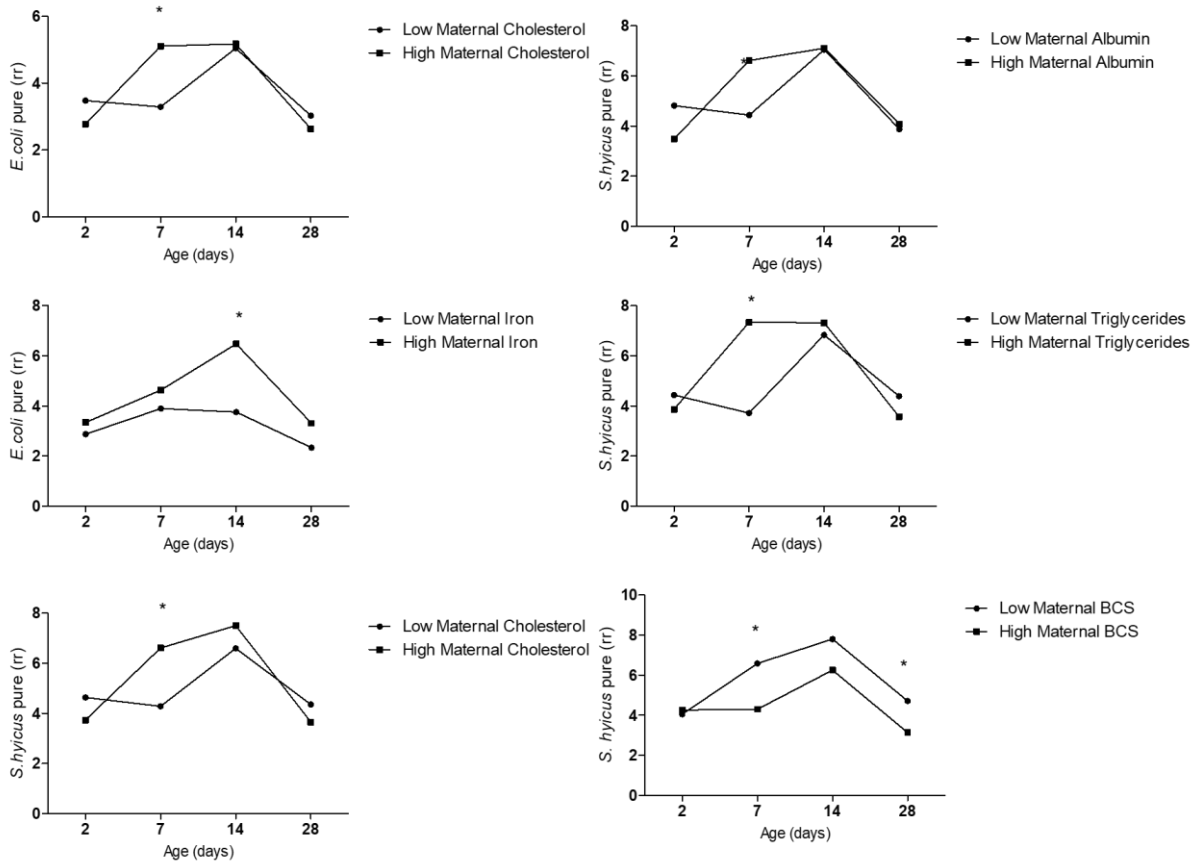
Groups were compared statistically with the t test.
* P<0.05
Source: SANTOS (2020).

Figure 18 – ROS production (response ratio) by neutrophils from calves divided by different maternal groups during the neonatal period.



Groups were compared statistically with the t test.
* P<0.05
Source: SANTOS (2020)

Figure 19 – ROS production (response ratio) by neutrophils from calves divided by different maternal groups during the neonatal period.



Groups were compared statistically with the t test.
* P<0.05
Source: SANTOS (2020)

5.7. IMPACT OF MATERNAL VARIABLES ON THE SERUM IgG LEVELS

The results about the relationship between the maternal groups and the serum IgG levels of the calves are in Table 20. Additional values (means and standard deviations) are among Tables 17 to 28 in Appendix A.

It was not founded any interaction between group and time was observed. The effect of time in IgG concentration was observed independently of maternal groups established birth (P< 0.0001).

Table 20. - Relationship between the high and low maternal groups maternal groups and the serum IgG levels in the neonatal period (result expressed by P value from mixed linear model).

Maternal groups	Calf IgG levels		
	Group	Time	Group x Time
NEFA	0.52	**<0.01**	0.80
BHB	0.27	**<0.01**	0.46
Glucose	0.37	**<0.01**	0.53
Total protein	0.41	**<0.01**	0.84
Albumin	0.08	**<0.01**	0.22
Triglycerides	0.42	**<0.01**	0.56
Cholesterol	0.31	**<0.01**	0.41
Hp	0.66	**<0.01**	0.36
Iron	0.14	**<0.01**	0.12
Thoracic perimeter	0.18	**<0.01**	0.25
BCS	0.57	**<0.01**	0.40

Mixed linear model was used to evaluate the main effects of group (low and high), time and group-time interaction. G = group; T = time; G x T = group vs time
P <0.05 was adopted for statistical differences.
Source: SANTOS (2020).

6. DISCUSSION

The current study was a retrospective observational study. As such, we may only determine the associations between maternal independent variables and dependent variables of calves. The main results of the present study will be discussed in the topics below.

6.1. DAMS STATUS AT CALVING

We hypothesized that in utero exposure to different maternal status during late gestation because the transition period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support fetal development and milk production (USDA, 2008), concurrently with the maturation of the fetus near birth with increased gluconeogenesis and hepatic glycogen stock (BATTAGLIA, 1978; FORHEAD et al., 2009), and the fastest proliferation of immune cells in the bovine fetuses (HIGGINS et al., 1983). For this, we measured biomarkers of the dams and classified their offspring according to the degrees of maternal metabolism and inflammatory experienced.

The lack of correlation among the maternal biomarkers demonstrated the dependency of some biomarkers. The correlation between NEFA, BHB, BCS and thoracic perimeter biomarkers was an expected finding, since these parameters are related to energetic metabolism (GRUMMER, 1995; DRACKLEY, 1999; HERDT, 2000). The dams when categorized into the groups with the highest concentrations for the NEFA, BHB, BCS and thoracic perimeter (high groups) showed higher concentrations of these same variables when compared to the cows from the groups with the lowest values for these biomarkers (low groups).

High NEFA and BHB concentrations are indicators of negative energy balance (ABDELLI et al., 2017). Similarly, BHB concentrations tend to increase as cows approach calving and as glucose concentrations decrease (FARAHANI et al., 2017).

The NEFA values in dairy cows with high lipomobilization may be up to 0.40 mmol/L when energy balance is not negative (OETZEL, 2004). Considering this value as a cut-off point, cows that were categorized in the low NEFA group were not in high lipomobilization, as they had a concentration of 0.35 mmol/L. However, the animals in the high NEFA group had an average of 0.79 mmol/L, in other words, the dams were in a state of high fat mobilization.

The BHB concentration should be less than 1.2 mmol/L, and above this cutoff point the cow has a greater risk of developing ketosis (OETZEL, 2004). For this study, the groups of high and low concentration of BHB showed average concentration of this biomarker below the cutoff point indicated. These results can be explained by the fact that the collection time to use BHB as a biomarker of metabolism in dairy cows is between the first and second postpartum weeks (LEBLANC, 2010).The serum BHB could be less efficient indicators of lipomobilization ate calving than serum NEFA in dairy cows during the late gestation. This agrees with Duffield (2000) who stated that the use of NEFA is a better indicator of energy imbalance in prepartum animals than BHB, but BHB is more useful postpartum.

The concentration of NEFA reflects the magnitude of mobilization of fat from storage and mirrors dry matter intake, while BHB reflects the completeness of oxidization of fat in the liver (LEBLANC, 2010). Thus, although both are related to energy metabolism, these biomarkers represent different pathways of metabolism. This fact may explain the moderate coefficient correlation (r = 0.50) found between NEFA and BHB.

Observing the maternal glucose concentrations, we see that it has a negative correlation with NEFA, BHB, BCS and thoracic perimeter. In other words, the dams with higher concentrations of NEFA and BHB, and greater values for BCS and thoracic perimeter had lower glycemia when compared to animals that had lower values for these variables. The serum levels of glucose are indicators of hepatic functionality (BOBE et al., 2004) and its low concentration may reflect fat infiltration in animals with high lipomobilization.

The indicators of the ability of the dams to deal with metabolic challenges in the transition period showed the highest concentrations of NEFA are found at the time of calving for animals with higher BCS and this may be associated with a lipolytic status of these animals. Lago and colleagues (2001) showed more intense weight loss and higher BHB levels in overweight Holstein cows when compared to cows with lower BCS. The authors concluded that animals with high thoracic perimeter presented more intense BEN considering that the glucose levels were lower.

In the present study, dams presented a minimum score of 2.00 and a maximum of 3.75 at the time of calving, but there is no information about the BCS of the cows in the previous weeks to understand the amount of weight mobilized by these dams. In a review, Garnsworthy (2006) suggested that Holstein cows are genetically programmed

to converge to BCS between 2.5 and 3 around 12 weeks postpartum, which is a body condition score range similar to that found at calving time in the present study.

Dams showing higher level of triglycerides also had higher values of cholesterol and lower values of albumin. For the relationship between triglycerides and cholesterol, there was a positive correlation and for the relationship between triglycerides and albumin, there was a negative correlation. Drackley (1999) indicated that extreme rates of lipid mobilization lead to increased uptake of NEFA by liver and increased triglyceride accumulation, but no significant correlation was found between triglycerides and NEFA or BHB, which means that triglyceride values may not be considered as an adequate indicator for lipomobilization.

The high group for total protein presented a low concentration of albumin, and these parameters showed a negatively correlation between them. This correlation can be explained by the fact that the periparturient inflammatory response is characterized by an increase in the production of positive acute phase proteins, with an increase of total protein, and a concomitant decrease in the production of negative acute phase protein such as albumin (BERTONI et al., 2008; PETERSEN et al. 2004). It was confirmed by the low concentration of albumin in the high Hp group, because the Hp is an acute phase protein in adult cattle (OSORIO et al., 2014; ECKERSALL, 2000).

Iron levels did not influence in the concentration of any other biomarker evaluated in this research. The high values for iron concentration in the high Hp were not expected, because the concentration of serum or plasma iron decreases rapidly in response to inflammation. It is important for the defense of the host, because iron is necessary for bacterial virulence and replication (MURATA et al., 2004; DETIVAUD et al., 2005).

6.2. IMPACT OF MATERNAL FACTORS ON DAIRY CALVES HEALTH

The variations in vital functions found probably are an effect of the age of the animals. Heart and respiratory rates decreased between the first to the 14th day of life, with a slight increase in D28. Silva et al. (2016) reported a similar profile for Holstein calves, with a decrease in values from D1 to D24, with an increase in subsequent days. Linke et al. (2013) reported that the lungs take at least two weeks to mature the gas exchange process. Piccione et al. (2007) reported that the systolic pressure of newborns is low, and the increase in heart rate is compensatory to increase cardiac

output. These findings are reflections of the newborn's incomplete physiological development.

Wittum and colleagues (1994) did not determine the influence of the cow body condition score at calving on the morbidity of the animals. However, in the present study, calves born from dams with high thoracic perimeter and with greater BCS showed a higher frequency of diarrhea. According to the maternal profile, the cows with the highest BCS showed high concentrations of NEFA. According to Ling et al. (2018), offspring born from cows that underwent excessive fat mobilization (high NEFA) during pre-partum had lower immune response than calves that had lower concentration of this parameter, with neonatal exposure to this maternal variable may influence the development of diseases in the first month of life.

Regarding the frequency of bovine respiratory disease, the influence of maternal factors has not been found. Although calves born from cows with higher thoracic perimeter and body condition score showed a higher occurrence of BRD, no statistical difference was determined. Result that can be attributed to the fact of the reduced sample size.

6.3. IMPACT OF MATERNAL FACTORS ON DAIRY CALVES PERFORMANCE

There are few studies that show data that report the effects of maternal factors on the performance of dairy calves. It was possible to prove the negative effects of some maternal factors in relation to the growth of the animals.

Calves born from dams with greater thoracic perimeter or greater BCS showed better performance in relation to the height of the withers and the rump width after the first week of life. Cows with greater thoracic perimeter and BCS are more able to meet the demands of the fetus and therefore have daughters with greater development. Considering that body condition is an indicator of energetic metabolism, the results in this study are in agreement with Gao et al. (2012): animals born from cows that did not undergo energy nutritional restriction showed greater body height and body length. The maternal-fetal environment has a great influence on the growth of calves (WATHES et al., 2007), since fetal programming occurs during pregnancy, characterized by the maternal stimulus that the fetus undergoes during pregnancy and which will affect, in the future, the physiology and development postnatal of offspring (BARKER et al., 1993). In contrast, thoracic perimeter of calves was not affected by

maternal factor during late gestation in the current study. Similarly, Martin et al. (2007) reported that the availability of maternal energy, characterized by adequate or inadequate nutrition during the final gestation period, did not influence the thoracic perimeter of calves.

In addition, results suggested that daughters of cows with higher concentrations of iron showed greater performance in relation to the rump width. Considering, blood iron levels in newborn calves, before suckling colostrum, was the same as the mother (ATYABI et al., 2006), animals born from cows with high iron had high concentrations of this mineral. It is known that higher iron levels in calves are characterized by increased performance in relation to thoracic perimeter and daily weight gain (KIRCHGESSNER et al., 1971). Regarding the maternal iron levels, the status of this mineral did not change appreciably during the dry period and peripartum period, being that much of the iron was provided from forages that were likely contaminated with soil (WEISS et al., 2010), so that the profile of the cows may have changed in relation to the forage that was provided by the farm.

6.4. IMPACT OF MATERNAL VARIABLES ON THE BIOCHEMICAL PARAMETERS OF DAIRY CALVES

Newborns must adapt to various environmental factors after birth, including nutrition, which changes from a primarily carbohydrate-based energy supply during the fetal period to a high fat and relatively low carbohydrate nutritional energy supply in colostrum (AYNSLEY-GREEN, 1988; FERRÉ et al., 1986; GIRARD, 1986; ODLE, 1997). When evaluating the design of the present study, we emphasized that the first collection occurred before the supply of colostrum, so that most maternal groups demonstrated to have influence on the biochemical parameters on D1.

Offspring from dams with low protein levels presented a high concentration of NEFA in D1. When interpreting data from cows that showed high concentrations of total protein, it is noted that high levels of this parameter were associated with high concentrations of Hp, which is an acute phase protein, and low level of albumin which is a negative acute phase protein (BERTONI et al., 2008; PETERSEN et al. 2004). A dysregulated inflammation in dairy cattle is highly implicated in the pathogenesis of metabolic stress during the transition period and inflammatory-based diseases (LEBLANC, 2014; SORDILLO; MAVANGIRA, 2014), thus, it is inferred that mothers

with high total protein were in an inflammatory state, which possibly may have generated less nutrient availability for the calf at the end of gestation. Calves have fat stores at birth and can mobilize them and provide non-esterified fatty acids for energy supply (STEINHOFF-WAGNER et al., 2011; HADORN et al., 1997).

BHB levels in the first few days after birth are low, but it is indicated as a marker of lipomobilization in calves (TODD et al., 2010; GIRARD et al., 1992). In the current study, we detected the BHB levels is influenced by the age of the calves. Possibly, the BHB levels increase according to the calf's ruminal development. According to Haga and colleagues (2008), BHB concentrations increase with age as animals begin to absorb considerable amounts of short-chain fatty acids and use them as an energy source.

Calves were born from dams with high concentration of iron presented high values of glucose on D1, without changing the level of iron. Similarly, Graham and colleagues (1994) founded maternal iron was not correlated with fetal iron. A gestational increase in fetal Fe could allow for development of in utero Fe provision (TENNANT et al., 1975). Still on D1, offspring from dams with high levels of iron had high concentrations of cholesterol and triglycerides. The mechanism by which prenatal exposure to high maternal iron concentrations in late gestation can be associated with an elevated glucose, triglycerides and cholesterol is unknown. The results of serum iron, preferably in combination with other inflammation biomarkers, may be a useful diagnostic tool for acute inflammation in cattle (BAYDAR, DABAK, 2014), but in this case it cannot be correlated with other inflammation biomarkers assessed.

Body condition score of dams also had effect on calf glucoses, observing low values in calves were born from dams with low BCS than animals born from cows with high BCS on D2. Maternal body composition is linked to maternal diet and feed intake and is important since the mother provides nutrition for the fetus from both her dietary intake and her own body reserves (COSTELLO et al., 2013), because fetal glycemia depends almost exclusively on placental supply of glucose (FOWDEN et al., 2009). It is possible that cows with higher body condition had a higher supply of glucose to their offspring, so that a difference was detected between calves born to mothers with high and low body condition. Another hypothesis is related to the quality of colostrum that cows with different body conditions produced, as studies have concluded that maternal nutritional status affects the quality of colostrum in ruminants (BANCHERO et al., 2006, PHOMVISITH et al., 2016). Thus, cows with lower body condition may have produced

colostrum with lower energy density, and the glycemia of the calves on D2 may have been influenced by the feeding of their own dams' colostrum.

This research identified three main metabolic markers for calves: glucose, cholesterol and triglycerides. The changes in biomarker profiles in calves starting after colostrum feeding were indicative of maternal effects in the colostrum and the calf response to those components. Colostrum contains proteins, essential and nonessential amino acids and fatty acids, lactose, vitamins, and minerals as well as non-nutrient substances, such as immunoglobulins, peptides, peptide, hormones, growth factors, cytokines, steroid hormones, thyroxine, nucleotides, polyamines, and enzymes (KOLDOVSKY, 1989), so that maternal factors may have influenced the composition of colostrum and it may have influenced the different calf profiles found in this study. In this study, the BHB levels demonstrated weak to determine the profile of the animals in the neonatal period, which can be explained by the low capacity for ketone body production in neonates. Plasma concentrations of BHB are low during the neonatal period (HAMMOM et al., 2012), but increase in older calves (SENN et al., 2000), because circulating concentrations of BHB are highly correlated with concentrate intake (QUIGLEY, 1996).

The results also demonstrate that the maternal effect was more accentuated in the moments immediately after birth and shortly after ingesting colostrum: since the mother with the highest body condition score or thoracic perimeter had calves with the highest concentration of cholesterol and triglycerides. After these first moments, the biochemical parameters may be more related to diet and management factors.

6.5. IMPACT OF MATERNAL VARIABLES ON THE INFLAMMATORY STATUS OF DAIRY CALVES

Haptoglobin is one of several acute phase proteins produced in response to inflammatory changes associated with infection, making it a potentially useful indicator of calf health (NONNECKE et al., 2009). In the current study, calves exposed to high maternal NEFA during late gestation had significantly higher serum Hp on D28. This finding is compatible with research realized by Ling and colleagues (2018), where high maternal NEFA levels were associated with higher inflammation on calves (expressed by Hp levels). These data suggest that prenatal exposure to elevated maternal lipomobilization (high NEFA) is associated with an increased level of inflammation on

the offspring. In addition, animals born to cows with low glucose had high levels of Hp than calves from dams with high glucose concentration. This result may be an indication that a low available energy, considering glucose as the main source of energy, is enough to induce differences in concentrations of haptoglobin after birth. The mechanism by which prenatal exposure to high maternal NEFA and low glucose concentrations in late gestation can be associated with an elevated Hp is unknown. Further research is required to determine whether offspring exposed to different maternal levels of NEFA and glucose during late gestation are at increased risk of diseases associated with inflammation.

The interaction between group and time was also observed for albumin levels founded in calves considering the groups categorized by maternal thoracic perimeter, with animals born from cows with high thoracic perimeter having a higher concentration of albumin when compared to calves born from cows with low thoracic perimeter on D2. Albumin is the most abundant of blood plasma proteins and constitutes about 50 to 65% of the total. Albumin is synthesized in the liver and its concentration can be altered by protein intake in the diet (LIMA et al., 2012), besides being a negative acute phase protein (BERTONI et al., 2008; PETERSEN et al. 2004). In this way, we can interpret that the results found suggest a relationship between the protein ingestion by the colostrum supplied to the calves, since the statistical difference was found in D2, that is, to consider that mothers with higher thoracic perimeter had better quality colostrum and this caused the calves to have a greater supply of proteins, which caused the difference soon after the consumption of colostrum in D2. The interpretation regarding the inflammatory profile of the calves is weak, since the acute phase protein (Hp) did not suffer an interaction effect between the animals 'age and the dams' thoracic perimeter.

6.6. IMPACT OF MATERNAL VARIABLES ON THE INNATE IMMUNE RESPONSE OF DAIRY CALVES

Calf total white blood cells (WBC) and the number of neutrophils were not affected for the maternal groups and it was not observed interaction between groups and time along the first month of life. Only the effect of age on the total leukocyte and neutrophil count was detected, which is expected, since physiological events occur over the neonatal period that contribute to changes over time. Among the important

events that cause differences in the leukogram profiles of calves over time is the influence of plasma cortisol at birth; also, the ingestion of colostrum, essential for the survival and performance of ruminant newborns, causes important changes in the biochemical profile of young calves and must be considered a physiological landmark in the evaluation of these animals (COLE et al. 1997).

Neonates are naturally predisposed to cellular oxidative stress, because they are exposed to high concentrations of oxygen in the tissues shortly after birth due to rapid blood perfusion (FRANK, 1985). In addition, they have reduced antioxidant defenses in the body, so that lower concentrations of superoxide dismutase (SOD) are found in red blood cells and plasma antioxidants. We can also mention that the immature immune system facilitates infections and inflammations, increasing the production of toxic free radicals (SAUGSTAD, 2003). All these conditions, added to the greater metabolic activity at this stage, cause an increase in the production of ROS (DAVIS; AUTEN, 2010; YIN et al., 2013). Considering the points cited, the objective of evaluating the production of reactive oxygen species (ROS) was to identify which maternal factors altered the immune response and cellular metabolism, causing a difference in the neutrophil profile of calves throughout the neonatal period.

Within WBC population, neutrophils are the main responsible for the ROS production. After phagocytosis, neutrophils kill invading pathogens depends on production of highly toxic ROS in the pathogen-containing vacuole and fusion of neutrophil granules, containing various antimicrobial mediators to the vacuole. Coincident with phagocytosis of bacteria, neutrophils produce an oxidative burst that is triggered by pro-inflammatory cytokines such as TNF-α or by binding of opsonized bacteria to phagocytic receptor. This process results in the rapid release of high levels of bactericidal reactive chemical species under the catalyzation of NADPH oxidase (NOX), myeloperoxidase (MPO), or nitric oxide (NO) synthetase. NADPH oxidase (NOX) is responsible for the generation of ROS, such as superoxide anion (O-2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (HO). Superoxide anion interacts spontaneously (dismutase) to generate one molecule of H2O2 under the influence of the SOD. Because this reaction occurs so rapidly, superoxide anion does not accumulate but H_2O_2 does. The hydrogen peroxide is converted to bactericidal compounds through the action of myeloperoxidase, the most significant respiratory burst enzyme in neutrophil granules. Myeloperoxidase catalyzes the reaction between

hydrogen peroxide and intracellular ions to produce bactericidal radicals as hypochlorite ions (OCl-) (JANEWAY, 2007; TIZARD, 2008):

The ROS production were presented in two different ways in this research by using the original unit emitted by the fluorescence equipment expressed in AFU and also it was showed the response ratio for ROS production by the division between the ROS stimulated (AFU) by basal ROS (AFU). The response ratio calculation identifies how many times the cells reacted in relation to the response of the unstimulated cell (basal response). The effect of maternal group or interaction between groups and time were more evident when the response ratio was presented, indicating that the maternal effect is more related to responses to antigens.

Calves from this research were naturally exposed to diarrhea-pathogen, manifested especially in D7 and D14. The most difference observed between maternal groups were detected observing that the offspring from dams with low maternal albumin, low maternal cholesterol and low maternal BCS presented high levels of basal ROS expressed in AFU (non-stimulated cells) on D7 and D28. The inflammatory response during the prepartum period is characterized by an increase in the production of positive acute phase proteins (Hp), and a concomitant decrease in the production of negative acute phase proteins (albumin) (BERTONI et al., 2008). Acute phase proteins concentrations were also demonstrated in the adipose tissue of cows with higher rates of lipolysis (ZACHUT et al.,2015). As previously mentioned, cholesterol is related to the nutritional condition of the animals (NDLOVU et al., 2007), and the decrease in serum/plasma cholesterol levels indicates an energy deficit (WITTER, 2000). Considering this information, we can interpret that the cows with less body condition were in a pro-inflammatory state with the tendency of having an energy deficit in the pre-calving period, and this maternal profile influenced the response of the neutrophils, who were more reactive and produced a greater amount of ROS.

The effect of the maternal group categorized by body condition was better perceived when we evaluated the different stimuli and response rates. Offspring born from dams classified with low BCS also had neutrophils more reactive for *S. aureus*, *S. hyicus* and *E. coli*, considering the AFU value. Also, the response ratio (RR) against *S. aureus* and *S. hyicus* also were higher in the offspring was born from the low maternal BCS. It is inferred that mothers with less body condition at the time of delivery had calves more reactive to different bacterial stimuli than animals born to dams with high BCS. The BCS determines the greater or lower predisposition to oxidative status

at calving, as the loss of body condition in the prepartum period accentuates this status (ABUELO et al., 2019). BCS loss is associated with fat and protein breakdown. Thus, increasing the catabolic pathways in order to generate energy from lipids and amino acids. Lipid peroxidation is one of the important consequences of oxidative stress (SORDILLO; AITKEN, 2009; CASTILLO et al., 2005; KONVIČNÁ et al., 2015). Research conducted by Ling and colleagues (2018) founded that calves exposed to higher maternal oxidative status showed a significantly higher plasma concentration of ROS. Increases in the concentration of ROS may cause immunosuppression as overproduction of free radicals compromise leukocyte function (SORDILLO; AITKEN, 2009). Thus, it is inferred that mothers with lower body condition were in oxidative stress, so that the calves born to these cows were exposed to this condition at the end of pregnancy, which may have contributed to the profile of ROS presented by these calves.

In relation to maternal biomarkers cut-off values, it was possible to observe some differences between groups when the offspring was categorized by cholesterol, triglycerides, albumin, and iron.

High maternal levels of cholesterol were associated with high response ratio against *S. aureus*, *S. hyicus* and *E. coli* in D7. Similar phenomenon was observed for maternal triglycerides group, observing that the high also had high response ratio against *S. hyicus*. On D7 and D14, calves were born from high maternal albumin and high maternal iron had high response ratio against *S. hyicus* and *E. coli*. As previously discussed, many of the maternal metabolic factors are related to oxidative status in the prepartum period. The degree of oxidative status experienced not only puts the dams at risk of subsequent diseases, but also has an impact on the offspring (ABUELO et al., 2019). In a recent study, it was demonstrated that serum concentrations of ROS were higher in calves exposed to higher maternal oxidative status when compared to calves born to cows with lower values of this biomarker (LING et al., 2018).

These findings probably were associated with the high frequency of diarrhea previously reported in this research, because the offspring was born from the high thoracic perimeter and high maternal albumin group had high frequencies of diarrhea. The results suggest that prenatal exposure to maternal parameters of metabolic stress (altered nutrient utilization) may adversely impact some immune responses of the offspring that could influence disease susceptibility.

7. CONCLUSION

Based on the presented results, the data suggest that prenatal exposure to different maternal factors during late gestation may adversely affect some metabolic, inflammatory and innate immune responses of the offspring that could influence disease susceptibility and performance. This is an important finding because it reinforces the perception that a successful neonatal period starts during the gestation. Further research is required to determine the mechanisms by which metabolic parameter during late gestation affects the immune and metabolic responses of the offspring and the clinical effect of these carryover effects on the health and growth of the offspring. This will allow the development of management practices that ultimately enhance the health and production of replacement heifers.

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APPENDIX A – RESULTS

Table 21.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by NEFA group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	58.9±21.07	67.75±24.25	54.5±15.37	57.5±12.46	49.2±13.8	45.25±10.42	38.1±13.42	43.5±23.07	48±9.06	56.5±19.53
Heart Rate (bpm)	134.8±21.56	155.5±15.92	127.68±20.59	139.5±15.18	118.2±23.52	109.75±11.97	94.6±13.32	117.5±44.79	124.3±18.67	125.5±15.85
RT (◦C)	38.46±0.47	38.39±0.22	38.83±0.23	38.71±0.18	38.76±0.39	38.78±0.28	38.79±0.46	38.96±0.29	38.88±0.34	38.99±0.27
Thoracic perimeter(cm)	76,21±3.51	77,57±3.41	76,10±4.09	77,77±3.30	80,50±3.08	81,21±2.72	82,64±2.95	82,64±2.71	88,86±2.51	90,79±3.09
Height at the withers (cm)	73.10±3.98	73.63±3.55	74.67±3.9	75.44±4.6	75.63±4.8	76.69±4.42	75.8±4.75	77.25±5.07	79.2±4.41	81.25±2.8
Rump width (cm)	22.1±1.81	23.69±2.05	23.17±1.99	23.94±1.57	23.8±2.63	24.44±2.76	23.05±2.57	24.5±2.05	25.08±1.68	25±2.39
NEFA (mmol/L)	0.45±0.36	0.42±0.26	0.23±0.22	0.35±0.23	0.34±0.33	0.3±0.12	0.2±0.08	0.47±0.85	0.47±0.81	0.2±0.06
BHB (mmol/L)	0.02±0.03	0.07±0.12	0.02±0.04	0.04±0.05	0.04±0.05	0.1±0.18	0.03±0.03	0.02±0.04	0.05±0.04	0.06±0.02
Glucose (mg/dL)	70.95±26.03	67.63±33.51	132.6±30.65	135.17±32.4	105.25±17.67	106.88±22.06	94.6±20.88	98.63±7.61	108.9±11.77	124.25±10.99
TP (µmol/L)	3.67±1.22	4.1±0.42	6.97±1.19	7.59±0.61	6.77±1.15	7.11±0.64	6.12±0.65	6.43±0.47	5.84±0.55	6.28±0.58
Albumin (g/dL)	2.19±0.58	2.5±0.22	2.06±0.3	2.24±0.36	2.34±0.31	2.41±0.18	2.45±0.11	2.53±0.2	2.77±0.13	2.9±0.14
Triglycerides (g/dL)	24.98±12.67	42.91±50.77	40.62±16.78	44.24±23.93	39.2±16.27	42.45±25.55	38.87±29.53	42.91±16.08	30.28±9.13	31.1±12.46
Cholesterol (mg/dL)	39.20±16.37	54.56±41.75	49.74±28.44	44.43±22.93	80.38±20.21	60.74±20.72	82.61±21.97	104.66±32.11	115.85±27.99	110.28±29.08
Hp (mg/dL)	8.18±8.11	3.96±2.11	5.92±9.01	4.26±1.85	3.94±2.96	2.57±0.71	2.58±0.75	3.52±1.09	7.93±10.18	13.3±10.84
Iron (mg/dL)	21.4±13.8	18.59±8.27	30.54±10.88	23.75±13.85	16.57±9.3	30.23±21.21	26.63±16.14	36.38±18.2	25.35±18.76	21.14±16.54
Serum IgG (mg/mL)	0.00±0.00	0.00±0.00	36.13±17.92	35.06±10.76	33.35±19.36	25.02±10.79	25.93±14.72	22.24±20.47	20.04±18.31	13.81±7.24

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; TP = total protein; Hp = haptoglobin; BCS = body condition score
Source: SANTOS (2020).

Table 22.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by BHB group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	61.14±24.44	61.71±20.06	52.71±15.76	58±13.01	52.14±14.39	44±10.05	44±20.34	35.29±10.4	51.43±15.36	49.43±10.91
Heart Rate (bpm)	151.71±22.16	129.71±15.9	136.86±17.34	125.25±20.79	116.14±20.19	115.43±22.57	106.86±36.37	95.43±13.3	121.14±19.24	128.14±15.78
RT (◦C)	38.34±0.49	38.54±0.31	38.76±0.23	38.83±0.21	38.87±0.35	38.66±0.34	38.97±0.35	38.71±0.46	38.88±0.25	38.94±0.39
Thoracic perimeter (cm)	75,69±4,15	78,21±2,26	76,92±2,43	77,20±5,01	80,57±2,95	81,14±2,88	82,57±2,65	82,71±3,00	90,00±3,09	89,64±2,87
Height at the withers (cm)	74.39±3.21	72.11±4.12	75.27±4.35	74.5±3.87	77.36±3.89	74.5±5.02	77.96±4.04	74.46±4.98	81.5±2.87	78.07±4.46
Rump width (cm)	22.86±1.55	22.25±2.36	23.38±1.52	23.5±2.31	24.54±2.53	23.43±2.71	24.39±2.1	22.54±2.56	25.71±2.17	24.39±1.24
NEFA (mmol/L)	0.54±0.38	0.33±0.24	0.26±0.12	0.26±0.3	0.42±0.38	0.24±0.11	0.18±0.08	0.37±0.64	0.38±0.7	0.41±0.71
BHB (mmol/L)	0.05±0.1	0.02±0.03	0.02±0.03	0.04±0.04	0.04±0.05	0.08±0.14	0.01±0.03	0.03±0.03	0.05±0.03	0.04±0.05
Glucose (mg/dL)	62.29±27.3	78.23±26.95	140.18±31.94	119±22.26	102.79±17.09	108.64±20.22	94.93±12.34	96.57±22.85	118.79±11.05	107.79±13.55
Iron (mg/dL)	18.01±8.09	23.18±15.46	27.85±14.39	29.35±9.39	20.87±15.2	20.06±14.81	32.76±19.27	26.06±14.34	24.29±16.67	23.99±19.78
Total Protein (µmol/L)	3.79±1.01	3.79±1.16	7.41±0.79	6.89±1.29	6.73±1.2	7.01±0.86	6.27±0.62	6.14±0.62	6.04±0.46	5.89±0.69
Albumin (g/dL)	2.31±0.55	2.25±0.51	2.25±0.33	1.96±0.25	2.37±0.31	2.35±0.25	2.48±0.18	2.48±0.11	2.78±0.11	2.83±0.17
Triglycerides (g/dL)	33.54±39.66	26.66±12.89	45.8±18.81	37.51±18.29	44.94±24.63	35.31±9.31	43.2±33.79	36.85±16.05	28.05±9.45	32.98±10.17
Cholesterol (mg/dL)	45.54±33.39	41.64±17.64	55.13±32.34	41.31±18.13	67.89±26.03	81.64±14.77	97.24±30.87	80.58±19.28	111.19±17.48	117.32±35.89
Hp (mg/dL)	7.44±9.74	6.5±3.33	5.83±8.49	4.12±2.59	3.54±3.19	3.56±1.94	2.92±1.11	2.78±0.78	12.4±12.84	6.53±6.64
Serum IgG (mg/dL)	0.00±0.00	0.00±0.00	33±17.34	38.65±14.64	26.81±14.68	35.13±19.7	23.48±16.78	26.27±16.21	13.18±9.74	20.66±16.84

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 23.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by glucose group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	56.57±23.22	66.29±20.24	54±14.44	56.71±14.83	50.71±15.52	45.43±9.36	43.14±21.23	36.14±9.23	56.14±13.04	44.71±10.8
Heart Rate (bpm)	141.43±25.13	140±19.28	130.57±13.09	131.54±25.17	116±20.58	115.57±22.22	103.43±34.04	98.86±20	127.71±21.77	121.57±12.31
RT (◦C)	38.41±0.39	38.46±0.45	38.71±0.21	38.88±0.2	38.73±0.35	38.8±0.37	38.86±0.38	38.81±0.47	39.04±0.34	38.78±0.25
Thoracic perimeter(cm)	77,79±4.02	76,00±2.66	76,62±4.17	77,60±3.03	80,71±3.34	81,00±2.45	82,43±3.30	82,86±2.25	90,57±2.93	89,07±2.84
Height at the withers (cm)	73.75±4.63	72.75±2.85	75.38±4.57	74.35±3.46	76.43±5.34	75.43±3.96	77.29±5.48	75.14±3.9	80.21±5.01	79.36±2.98
Rump width (cm)	22.18±1.79	22.93±2.16	22.96±1.71	24.05±1.94	24.18±2.66	23.79±2.69	23.54±2.81	23.39±2.22	25.39±2.21	24.71±1.44
NEFA (mmol/L)	0.4±0.25	0.48±0.41	0.35±0.28	0.18±0.1	0.42±0.37	0.24±0.11	0.19±0.04	0.37±0.65	0.36±0.69	0.43±0.71
BHB (mmol/L)	0.05±0.1	0.03±0.03	0.03±0.04	0.02±0.03	0.08±0.14	0.04±0.04	0.02±0.02	0.03±0.04	0.05±0.03	0.05±0.04
Glucose (mg/dL)	66.64±29.21	73.54±26.94	130.5±29.81	138.67±33.1	103.36±17	108.07±20.45	97.64±14.74	93.86±21.23	116.5±12.46	110.07±13.91
Iron (mg/dL)	18.74±8.83	22.46±15.27	26.41±11.52	30.79±12.39	21.59±15.32	19.34±14.61	34.48±17.72	24.34±15.21	21.17±12.2	27.11±22.39
Total Protein (µmol/L)	3.86±0.78	3.72±1.32	7.4±1.05	6.9±1.09	6.84±1.16	6.9±0.93	6.39±0.53	6.03±0.66	6.14±0.54	5.79±0.59
Albumin (g/dL)	2.36±0.43	2.2±0.6	2.18±0.38	2.04±0.26	2.38±0.33	2.35±0.22	2.52±0.16	2.44±0.11	2.84±0.14	2.78±0.15
Triglycerides (g/dL)	23.01±11.6	37.19±39	45.37±18.43	37.94±18.88	41.04±21.51	39.21±16.68	47.69±32.84	32.36±14.7	30.97±9.94	30.06±10.33
Cholesterol (mg/dL)	37.44±11.5	49.74±34.93	51.12±28.76	45.32±25.14	69.74±21.08	79.79±22.33	99.06±27.23	78.76±22.59	107.69±22.06	120.82±32.16
Hp (mg/dL)	6.12±6.52	7.82±7.89	5.95±8.94	4.22±2.39	2.97±1.31	4.13±3.39	2.95±0.66	2.74±1.18	13.81±12.67	5.12±5.14
Serum IgG (mg/dL)	0±0	0±0	34.46±15.83	37.19±16.67	30.33±17.14	31.61±18.61	22.19±11.17	27.56±20.2	12.23±6.12	21.61±17.99

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 24.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by total protein group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	69.57 ±24.93	53.29 ±15.34	54.71 ±12.39	56.00 ±16.68	49.86 ±15.70	46.29 ±9.51	44.00 ±18.43	35.29 ±13.49	50.86 ±15.70	50.00 ± 10.50
Heart Rate (bpm)	140.00 ±22.95	141.43 ±21.83	134.11 ±25.05	128.00 ±12.55	111.43 ±13.75	120.14 ±26.21	107.14 ±32.27	95.14±21.23	123.86 ±16.61	125.43 ±19.19
RT (◦C)	38.60 ±0.31	38.27 ±0.45	38.77 ±0.19	38.81 ±0.26	38.85 ±0.39	38.68 ±0.31	38.79 ±0.46	38.89±0.39	38.94 ±0.37	38.88 ±0.27
Thoracic perimeter (cm)	76,93±2,89	76,86±4,07	78,00±2,72	76,17±4,30	81,36±2,47	80,36±3,25	82,86±2,48	82,43±3,13	90,00±2,32	89,64±3,52
Height at the withers (cm)	73.11 ±4.86	73.39 ±2.54	75.36 ±5.03	74.54 ±3.14	75.32 ±5.42	76.54 ±3.82	76.18 ±5.78	76.25±3.79	79.79 ±5.17	79.79 ±2.77
Rump width (cm)	22.46 ±2.22	22.64 ±1.79	22.95 ±2.03	23.88 ±1.64	24.39 ±3.09	23.57 ±2.12	23.50 ±2.43	23.43±2.64	25.14 ±1.98	24.96 ±1.81
NEFA (mmol/L)	0.31 ±0.22	0.56 ±0.38	0.19 ±0.12	0.33 ±0.28	0.25 ±0.11	0.41 ±0.38	0.33 ±0.65	0.22±0.09	0.20 ±0.06	0.59 ±0.95
BHB (mmol/L)	0.04 ±0.06	0.04 ±0.09	0.02 ±0.02	0.04 ±0.05	0.05 ±0.04	0.07 ±0.14	0.02 ±0.04	0.02±0.03	0.05 ±0.05	0.04 ±0.03
Glucose (mg/dL)	72.00 ±28.75	68.07 ±27.87	130.14 ±29.76	136.22 ±32.15	111.36 ±17.84	100.07 ±18.23	98.50 ±19.48	93.00 ±16.73	115.29 ±15.31	111.29 ±11.31
Iron (mg/dL)	23.71 ±14.83	17.49 ±8.83	30.20 ±11.90	27.00 ±12.23	26.88 ±17.05	14.06 ±8.45	34.62 ±20.62	24.20 ±10.83	23.66 ±20.82	24.63 ±15.34
Total Protein (µmol/L)	3.84 ±1.20	3.75 ±0.96	7.33 ±0.75	6.97 ±1.34	7.09 ±0.69	6.65 ±1.28	6.29 ±0.58	6.12 ±0.65	5.91 ±0.52	6.01 ±0.65
Albumin (g/dL)	2.26 ±0.52	2.30 ±0.54	2.15 ±0.36	2.06 ±0.30	2.40 ±0.15	2.32 ±0.36	2.49 ±0.15	2.46 ±0.14	2.81 ±0.09	2.80 ±0.19
Triglycerides (g/dL)	34.88 ±39.69	25.32 ±11.83	33.47 ±13.71	49.84 ±19.84	43.43 ±19.56	36.82 ±18.36	36.51 ±18.57	43.54 ±32.40	30.61 ±10.9	30.42 ±9.31
Cholesterol (mg/dL)	49.68 ±34.65	37.50 ±12.39	46.00 ±30.92	50.44 ±22.60	73.59 ±18.84	75.94 ±25.29	90.98 ±28.95	86.84 ±25.03	106.18 ±29.76	122.34 ±24.24
Hp (mg/dL)	6.24 ±6.74	7.70 ±7.74	4.57 ±2.62	5.87 ±9.42	3.99 ±3.37	3.11 ±1.47	2.75 ±0.70	2.94 ±1.16	11.64 ±13.14	7.29 ±6.68
Serum IgG (mg/dL)	0 ± 0	0±0	37.17±15.73	34.48±16.76	32.56 ±15.48	29.37±19.90	28.32±17.77	21.43±14.40	19.12±17.51	14.73±9.57

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 25.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by albumin group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	67.86 ±25.43	55.00 ± 16.26	53.86 ± 13.73	56.86 ± 15.47	53.86 ± 14.17	42.29 ± 8.41	40.57 ± 16.14	38.71 ±17.32	52.57±13.37	48.29±12.98
Heart Rate (bpm)	147.14±17.06	134.29±24.99	136.39 ±23.03	125.71±14.61	117.14±21.36	114.43±21.37	98.57 ±23.25	103.71±31.86	130.00±17.94	119.29±16.18
RT (◦C)	38.56 ±0.35	38.31 ±0.45	38.71 ±0.22	38.88 ±0.20	38.90 ±0.39	38.63 ±0.27	38.71 ±0.39	38.97 ±0.42	38.96 ±0.37	38.86 ±0.27
Thoracic perimeter (cm)	76,07±3.47	77,71±3.38	76,50±4.01	77,46±3.50	80,93±3.32	80,79±2.49	81,86±2.88	83,43±2.53	89,43±2.53	90,21±3.33
Height at the withers (cm)	73.71 ±4.47	72.79 ±3.11	74.55 ±4.70	75.23 ±3.69	75.71 ±5.24	76.14 ±4.14	76.75 ±5.65	75.68 ±3.91	79.32 ±4.90	80.25 ±3.16
Rump width (cm)	22.04 ±1.39	23.07 ±2.38	22.45 ±1.30	24.19 ±1.90	24.04 ±2.96	23.93 ±2.37	23.29 ±2.27	23.64 ±2.76	25.21 ±2.15	24.89 ±1.58
NEFA (mmol/L)	0.32 ±0.26	0.55 ±0.36	0.26 ±0.25	0.27 ±0.20	0.37 ±0.38	0.29 ±0.14	0.17 ±0.04	0.39 ±0.64	0.19 ±0.07	0.60 ±0.94
BHB (mmol/L)	0.06 ±0.10	0.01 ±0.01	0.02 ±0.04	0.03 ±0.04	0.04 ±0.05	0.07 ±0.14	0.01 ±0.02	0.03 ±0.04	0.06 ±0.04	0.04 ±0.03
Glucose (mg/dL)	79.00 ±34.01	61.57 ±17.96	128.63±32.53	138.5 ±29.11	109.71±13.62	101.71±22.35	99.57 ±17.70	91.93 ±18.20	113.07±15.17	113.50±11.87
Iron (mg/dL)	20.74 ±15.47	20.45 ±8.91	25.96 ±11.04	31.24 ±12.64	20.93 ±15.16	20.01 ±14.85	27.16 ±17.91	31.66 ±16.40	24.53 ±20.66	23.76 ±15.56
Total Protein (µmol/L)	3.93 ±1.20	3.66 ±0.94	7.26 ±0.99	7.04 ±1.19	6.96 ±1.25	6.78 ±0.80	6.44 ±0.54	5.98 ±0.61	6.07 ±0.42	5.86 ±0.71
Albumin (g/dL)	2.31 ±0.53	2.25 ±0.53	2.05 ±0.35	2.17 ±0.29	2.33 ±0.30	2.40 ±0.26	2.45 ±0.17	2.50 ±0.12	2.81 ±0.09	2.81 ±0.19
Triglycerides (g/dL)	36.30 ±39.75	23.90 ±10.05	36.41 ±16.01	46.89 ±20.25	38.85 ±21.93	41.40 ±16.07	39.62 ±36.03	40.43 ±11.07	29.20 ±11.09	31.83 ±8.90
Cholesterol (mg/dL)	51.00 ±34.55	36.18 ±11.03	39.69 ±13.23	56.76 ±33.85	68.70 ±21.75	80.83 ±21.10	87.99 ±32.01	89.84 ±21.16	107.39 ±26.4	121.13±28.51
Hp (mg/dL)	6.11 ±6.68	7.83 ±7.76	3.65 ±2.82	6.95 ±9.70	3.92 ±3.40	3.17 ±1.43	2.74 ±0.71	2.95 ±1.16	10.27 ±12.91	8.65 ±7.70
Serum IgG (mg/dL)	0.00 ± 0,00	0.00 ± 0.00	40.19± 17.95	31.45 ± 13.00	37.88 ± 18.64	24.06± 13.81	30.59 ± 17.22	19.16± 13.48	19.14±17.49	14.70 ± 9.60

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 26.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by triglyceride group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	66.00±22.44	56.86±21.25	57.14±12.79	53.57±16.19	51.57±13.50	44.57±11.62	39.71±13.81	39.57±19.28	51.00±14.65	49.86±11.91
Heart Rate (bpm)	144.00±16.23	137.43±26.79	131.82±21.45	130.29±18.54	114.14±15.66	117.43±25.81	102.00±26.84	100.29±29.12	127.43±16.44	121.86±18.93
RT (◦C)	38.53±0.36	38.34±0.45	38.77±0.20	38.81±0.25	38.89±0.22	38.64±0.43	38.79±0.37	38.89±0.47	38.91±0.24	38.91±0.40
Thoracic perimeter (cm)	75,64±3,93	78,14±2,48	77,00±4,74	77,08±2,81	80,86±3,25	80,86±2,57	82,29±2,87	83,00±2,75	89,93±3,22	89,71±2,73
Height at the withers (cm)	74.00±2.97	72.50±4.48	74.25±5.02	75.46±3.29	75.29±4.64	76.57±4.72	76.36±5.06	76.07±4.70	79.57±2.96	80.00±5.06
Rump width (cm)	21.96±1.62	23.14±2.19	23.30±1.78	23.54±1.97	23.93±2.72	24.04±2.65	23.14±2.27	23.79±2.74	24.64±1.88	25.46±1.82
NEFA (mmol/L)	0.37±0.29	0.50±0.37	0.30±0.29	0.23±0.13	0.29±0.10	0.37±0.40	0.17±0.05	0.38±0.64	0.19±0.06	0.59±0.95
BHB (mmol/L)	0.07±0.10	0.01±0.01	0.04±0.04	0.02±0.03	0.08±0.14	0.04±0.05	0.01±0.01	0.04±0.04	0.06±0.04	0.03±0.03
Glucose (mg/dL)	75.54±32.84	64.79±22.19	128.25±31.91	138.88±29.63	105.71±15.47	105.71±21.91	102.79±14.41	88.71±19.02	111.21±15.31	115.36±11.28
Iron (mg/dL)	20.86±15.83	20.34±8.23	24.74±9.91	32.46±12.90	22.11±15.34	18.83±14.48	30.50±17.27	28.32±17.33	27.87±22.07	20.41±12.34
Total Protein (µmol/L)	4.10±1.08	3.49±0.99	7.18±1.12	7.12±1.08	7.09±0.78	6.64±1.22	6.44±0.50	5.97±0.64	6.19±0.57	5.74±0.52
Albumin (g/dL)	2.40±0.46	2.16±0.57	2.07±0.34	2.14±0.32	2.36±0.25	2.36±0.31	2.52±0.15	2.43±0.13	2.87±0.14	2.74±0.12
Triglycerides (g/dL)	35.74±39.76	24.46±10.69	37.34±18.30	45.97±18.72	39.07±21.23	41.18±17.03	34.40±16.79	45.65±32.73	32.42±11.95	28.61±7.43
Cholesterol (mg/dL)	50.41±34.46	36.77±12.10	40.72±17.91	55.72±32.18	70.86±23.09	78.67±20.77	89.51±31.69	88.31±21.66	119.19±34.17	109.32±19.83
Hp (mg/dL)	7.15±7.10	6.79±7.48	3.95±2.68	6.65±9.85	3.38±1.79	3.72±3.26	3.11±1.07	2.58±0.75	11.30±12.94	7.63±7.26
Serum IgG (mg/dL)	0.00±0.00	0.00±0.00	35.22±14.46	36.43±17.96	34.40±19.76	27.54±15.01	27.97±18.66	21.79±13.40	19.33±17.85	14.51±8.81

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 27.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by cholesterol group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	64.77±25.79	58.53±18.42	57.54±11.49	53.47±16.74	53.38±15.00	43.47±8.80	42.77±15.78	36.93±17.09	52.62±13.91	48.53±12.55
Heart Rate (bpm)	142.46±16.13	139.20±26.54	130.58±20.34	131.47±19.82	115.08±14.53	116.40±25.88	100.62±23.43	101.60±31.42	128.92±19.88	120.93±15.13
RT (◦C)	38.54±0.39	38.35±0.42	38.72±0.22	38.85±0.21	38.83±0.34	38.71±0.37	38.72±0.41	38.94±0.41	38.94±0.38	38.88±0.27
Thoracic perimeter(cm)	75,15±3.29	78,40±3.02	75,67±4.27	77,93±3.20	80,69±3.30	81,00±2.64	81,69±2.87	83,47±2.49	89,69±2,56	89,93±3,23
Height at the withers (cm)	72.96±4.42	73.50±3.33	74.06±4.70	75.50±3.68	74.62±5.23	77.07±3.89	76.12±6.06	76.30±3.59	78.35±4.81	81.03±2.92
Rump width (cm)	21.62±1.50	23.37±2.03	22.39±1.36	24.11±1.85	23.96±2.76	24.00±2.61	22.65±2.18	24.17±2.59	25.04±1.93	25.07±1.87
NEFA (mmol/L)	0.32±0.25	0.53±0.36	0.27±0.26	0.26±0.19	0.27±0.10	0.38±0.38	0.18±0.03	0.36±0.62	0.21±0.06	0.56±0.92
BHB (mmol/L)	0.07±0.10	0.01±0.01	0.03±0.04	0.02±0.04	0.04±0.05	0.07±0.13	0.01±0.02	0.03±0.04	0.06±0.05	0.04±0.03
Glucose (mg/dL)	78.33±34.50	63.27±19.84	128.14±35.11	137.78±27.31	109.69±14.30	102.27±21.57	100.92±17.44	91.27±17.90	111.62±15.86	114.73±11.14
Iron (mg/dL)	21.48±16.17	19.83±8.39	26.54±10.61	30.39±13.10	21.34±15.41	19.71±14.62	28.15±16.95	30.51±17.58	25.49±21.21	22.97±15.25
Total Protein (µmol/L)	4.07±1.09	3.55±1.02	7.23±1.20	7.08±1.01	7.05±0.74	6.71±1.24	6.37±0.53	6.07±0.66	6.02±0.46	5.91±0.68
Albumin (g/dL)	2.39±0.46	2.18±0.57	2.07±0.32	2.13±0.34	2.33±0.25	2.39±0.30	2.49±0.16	2.46±0.13	2.83±0.10	2.79±0.17
Triglycerides (g/dL)	26.85±15.43	32.91±37.62	38.15±15.07	44.69±21.39	38.99±21.97	41.11±16.54	42.72±36.31	37.69±13.31	31.58±9.72	29.59±10.40
Cholesterol (mg/dL)	43.57±17.62	43.61±32.64	38.99±11.87	56.22±33.22	71.10±23.21	77.94±21.00	87.43±32.22	90.19±21.80	112.08±33.06	116.14±23.53
Hp (mg/dL)	7.51±7.25	6.51±7.30	3.60±3.05	6.62±9.13	3.29±1.87	3.78±3.13	2.79±0.73	2.89±1.13	10.71±13.34	8.38±7.49
Serum IgG (mg/dL)	0.00±0.00	0.00±0.00	36.05±15.28	35.63±17.15	36.54±19.48	26.14±14.70	27.30±14.42	22.77±17.91	19.91±17.99	14.34±9.31

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 28.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by haptoglobin group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	59.54±23.62	63.07±21.07	54.46±17.17	56.13±12.15	47.85±10.21	48.27±15.15	44.46±19.87	35.47±11.99	52.92±14.89	48.27±11.44
Heart Rate (bpm)	139.38±23.09	141.87±21.74	129.85±14.75	132.10±23.64	115.85±22.16	115.73±20.76	110.46±36.57	93.07±12.69	119.23±15.27	129.33±18.68
RT (◦C)	38.46±0.48	38.41±0.36	38.83±0.18	38.76±0.26	38.74±0.35	38.79±0.38	38.95±0.52	38.74±0.30	38.83±0.25	38.97±0.37
Thoracic perimeter (cm)	77,69±2,87	76,20±3,97	77,70±2,45	76,54±4,40	81,54±2,93	80,27±2,82	83,08±2,63	82,27±3,05	90,08±2,84	89,60±3,20
Height at the withers (cm)	73.58±3.29	72.97±4.30	76.30±3.90	73.88±4.03	76.04±3.94	75.83±5.31	76.42±4.23	76.03±5.38	80.81±3.00	78.90±4.74
Rump width (cm)	22.81±1.60	22.33±2.30	24.00±2.07	23.00±1.62	24.08±2.53	23.90±2.80	23.69±2.00	23.27±2.90	25.12±1.29	25.00±2.29
NEFA (mmol/L)	0.50±0.41	0.38±0.24	0.24±0.13	0.28±0.28	0.41±0.40	0.26±0.10	0.21±0.10	0.34±0.62	0.61±0.98	0.21±0.08
BHB (mmol/L)	0.03±0.05	0.04±0.09	0.02±0.04	0.03±0.04	0.04±0.05	0.07±0.13	0.02±0.03	0.02±0.03	0.06±0.05	0.04±0.03
Glucose (mg/dL)	63.85±23.21	75.64±31.28	139.75±38.13	127.38±20.49	101.31±19.43	109.53±17.63	93.92±20.56	97.33±16.11	114.23±15.73	112.47±11.44
Iron (mg/dL)	20.25±8.41	20.90±15.33	28.74±14.26	28.48±10.06	21.41±13.90	19.65±15.85	30.07±20.03	28.84±14.61	24.68±18.03	23.67±18.50
Total Protein (µmol/L)	3.90±1.23	3.70±0.94	7.07±0.94	7.21±1.22	6.41±1.17	7.27±0.72	6.13±0.76	6.27±0.47	5.81±0.56	6.10±0.59
Albumin (g/dL)	2.30±0.55	2.26±0.51	2.21±0.34	2.02±0.30	2.36±0.32	2.36±0.24	2.51±0.13	2.45±0.15	2.79±0.12	2.82±0.16
Triglycerides (g/dL)	23.15±12.02	36.12±37.82	41.07±17.23	42.16±20.46	44.90±24.78	35.99±11.13	37.12±18.17	42.55±31.97	32.70±8.86	28.62±10.75
Cholesterol (mg/dL)	39.22±17.37	47.37±32.25	55.32±33.12	42.07±18.56	79.44±25.27	70.71±18.46	93.19±26.76	85.20±26.90	113.29±30.60	115.09±26.35
Hp (mg/dL)	6.87±7.83	7.06±6.80	7.74±9.42	2.85±2.42	3.50±2.07	3.59±3.04	2.82±1.15	2.86±0.76	9.81±9.50	9.17±11.56
Serum IgG (mg/dL)	0.00±0.00	0.00±0.00	34.56±19.59	36.92±12.75	28.49±18.64	33.12±16.93	20.90±17.61	28.32±14.70	18.87±20.16	15.24±4.93

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 29.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by iron group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	63.43±22.00	59.43±22.52	53.86±15.10	56.86±14.14	51.14±14.31	45.00±10.89	45.86±20.76	33.43±6.95	52.29±14.16	48.57±12.21
Heart Rate (bpm)	147.14±24.37	134.29±17.94	132.00±17.68	130.11±22.15	118.43±20.52	113.14±21.93	107.43±35.91	94.86±13.98	121.86±16.58	127.43±18.82
RT (◦C)	38.34±0.50	38.54±0.28	38.76±0.20	38.82±0.24	38.75±0.44	38.78±0.27	39.05±0.44	38.63±0.28	38.86±0.26	38.96±0.38
Thoracic perimeter(cm)	78,50±2,79	75,29±3,41	77,62±3,52	76,30±3,92	80,93±2,59	80,79±3,24	83,36±2,44	81,93±3,00	90,43±3,34	89,21±2,42
Height at the withers (cm)	73.57±3.27	72.93±4.38	75.15±3.92	74.65±4.45	77.46±4.37	74.39±4.53	76.96±4.16	75.46±5.40	81.18±3.07	78.39±4.56
Rump width (cm)	22.75±1.60	22.36±2.35	24.15±1.86	22.50±1.43	24.96±2.27	23.00±2.68	24.39±2.59	22.54±2.07	25.68±2.04	24.43±1.48
NEFA (mmol/L)	0.54±0.38	0.33±0.24	0.33±0.18	0.20±0.24	0.39±0.38	0.27±0.14	0.20±0.09	0.36±0.64	0.39±0.72	0.40±0.69
BHB (mmol/L)	0.02±0.05	0.05±0.09	0.03±0.04	0.03±0.04	0.06±0.14	0.05±0.05	0.02±0.03	0.02±0.03	0.04±0.03	0.05±0.04
Glucose (mg/dL)	57.07±20.23	83.85±28.82	135.80±29.83	129.83±33.43	103.79±14.61	107.64±22.31	93.21±17.38	98.29±18.97	116.21±12.39	110.36±14.11
Iron (mg/dL)	19.37±8.40	21.82±15.65	28.82±13.47	28.38±10.73	20.84±15.79	20.10±14.19	33.01±19.44	25.81±13.96	25.92±16.76	22.36±19.53
Total Protein (µmol/L)	3.69±0.98	3.89±1.18	7.34±0.99	6.96±1.17	6.59±1.19	7.15±0.80	6.16±0.70	6.26±0.53	6.01±0.73	5.92±0.41
Albumin (g/dL)	2.25±0.52	2.31±0.54	2.22±0.30	1.99±0.32	2.36±0.31	2.36±0.24	2.47±0.15	2.48±0.14	2.80±0.17	2.81±0.12
Triglycerides (g/dL)	21.44±10.11	38.76±38.73	50.14±18.17	33.16±15.50	46.47±23.32	33.78±10.60	48.60±33.14	31.45±12.83	30.40±10.72	30.63±9.53
Cholesterol (mg/dL)	34.68±10.07	52.50±34.08	59.02±31.11	37.42±16.08	75.39±22.58	74.14±22.06	95.79±27.18	82.03±25.17	114.51±24.80	114.01±31.60
Hp (mg/dL)	8.04±9.50	5.90±3.71	3.25±1.63	8.72±11.16	3.54±3.12	3.56±2.05	2.95±1.16	2.74±0.69	11.46±12.17	7.47±8.41
Serum IgG (mg/dL)	0.00±0.00	0.00±0.00	34.91±16.89	36.73±15.65	25.43±14.01	36.51±19.46	19.67±12.53	30.08±18.26	12.53±9.21	21.32±16.79

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 30.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by thoracic perimeter group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	57.71±21.99	65.14±22.06	56.57±13.93	54.14±15.34	45.00±11.11	51.14±14.14	34.14±10.36	45.14±19.75	48.29±9.95	52.57±15.76
Heart Rate (bpm)	128.29±15.00	153.14±21.13	128.29±15.65	133.82±23.32	113.71±23.93	117.86±18.30	91.71±10.69	110.57±35.56	127.86±16.50	121.43±18.74
RT (◦C)	38.46±0.47	38.41±0.37	38.79±0.17	38.80±0.27	38.69±0.36	38.84±0.35	38.74±0.48	38.94±0.34	38.99±0.39	38.83±0.23
Thoracic perimeter(cm)	76,36±3,84	77,43±3,11	76,78±4,29	77,21±3,38	81,71±3,20	80,00±2,32	83,07±3,02	82,21±2,55	90,00±2,42	89,64±3,46
Height at the withers (cm)	72.39±4.62	74.11±2.68	75.11±4.88	74.82±3.65	74.11±4.85	77.75±3.75	74.14±5.45	78.29±2.97	77.93±4.48	81.64±2.64
Rump width (cm)	22.43±2.25	22.68±1.75	23.67±2.22	23.29±1.65	22.75±2.16	25.21±2.55	22.00±2.03	24.93±2.03	24.36±1.13	25.75±2.21
NEFA (mmol/L)	0.36±0.28	0.52±0.37	0.22±0.25	0.31±0.19	0.33±0.39	0.33±0.12	0.38±0.64	0.17±0.06	0.39±0.71	0.40±0.69
BHB (mmol/L)	0.02±0.03	0.05±0.10	0.04±0.04	0.02±0.04	0.04±0.04	0.08±0.14	0.04±0.04	0.01±0.02	0.04±0.05	0.05±0.02
Glucose (mg/dL)	81.64±24.01	57.38±26.86	130.00±53.56	134.38±25.73	115.21±16.62	96.21±15.77	97.50±23.08	94.00±11.69	108.71±14.10	117.86±11.29
Iron (mg/dL)	22.52±14.45	18.67±10.10	28.84±10.32	28.36±13.79	20.74±14.33	20.19±15.66	25.00±14.20	33.82±18.90	23.66±20.00	24.63±16.39
Total Protein (µmol/L)	3.96±1.00	3.62±1.14	6.73±1.14	7.57±0.86	6.59±1.23	7.14±0.74	6.14±0.65	6.27±0.58	5.74±0.51	6.19±0.58
Albumin (g/dL)	2.36±0.41	2.20±0.62	1.90±0.26	2.31±0.25	2.27±0.34	2.46±0.15	2.47±0.12	2.48±0.17	2.79±0.11	2.83±0.17
Triglycerides (g/dL)	28.22±13.53	31.98±39.67	32.59±12.40	50.71±19.87	37.24±14.67	43.01±22.58	36.99±15.01	43.06±34.29	31.34±8.89	29.69±11.20
Cholesterol (mg/dL)	43.26±16.71	43.92±33.97	33.25±8.70	63.19±30.32	81.86±17.68	67.66±24.01	80.42±21.96	97.40±28.93	110.48±32.99	118.04±22.24
Hp (mg/dL)	6.34±3.48	7.60±9.67	5.02±2.45	5.36±7.89	3.62±1.87	3.47±3.23	2.54±0.52	3.15±1.17	5.89±7.73	13.04±11.84
Serum IgG (mg/dL)	0.00±0.00	0.00±0.00	38.08±17.44	33.57±14.73	37.24±19.63	24.70±13.08	26.04±15.97	23.71±17.05	20.85±17.92	13.00±7.36

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 31.- Mean and standard deviation of calf parameters (vital functions, performance, metabolism and inflammation markers) divided by body condition score group (high and low)

Parameters	D1		D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High	Low	High
Respiratory rate (bpm)	60.93±26.12	62.00±16.93	54.93±14.69	55.85±14.71	43.47±10.24	53.38±13.89	39.33±16.73	40.00±16.81	48.80±14.26	52.31±11.94
Heart Rate (bpm)	127.73±17.85	155.69±16.20	123.30±20.25	140.00±15.23	112.80±18.28	119.23±24.08	97.33±23.74	105.54±31.69	122.53±17.54	127.08±18.12
RT (◦C)	38.45±0.48	38.42±0.33	38.82±0.17	38.76±0.27	38.69±0.35	38.85±0.36	38.81±0.52	38.87±0.29	38.95±0.37	38.85±0.26
Thoracic perimeter (cm)	76,33±3,42	77,54±3,55	77,00±3,79	77,09±3,73	81,33±2,55	80,31±3,22	83,07±2,74	82,15±2,85	90,33±2,29	89,23±3,54
Height at the withers (cm)	72.40±4.38	74.23±2.87	74.58±3.96	75.32±4.34	74.27±4.43	77.85±4.25	74.67±4.91	78.00±4.15	78.47±4.68	81.31±2.65
Rump width (cm)	22.07±2.44	23.12±1.12	23.17±2.11	23.73±1.57	23.40±2.78	24.65±2.38	22.17±1.98	24.96±2.19	24.30±1.25	25.92±2.11
NEFA (mmol/L)	0.41±0.28	0.47±0.39	0.24±0.24	0.29±0.20	0.29±0.14	0.38±0.39	0.38±0.62	0.16±0.06	0.54±0.92	0.22±0.13
BHB (mmol/L)	0.03±0.06	0.04±0.09	0.03±0.04	0.02±0.04	0.05±0.05	0.07±0.14	0.04±0.04	0.01±0.01	0.05±0.05	0.05±0.02
Glucose (mg/dL)	77.57±24.82	61.77±29.48	113.50±19.35	145.60±29.80	110.73±20.17	99.92±15.39	94.60±23.86	97.08±8.02	113.27±14.49	113.31±12.53
Iron (mg/dL)	20.02±15.52	21.26±7.96	30.64±8.99	26.25±14.69	21.77±17.28	18.96±11.63	31.34±18.18	27.18±15.98	24.00±19.16	24.31±17.22
Total Protein (µmol/L)	3.43±0.96	4.21±1.07	6.84±1.09	7.51±0.99	6.86±0.73	6.88±1.33	6.05±0.55	6.39±0.65	5.75±0.51	6.22±0.58
Albumin (g/dL)	2.14±0.56	2.44±0.44	2.03±0.31	2.20±0.33	2.36±0.25	2.36±0.32	2.49±0.12	2.46±0.17	2.81±0.13	2.80±0.16
Triglycerides (g/dL)	24.68±13.80	36.35±40.12	35.72±12.53	48.50±22.54	43.79±20.21	35.90±17.09	40.56±16.79	39.41±34.79	33.45±8.60	27.12±10.65
Cholesterol (mg/dL)	37.79±16.38	50.28±33.91	45.20±28.63	51.71±24.89	79.01±18.59	69.86±25.07	81.37±19.69	97.61±31.49	112.13±32.74	116.72±22.04
Hp (mg/dL)	6.00±3.41	8.09±9.96	9.01±10.82	3.07±2.13	3.55±1.97	3.55±3.25	2.58±0.73	3.16±1.10	6.13±6.18	13.31±13.12
Serum IgG (mg/dL)	0.00±0.00	0.00±0.00	34.79±15.73	37.01±16.88	36.26±20.37	24.86±11.61	25.86±15.19	23.74±17.95	16.81±11.91	17.05±16.65

RT = rectal temperature; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; Hp = haptoglobin; BCS = body condition score.
Source: SANTOS (2020).

Table 32.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by NEFA group (high and low)

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	60.81±40.7	93.31±46.83	38.67±31.42	52.65±36.56	25.04±19.07	32.26±16.11	51.53±33.17	65.94±48.95
Cells stimulated by PMA 10^{-7}M (AFU)	326.38±236.43	399.27±182.52	129.17±116.19	206.18±154.07	131.53±129.47	202.5±131.23	169.74±144.06	267.08±146.72
S. aureus pure (AFU)	232.61±138.88	227.21±62.77	171.18±40.94	145.74±55.11	158.06±44.5	146.64±55.2	179.64±54.71	162.16±48.6
E. coli pure (AFU)	184.38±155.15	224.23±109.68	148.09±55.05	110.48±41.61	119.4±51.18	95.91±44.58	130.06±48.35	120.7±41.34
S. hyicus pure (AFU)	223.7±132.03	273.05±95.74	177.8745.86	142.52±53.93	160.52±53.34	147.64±65.5	181.71±59.2	167.59±56.95
Cells stimulated by PMA 10-7M (RR)	5.69±3.56	6.10±3.47	3.72±3.18	3.73±1.83	5.04±4.40	5.23±3.34	3.05±1.85	4.16±2.03
S. aureus pure (RR*)*	4.43±2.01	3.75±1.83	6.35±2.82	4.32±2.37	7.29±1.83	6.82±4.02	3.72±0.96	4.26±2.69
E. coli pure (RR)	3.25±1.47	2.97±1.14	5.11±1.66	3.42±2.21	5.10±1.03	5.13±3.97	2.57±0.68	3.07±1.68
S.hyicus pure (RR*)*	4.71±2.11	3.60±1.58	6.75±3.15	4.31±2.46	7.90±1.90	6.26±3.86	3.76±0.91	4.20±2.64

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 33.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by BHB group (high and low)

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	73.69±44.7	66.51±45.17	48.85±36.64	36.48±28.7	35.26±22.29	18.95±7.36	61.57±40.63	49.73±35.54
Cells stimulated by PMA 10^{-7}M (AFU)	378.76±235.26	315.64±210.9	189.05±139.19	113.29±112.49	202.31±132.61	101.3±113.61	265.1±156.99	126.94±103.49
S. aureus puro (AFU)	231.75±115.38	230.39±130.43	166.15±38.02	161.68±53.97	159.91±44.09	149.69±50.91	181.3±60.26	167.99±45.32
E. coli pure (AFU)	196.16±129.31	195.37±160.12	141.27±54.1	133.41±54.98	115.75±44.66	109.63±55.92	134.97±51.04	119.81±40.59
S.hyicus pure (AFU)	239.02±105.55	236.58±142.62	162.39±45.74	173.15±55.09	169.96±52.85	143.72±58.11	185.97±62.13	169.38±54.29
Cells stimulated by PMA 10-7M (RR)	5.98±3.59	5.81±3.45	4.03±2.18	3.43±2.92	5.71±3.26	4.56±4.38	4.58±1.86	2.60±1.63
S.aureus pure (RR*)*	3.82±1.97	4.35±1.90	4.76±2.50	5.90±2.97	5.87±3.21	8.23±2.52	3.68±1.7	4.30±2.24
E.coli pure (RR)	2.89±1.15	3.33±1.44	3.97±2.41	4.56±1.79	4.32±3.05	5.91±2.48	2.74±1.39	2.90±1.21
S.hyicus pure (RR*)*	3.92±2.01	4.39±1.86	4.60±2.46	6.45±3.36	6.40±4.03	7.76±1.66	3.71±1.58	4.25±2.29

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 34.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by glucose group (high and low)

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	83.21±50.99	56.98±33.1	50.93±41.9	34.39±18.59	28.89±21.57	25.31±14.91	64.56±49.61	46.73±18.91
Cells stimulated by PMA 10^{-7}M (AFU)	435.97±271.35	258.43±106.61	192.73±143.95	109.62±103.34	171.12±121.39	132.5±142.99	252.59±162.26	148.43±120.43
S.aureus puro (AFU)	286.61±144.92	175.53±51.75	164.86±45.99	162.97±47.46	142.43±27.82	167.17±59.06	170.75±60.58	178.54±45.58
E.coli pure (AFU)	242.36±177.05	149.16±79.59	131.34±59.59	143.35±48.52	93.08±32.13	132.3±57.28	135.58±57.27	119.19±30.85
S.hyicus pure (AFU)	279.7±152.22	195.9±67.32	165.72±46.12	169.82±55.26	139.32±32.29	174.35±69.55	173.4±66.2	181.95±50.34
Cells stimulated by PMA 10-7M (RR)	6.16±3.73	5.63±3.28	4.07±2.15	3.38±2.93	6.08±4.34	4.19±3.13	4.23±2.22	3.07±1.64
S.aureus pure (RR)	4.20±2.02	3.97±1.88	5.18±3.39	5.49±2.05	6.45±3.14	7.66±3.01	3.65±1.89	4.33±2.13
E.coli pure (RR)	3.14±1.36	3.08±1.28	3.86±2.54	4.67±1.54	4.34±2.99	5.89±2.57	2.80±1.44	2.84±1.16
S.hyicus pure (RR)	3.98±1.97	4.33±1.92	5.38±3.89	5.67±1.98	6.44±3.66	7.73±2.39	3.60±1.72	4.36±2.15

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 35.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by total protein group (high and low)

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	168.38±178.90	152.53±85.33	148.82±136.31	102.28±52.67	75.56±45.35	105.49±125.27	124.19±58.30	183.27±94.25
Cells stimulated by PMA 10^{-7}M (AFU)	1011.10±1034.28	717.72±560.03	428.36±361.90	371.46±220.62	356.26±298.68	500.95±435.01	548.90±380.04	638.80±397.42
S.aureus puro (AFU)	573.77±552.65	485.43±295.14	448.12±221.76	567.87±304.62	407.90±158.71	720.12±1028.67	439.41±182.34	500.52±143.87
E.coli pure (AFU)	532.80±685.18	364.93±217.85	381.33±222.34	483.64±327.16	270.35±103.92	486.88±567.64	317.87±103.32	385.29±127.01
S.hyicus pure (AFU)	633.81±832.34	447.60±192.31	455.59±218.65	579.43±328.89	395.15±149.60	684.34±829.40	439.73±200.44	521.19±167.50
Cells stimulated by PMA 10-7M (RR)	6.17±2.86	5.62±4.06	3.29±2.00	4.16±3.01	4.58±3.50	5.69±4.20	3.89±2.24	3.35±1.74
S.aureus pure (RR)	4.02±1.51	4.15±2.31	4.55±2.68	6.12±2.70	6.78±3.09	7.32±3.15	3.93±1.10	4.05±2.67
E.coli pure (RR)	3.21±1.00	3.01±1.57	3.64±1.93	4.89±2.16	4.68±2.63	5.56±3.08	2.77±0.72	2.87±1.70
S.hyicus pure (RR)	4.32±1.78	3.99±2.10	4.81±2.85	6.24±3.15	6.68±2.97	7.48±3.28	3.86±1.06	4.11±2.60

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 36.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by albumin group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	97.09±49.33	228.44±166.28	161.90±132.98	90.13±45.32	68.67±41.67	115.21±128.31	148.42±78.16	164.41±92.12
Cells stimulated by PMA 10^{-7}M (AFU)	680.45±425.39	1051.47±1088.67	426.84±341.34	372.88±249.37	328.53±248.25	541.94±462.19	617.89±340.30	577.98±437.55
S.aureus puro (AFU)	405.62±206.74	660.24±566.98	496.33±245.62	523.11±299.27	385.91±136.19	767.82±1060.31	497.31±183.22	448.18±140.82
E.coli pure (AFU)	317.27±218.80	584.43±666.72	435.48±250.27	433.35±316.66	258.49±88.40	516.30±583.43	366.00±124.71	343.30±118.31
S.hyicus pure (AFU)	408.47±199.34	676.20±817.79	488.53±237.29	548.84±326.18	386.17±132.14	716.25±857.02	491.56±201.01	476.77±173.53
Cells stimulated by PMA 10-7M (RR)	7.24±3.75	4.55±2.62	3.18±2.02	4.28±2.96	4.77±3.86	5.50±3.92	3.92±2.15	3.31±1.84
S.aureus pure (RR)	4.63±1.95	3.54±1.79	4.36±2.36	6.31±2.85	7.08±3.29	7.02±2.97	3.96±1.25	4.02±2.60
E.coli pure (RR)	3.42±1.33	2.80±1.24	3.53±1.79	5.00±2.20	4.86±2.66	5.37±3.10	2.85±0.86	2.79±1.64
S.hyicus pure (RR)	4.82±1.82	3.49±1.83	4.44±2.57	6.62±3.16	7.05±3.28	7.11±3.03	3.88±1.24	4.09±2.51

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 37.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by triglyceride group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	130.32±90.23	187.66±166.07	164.42±130.14	87.79±48.66	67.53±42.37	116.44±127.56	152.99±84.59	158.79±86.57
Cells stimulated by PMA 10^{-7}M (AFU)	744.00±393.02	964.27±1082.66	443.45±340.27	357.45±246.04	356.58±272.86	511.73±460.21	634.60±283.82	572.55±448.74
S.aureus puro (AFU)	417.66±205.73	629.53±555.87	446.97±205.66	568.94±314.43	370.65±86.47	784.26±1059.29	477.36±189.68	469.55±146.95
E.coli pure (AFU)	346.09±222.64	537.27±654.23	383.44±231.40	481.68±321.96	249.51±68.01	525.98±581.59	349.61±114.92	358.13±126.58
S.hyicus pure (AFU)	433.30±199.94	632.69±795.45	438.69±213.48	595.12±324.81	358.41±95.53	746.15±848.68	479.72±193.90	487.24±183.78
Cells stimulated by PMA 10-7M (RR)	6.61±3.32	5.18±3.56	3.20±2.02	4.25±2.96	5.05±3.85	5.22±3.97	3.96±2.24	3.32±1.77
S.aureus pure (RR)	4.27±1.92	3.90±1.97	3.74±1.83	6.92±2.64	7.23±3.67	6.87±2.47	4.39±2.27	3.59±1.69
E.coli pure (RR)	3.25±1.48	2.97±1.13	2.93±1.28	5.60±1.93	5.07±3.18	5.16±2.59	3.00±1.28	2.65±1.31
S.hyicus pure (RR)	4.44±1.82	3.87±2.03	3.72±1.75	7.34±3.00	6.84±3.18	7.33±3.12	4.39±2.31	3.58±1.47

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 38.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by cholesterol group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	102.96±46.77	212.91±168.70	173.35±132.04	85.75±46.82	69.65±42.97	110.98±124.36	147.71±87.28	162.44±84.29
Cells stimulated by PMA 10^{-7}M (AFU)	708.51±418.39	997.04±1065.47	458.44±336.99	351.19±253.59	326.19±253.28	528.87±449.35	615.10±373.13	586.05±404.46
S.aureus puro (AFU)	418.81±204.91	628.47±556.59	520.46±240.74	502.02±299.21	396.31±145.15	730.88±1026.88	482.17±198.10	466.22±139.04
E.coli pure (AFU)	336.54±219.75	546.09±652.20	445.37±240.34	425.58±318.16	265.48±99.48	491.40±566.47	357.24±134.72	352.85±112.92
S.hyicus pure (AFU)	419.55±203.52	645.38±790.93	514.10±229.53	524.37±327.66	374.59±139.88	703.43±823.29	466.05±207.26	496.71±172.66
Cells stimulated by PMA 10-7M (RR)	6.94±3.19	4.99±3.52	3.25±2.06	4.14±2.91	4.72±3.95	5.50±3.84	3.70±2.37	3.57±1.72
S.aureus pure (RR)	4.53±1.65	3.70±2.10	4.14±2.30	6.37±2.76	7.24±3.60	6.89±2.67	4.42±2.29	3.62±1.71
E.coli pure (RR)	3.48±1.31	2.78±1.24	3.29±1.77	5.11±2.06	5.05±3.13	5.18±2.69	3.03±1.27	2.64±1.31
S.hyicus pure (RR)	4.64±1.63	3.74±2.09	4.28±2.63	6.61±3.03	6.60±2.61	7.50±3.50	4.36±2.39	3.66±1.48

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 39.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by haptoglobin group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	197.96±177.02	125.22±72.64	127.08±84.18	122.46±120.40	76.00±44.44	103.14±121.92	125.45±62.01	182.22±93.01
Cells stimulated by PMA 10^{-7}M (AFU)	1124.32±1066.02	613.21±401.78	427.11±252.54	372.62±332.92	458.90±392.60	409.19±374.13	588.96±404.61	605.41±382.16
S.aureus puro (AFU)	640.43±571.21	423.90±218.33	532.25±317.56	489.76±227.16	402.08±185.63	703.96±990.27	439.59±142.72	500.38±176.79
E.coli pure (AFU)	545.76±682.91	352.97±213.64	492.72±333.15	380.20±221.54	288.26±152.41	458.11±546.84	323.61±97.05	380.51±133.45
S.hyicus pure (AFU)	659.12±825.04	424.24±193.42	549.84±339.33	491.91±228.54	433.61±201.95	634.29±804.36	471.61±176.24	494.62±196.32
Cells stimulated by PMA 10-7M (RR)	6.54±3.75	5.34±3.20	3.82±2.43	3.64±2.72	5.49±3.59	4.83±4.14	3.99±2.36	3.30±1.60
S.aureus pure (RR)	4.03±2.13	4.13±1.79	5.00±2.78	5.62±2.80	6.42±2.75	7.60±3.33	3.99±1.56	3.99±2.38
E.coli pure (RR)	2.86±0.99	3.32±1.52	4.49±2.55	4.07±1.70	4.75±2.90	5.44±2.86	2.91±1.33	2.74±1.28
S.hyicus pure (RR)	3.97±2.02	4.32±1.88	5.10±2.78	5.90±3.29	7.22±3.47	6.96±2.86	4.10±1.30	3.88±2.42

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 40.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by iron group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	203.35±181.20	120.25±55.34	133.91±109.99	114.75±97.41	87.23±48.20	94.66±126.02	164.34±84.36	146.91±86.54
Cells stimulated by PMA 10^{-7}M (AFU)	883.20±1044.32	835.78±581.46	472.17±329.38	319.91±234.44	515.08±344.24	353.47±399.38	654.51±335.51	530.04±442.04
S.aureus puro (AFU)	533.08±553.51	522.99±300.41	484.31±245.80	538.11±301.13	388.75±172.29	737.91±1020.46	445.49±134.26	505.44±191.31
E.coli pure (AFU)	515.94±690.10	380.49±215.33	409.42±232.07	461.25±333.78	274.57±146.98	482.96±560.38	343.66±103.89	367.83±140.00
S.hyicus pure (AFU)	624.09±835.12	456.57±190.18	481.94±227.97	560.59±337.39	404.81±190.62	675.36±824.89	474.37±162.51	495.91±214.31
Cells stimulated by PMA 10-7M (RR)	4.85±3.54	6.94±3.15	3.86±2.18	3.60±2.94	5.91±3.14	4.36±4.40	4.20±1.95	3.10±1.95
S.aureus pure (RR)	3.60±2.11	4.57±1.63	4.64±2.32	6.02±3.06	5.33±1.94	8.78±3.08	3.18±1.03	4.80±2.42
E.coli pure (RR)	2.87±1.27	3.34±1.33	3.89±2.00	4.64±2.22	3.76±1.58	6.48±3.22	2.33±0.72	3.31±1.54
S.hyicus pure (RR)	3.96±2.23	4.35±1.61	4.66±2.18	6.40±3.57	5.81±2.68	8.35±3.05	3.33±1.15	4.63±2.38

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 41.- Mean and standard deviation of production of reactive oxygen species (AFU and RR) by calves divided by thoracic perimeter group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	120.20±51.99	197.00±176.65	120.20±92.53	129.52±116.09	97.35±129.64	85.25±49.65	137.83±65.56	169.29±94.73
Cells stimulated by PMA 10^{-7}M (AFU)	643.22±323.55	1057.30±1074.17	274.02±209.39	533.30±316.63	276.61±249.06	574.91±423.12	399.98±326.30	734.98±368.87
S.aureus puro (AFU)	443.15±179.61	606.01±572.51	537.34±236.54	481.00±308.80	718.86±1061.42	431.38±221.73	496.08±200.01	456.59±135.08
E.coli pure (AFU)	363.87±227.37	520.86±657.47	477.59±260.70	387.84±305.14	458.70±581.21	311.98±183.05	370.07±145.00	343.97±102.67
S.hyicus pure (AFU)	430.53±200.70	635.24±794.57	554.06±255.12	482.91±316.64	655.33±840.60	442.74±276.11	497.26±221.27	475.10±161.27
Cells stimulated by PMA 10-7M (RR)	6.08±3.75	5.71±3.27	2.60±1.88	4.86±2.67	3.92±4.05	6.35±3.32	2.68±1.92	4.51±1.67
S.aureus pure (RR)	4.54±2.05	3.63±1.73	5.86±3.06	4.80±2.40	7.65±2.50	6.46±3.56	4.33±2.11	3.65±1.91
E.coli pure (RR)	3.38±1.37	2.83±1.20	4.81±1.91	3.72±2.22	5.29±1.99	4.94±3.58	2.93±1.14	2.71±1.45
S.hyicus pure (RR)	4.54±2.01	3.76±1.81	6.12±3.42	4.94±2.59	7.75±2.23	6.41±3.74	4.32±2.19	3.64±1.69

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 42.- Mean and standard deviation of production of reactive oxygen species (average rate of fluorescence unit and response ratio) by calves divided by body condition score group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Unstimulated cells (AFU)	142.85±61.47	178.86±187.71	118.72±91.80	131.10±116.56	91.05±126.48	91.11±47.22	109.23±70.02	203.60±70.24
Cells stimulated by PMA 10^{-7}M (AFU)	867.05±577.90	849.33±1046.99	350.54±249.48	450.89±335.31	278.72±232.90	595.58±436.81	314.14±241.12	881.72±269.84
S.aureus puro (AFU)	561.94±298.19	490.89±552.28	591.26±310.98	422.94±192.55	688.78±1025.95	441.66±227.39	420.40±188.45	525.09±114.83
E.coli pure (AFU)	427.41±228.57	465.11±692.41	514.52±337.15	348.06±180.43	451.64±558.29	308.30±192.33	314.57±133.93	394.72±91.44
S.hyicus pure (AFU)	455.50±193.29	625.26±834.08	622.80±330.06	408.89±172.88	612.11±821.23	472.94±270.36	417.07±199.86	551.25±144.19
Cells stimulated by PMA 10-7M (RR)	6.22±3.58	5.51±3.40	3.43±2.19	4.07±2.95	4.57±4.61	5.78±2.73	3.03±2.28	4.27±1.47
S.aureus pure (RR)	4.45±1.79	3.66±2.04	6.19±3.11	4.34±1.95	8.29±3.07	5.63±2.48	4.80±2.27	3.05±1.13
E.coli pure (RR)	3.35±1.34	2.83±1.24	4.96±2.33	3.46±1.52	6.20±3.16	3.86±1.84	3.35±1.45	2.21±0.70
S.hyicus pure (RR)	4.06±1.90	4.27±2.00	6.59±3.36	4.31±2.13	7.80±2.61	6.25±3.50	4.71±2.24	3.14±1.13

AFU: average rate of fluorescence unit; RR: response ratio.
Source: SANTOS (2020).

Table 43.- Mean and standard deviation of calf leucogram parameters divided by NEFA group (high and low).

Parameters	D2		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	7.61± 2.57	8.82 ± 2.77	8.08± 1.82	9.22± 3.16	11.31± 3.32	10.02± 3.59	10.55± 2.83	9.77± 3.65
Neutrophils (x 10^3/µL)	5.15± 2.69	5.46± 3.98	2.67± 1.55	3.22± 1.87	5.05± 2.71	3.93± 1.78	4.91± 1.93	3.52± 1.97

Source: SANTOS (2020).

Table 44.- Mean and standard deviation of calf leucogram parameters divided by BHB group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.47± 2.99	7.97± 2.45	8.88± 2.87	8.42± 2.37	10.34± 3.40	10.99± 3.60	9.73± 3.76	10.60± 2.64
Neutrophils (x 10^3/µL)	6.16± 4.17	4.45± 2.03	3.08± 2.00	2.81± 1.41	4.02± 1.84	4.95± 2.70	3.33± 1.96	4.79± 1.93

Source: SANTOS (2020).

Table 45.- Mean and standard deviation of calf leucogram parameters divided by glucose group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.74± 2.54	7.70± 2.83	9.00± 2.67	8.30± 2.56	11.60± 4.06	9.73± 2.54	10.04± 4.04	10.24± 2.47
Neutrophils (x 10^3/µL)	5.43± 3.78	5.18± 2.96	3.05± 1.82	2.84± 1.64	4.73± 2.84	4.24± 1.72	4.32± 2.31	3.95± 1.81

Source: SANTOS (2020).

Table 46.- Mean and standard deviation of calf leucogram parameters divided by total protein group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.71± 2.18	7.72± 3.13	8.54± 2.64	8.76± 2.64	10.42± 3.62	10.91± 3.40	9.45± 3.19	11.08± 3.21
Neutrophils (x 10^3/µL)	5.39± 3.81	5.23± 2.93	2.60± 1.70	3.29± 1.69	4.37± 2.86	4.60± 1.73	3.86± 2.17	4.70± 1.74

Source: SANTOS (2020).

Table 47.- Mean and standard deviation of calf leucogram parameters divided by albumin group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.41± 2.46	8.02± 2.99	7.91± 2.26	9.39± 2.77	11.35± 3.52	9.98± 3.38	9.10± 3.17	11.30± 3.02
Neutrophils (x 10^3/µL)	4.92± 2.74	5.70± 3.91	2.77± 1.67	3.12± 1.78	4.61± 2.90	4.37± 1.65	3.89± 2.29	4.52± 1.60

Source: SANTOS (2020).

Table 48.- Mean and standard deviation of calf leucogram parameters divided by triglycerides group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.11± 3.10	8.32± 2.34	8.15± 2.64	9.15± 2.54	10.30± 2.59	11.02± 4.22	9.23± 2.88	10.97± 3.43
Neutrophils (x 10^3/µL)	4.40± 3.00	6.21± 3.51	2.74± 1.62	3.15± 1.82	3.72± 1.39	5.26± 2.82	3.83± 2.11	4.52± 1.99

Source: SANTOS (2020).

Table 49.- Mean and standard deviation of calf leucogram parameters divided by cholesterol group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.42± 2.63	8.04± 2.82	8.73± 2.62	8.58± 2.66	11.87± 3.32	9.62± 3.32	8.82± 3.47	11.14± 2.76
Neutrophils (x 10^3/µL)	4.65± 2.92	5.88± 3.65	2.76± 1.74	3.10± 1.72	4.78± 2.92	4.24± 1.70	3.83± 2.45	4.44± 1.59

Source: SANTOS (2020).

Table 50.- Mean and standard deviation of calf leucogram parameters divided by haptoglobin group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	7.47± 3.01	8.87± 2.29	9.18± 2.36	8.19± 2.77	10.94± 3.19	10.43± 3.76	10.31± 4.32	10.02± 2.30
Neutrophils (x 10^3/µL)	5.86± 4.25	4.83± 2.32	2.86± 1.91	3.02± 1.57	4.68± 1.92	4.32± 2.67	3.56± 2.36	4.43± 1.88

Source: SANTOS (2020).

Table 51.- Mean and standard deviation of calf leucogram parameters divided by iron group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	7.89± 2.91	8.54± 2.53	9.31± 2.57	7.99± 2.53	9.92± 2.59	11.41± 4.11	9.17± 3.13	11.03± 3.19
Neutrophils (x 10^3/µL)	5.75± 4.31	4.87± 2.03	3.22± 2.01	2.68± 1.36	4.14± 1.80	4.83± 2.77	3.62± 1.95	4.55± 2.08

Source: SANTOS (2020).

Table 52.- Mean and standard deviation of calf leucogram parameters divided by thoracic perimeter group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.08± 2.51	8.35± 2.96	8.35± 2.31	8.95± 2.91	11.63± 3.28	9.69± 3.46	10.78± 2.96	9.67± 3.46
Neutrophils (x 10^3/µL)	4.78± 1.91	5.84± 4.34	2.68± 1.02	3.21± 2.20	5.20± 2.56	3.78± 1.87	4.66± 2.22	3.72± 1.87

Source: SANTOS (2020).

Table 53.- Mean and standard deviation of calf leucogram parameters divided by BCS group (high and low).

Parameters	D3		D7		D14		D28	
	Low	High	Low	High	Low	High	Low	High
Leucocyte total count (x 10^3/µL)	8.72± 2.81	7.64± 2.54	9.01± 2.68	8.23± 2.52	11.37± 4.12	9.85± 2.39	11.40± 3.46	9.00± 2.64
Neutrophils (x 10^3/µL)	4.72± 2.86	5.98± 3.82	2.76± 1.52	3.16± 1.94	4.95± 2.67	3.96± 1.79	4.61± 1.91	3.76± 2.13

Source: SANTOS (2020).