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Protein, energetic, enzymatic and mineral profile of Nellore cows during the pregnancy, parturition and postpartum

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ABSTRACT. The aim of this study was to evaluate the protein energetic, enzymatic and mineral profile of Nellore cows during the pregnancy, parturition and postpartum. Fifteen multiparous cows with 4 ± 1 years of age and live weight of 400 ± 50 kg were used at different stages (non-pregnant, in the initial, middle and late pregnancy, at birth, one day postpartum, 30 and 60 days postpartum). Blood collections were performed every 30 days and assayed for the following blood biomarkers: Protein (total proteins, albumin, urea and creatinine), energetic (cholesterol, triglycerides, glucose and beta hydroxybutyrate), mineral (calcium, phosphorus, magnesium) and enzymatic (alkaline phosphatase and aspartate aminotransferase). Calcium had the lowest concentrations ($p < 0.05$) in the initial pregnancy, while phosphorus had the highest concentration at parturition ($p < 0.05$). Triglycerides, glucose and beta hydroxybutyrate were influenced by the stages of pregnancy, reducing in the late pregnancy and at parturition. Glucose had a reduction in the late pregnancy and elevation in the postpartum. Beta hydroxybutyrate showed increase at the late pregnancy. Although lipomobilization occurred in the phases of higher metabolic demands in the attempt to maintain homeostatic conditions. Nellore cows did not present negative energy balance in the late pregnancy period, maintaining normal variation in blood markers throughout the experimental period.

Keywords: biochemistry; blood; ketone bodies; metabolites; physiology.

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Introduction

Pregnancy represents one of the most important reproductive stages of cattle, because of the changes that can occur directly in animal metabolism in the search for adaptation to physiological changes. Changes that occur mainly in the final third of pregnancy and in the postpartum, in which nutritional requirements are intensified as a consequence of the nutrient demand for the maintenance, development of the foetus and preparation for lactation (Roberts et al., 2012).

However, in order to meet their nutritional requirements during the periods of higher demands, ruminants can mobilize their body reserves. This mobilization occurs mainly in the last weeks of pregnancy and early lactation in an attempt to supply the energy deficit that is affected by low feed intake in these periods (Chapinal et al., 2011) representing a challenge for the animal organism.

The metabolism of dairy animals is different when compared to beef cattle, once during pregnancy and early lactation the dairy cows undergo more evident transition period (Lima et al., 2015), in which the negative energy balance becomes more accentuated by the high requirement in nutrients, and can trigger a series of metabolic disorders, affecting the productive efficiency of these animals.

Assuming that the metabolism among breeds may be different, the knowledge of the mechanisms used in beef cows in response to metabolic needs during gestation and postpartum periods are becoming increasingly necessary. The study is necessary because there are few literary data, being of great importance the knowledge of the serum constituents that provide homeorrheic responses, in the periods of metabolic overload.

Therefore, the evaluation of the metabolic profile emerges in recent years as a tool of great utility as an indicator of the metabolic balance, allowing not only the diagnosis of metabolic disorders, but also

assessing the nutritional condition, aiding in the adequacy of nutritional management (Roberts et al., 2012). Thus, it was hypothesized that the nutrient mobilization as well as the nutritional serum concentrations of Nellore cows varies with the physiological state, being different from what occurs with dairy cows.

Thus, in view of the scarcity of information about the serum biochemistry of Nellore cows in the different reproductive stages, the objective of this study was to evaluate the influence of pregnancy, parturition and puerperium on the protein, energetic, enzymatic and mineral profile of Nellore cows.

Material and methods

Study location and ethical committee

The experiment was carried out at the experimental bovine farm of Campus Professora Cinobelina Elvas - Federal University of Piauí (UFPI), located in the city of Alvorada do Gurguéia, State of Piauí. This work followed all ethical principles involving animals in research and All experimental procedures and animal handling were perfomed after submission and approval by the Bioethics Committee of Federal University of Piauí (protocol ID: 048/2012).

Animals, estrus synchronization, handling, and feeding

Thirty female Nellore cows were used, in which were submitted to the previous sanitary management, being dewormed, three months before the experimental start, with Ivermectin (Invomec - Merial®, São Paulo, Brasil) and vaccinated against foot-and-mouth disease and Clostridiosis (Biocell da Vallé® e SintoxanT da Merial®, São Paulo, Brasil) respectively.

For this study, the cows were submitted to estrus synchronization and artificially inseminated at fixed time. After 30 days, the pregnancy diagnosis was performed by trans-rectal ultrasonography, selecting 15 pregnant cows, aged 4±1 years and body condition score (BCC) 7±1 and body weight of 400 ± 50 kg. During the experimental period the cows remained in pasture paddocks with an area of 8 hectares, formed by pastures of *Panicum maximum* cv. Mombaça and *Brachiaria brizantha* cv. Marandu and Xaraés. In the late afternoon, the cows were collected, and offered concentrate supplementation in a collective trough at the rate of 2 kg for each animal. The supplementation consisted of ground corn, soybean meal and mineral supplementation (Fosbovi 20®, Tortuga, São Paulo, Brasil) for beef cattle, based on dry matter (Table 1).

Table 1. Chemical composition and proportion of ingredients in the concentrate offered cows.

Ingredients (g kg^{-1} of dry matter)	
Ground Corn	700.00
Soybean meal	250.00
Mineral Supplement[1]	50.00
Concentrate composition[2] (g kg^{-1} de MS)	
Dry matter	83,56
Crude protein	18,56
ether extract	3,36
Neutral detergent fiber	11,90
Acid Detergent Fiber	7,37
Calcium	10,00
Phosphorus	5,00

[1]Supplement mineral: zinc: 1.300 mg kg^{-1}; copper: 1.530 mg kg^{-1}; manganese: 1.300 mg kg^{-1}; iron: 1.800; iodine: 75.00 mg kg^{-1}; sulfur: 12.00 g kg^{-1}.

Blood collection and analysis

Blood samples were collected monthly, totaling 14 collections, always in the morning, before the cows were conducted to pasture. The first blood collection was performed for the formation of the control group (not pregnant) and the others were performed to monitor the blood parameters, in the periods in which the animals were pregnant (initial third, middle and final), at parturition, 1 day postpartum and 1st and 2nd month of puerperium. Blood samples were collected by jugular venipuncture, using disposable needles 25 x 8 mm (21x *g*) and deposition in vacutainer tubes, except for the glucose dosage collections that were performed in vacutainer tube containing fluoride. Serum blood samples were centrifuged at 3500 rpm 15 $minutes^{-1}$ and packed as serum aliquots into Eppendorf tubes and stored in a freezer at -20°C for subsequent biochemical analysis.

The laboratory analyzes were carried out at the Laboratory of Veterinary Clinical Pathology of the University Veterinary Hospital (UFPI-CPCE), located in the city of Bom Jesus, Piauí, by the colorimetric method in a semiautomatic biochemical analyzer (Spectrum®, São Paulo, Brazil). The metabolites evaluated were creatinine (Labtest Diagnóstica® S.A); Urea (Urea liquiform Labtest Diagnóstica® S.A) by the colorimetric enzymatic method; cholesterol (cholesterol liquiform Labtest Diagnóstica®S.A), triglycerides (triglycerides liquiform Labtest Diagnóstica® S.A). The enzymes evaluated were aspartate Aminotransferase by the UV kinetic method (AST/GOT Liquiform Labtest Diagnóstica® S.A) and alkaline phosphatase (alkaline phosphatase Liquiform Labtest Diagnóstica® S.A). For the protein profile the biuret method was used, (Labtest Diagnóstica® S.A), in which for total serum proteins and serum albumin by the bromocresol green method (Labtest Diagnóstica® S.A).

Regarding the mineral profile, the serum calcium was measured by the method of purple Phthalein (Labtest Diagnóstica® S.A., Brasil); Inorganic phosphorus by the ammonium molybdate method (Labtest Diagnóstica® S.A., Brasil) and serum magnesium by the sulphonated magon method (Labtest Diagnóstica® S.A., Brasil). For the analysis of the serum concentrations of beta hydroxybutyrate (BHB) the D-3-Hydroxybutyrate (Ranbut) assay method was used (RANBUT) Randox® in automatic biochemical analyzer (BIOPLUS 2000®), conducted in the laboratory of Veterinary Pathology of the University Veterinary Hospital of the Federal University of Campina Grande, in the Center for Health and Rural Technology (UFCG-CSTR), in the city of Patos, Paraíba.

Statistical analysis

The experiment was conducted in a completely randomized design in which the treatment effect was the physiological phases (not pregnant, initial third, middle third, final third, parturition, 1 day postpartum, 1 and 2 months postpartum), with 15 repetitions and measures repeated over time. The data were analyzed by the GLM Procedure and the means were compared by the Tukey test at 5% probability (Statistical Analysis System [SAS], 2002).

Results and discussion

The serum mineral profile was influenced by the reproductive stages, which there were changes in the serum concentrations of calcium ($p < 0.05$) and phosphorus ($p < 0.05$), but the serum concentrations of magnesium did not change ($p > 0.05$) (Table 2).

Table 2. Serum mineral profile of Nellore cows during the reproductive periods.

Reproductive stage	Mineral Metabolites (mg dL^{-1})		
	Calcium	Phosphorus	Magnesium
Non-pregnant	10.64[a]	6.25[b]	2.55[a]
Initial pregnancy	9.45[abc]	6.40[b]	2.40[a]
Middle pregnancy	10.65[a]	6.30[b]	2.37[a]
Late pregnancy	8.71[c]	7.20[a]	2.58[a]
Parturition	10.05[ab]	7.43[a]	2.32[a]
1 day postpartum	8.28[c]	6.30[b]	2.30[a]
30 days postpartum	8.27[c]	6.40[b]	2.67[a]
60 days postpartum	9.45[bc]	7.17[a]	2.38[a]
Reference[1]	9.7-12.4	5.6-6.5	1.8-2.3
P-value	<0.0001	<0.0001	0.0576
SEM[2]	0.1464	0.1980	0.0340

*Means followed by different letters in the same column were statistically different ($p < 0.05$) by the Tukey test; [1]Reference range for adult cattle (Kaneko et al. 2008); [2]SEM= standard error of mean.

Serum calcium levels remained similar during prepartum period. However, in the initial pregnancy and late pregnancy were observed values below that described by Kaneko, Harvey, and Bruss (2008) (Table 2). However, occurred a rapid increase in serum concentrations during the parturition, remaining within the limit described for the species (Kaneko et al., 2008). Reduction of calcium was verified 1 day postpartum and in the first month postpartum, presenting hypocalcemia in some cows, however without apparent clinical signs.

The homeostatic mechanism involving calcium is directly influenced by the action of hormones calcitonin and parathormone that act in an antagonistic way. When calcium concentrations are lower than

the reference range, as observed in the animals during the initial third of pregnancy, one day after parturition and in the first month postpartum, the parathyroid hormone release occurs, increasing bone resorption and activating 1,25-dihydroxyvitamin D, which acts to increase renal tubular reabsorption and intestinal calcium absorption (Goff, 2009).

The reduction in blood calcium concentrations, evidenced in the final third of gestation (Table 2), probably can be attributed to the high requirement of minerals presented by animals at this stage, in consequence of decrease in feed intake and the adaptation to the homeostatic mechanisms of calcium in this period, with great mobilization of nutrients from the maternal body, to support the fetal development and the high necessity of this mineral for the synthesis of colostrum (Degaris & Lean, 2008; Brozos Mavrogianni, & Fthenaskys, 2011).

Plasma calcium was reduced in the final third of gestation and in the immediate postpartum, but with a higher mobilization at parturition, which was similar to non-pregnant cows. Similar behavior occurred in the immediate postpartum, being the uterine involution, a factor that also contributes to this puerperal hypocalcemia. However, at parturition, serum calcium concentrations in the present experiment were higher than reported in previews research (Moreira et al., 2015).

The phosphorus concentration in blood are shown in Table 2, being directly associated with the homeostatic mechanism of calcium for the control of this mineral. Variations in serum phosphorus concentrations were similar to calcium during the periods prior to parturition, except for the late pregnancy, presenting higher values than reported by Kaneko et al. (2008), characterizing a hyperphosphatemia, which may interfere with the renal conversion of vitamin D to 1,25-dihydroxyvitamin D, decreasing its concentration and impairing intestinal absorption of calcium (Degaris & Lean, 2008), reducing the concentrations serum calcium levels, evidenced during the final third of gestation.

During the postpartum period, there was reduction of phosphorus, with lower concentrations than prepartum, but within the established values for the species (Kaneko et al., 2008) (Table 2). The blood concentration of this mineral undergoes many variations in ruminants, caused by both the amount recycled through saliva, and by the absorption at the rumen and intestine level.

Researches carried out under different breeding conditions, different climatic conditions and different cattle patterns presented different results (Moreira et al., 2015). This shows the need of the present study to characterize the blood biodynamics of Nellore cows during the transition period.

Serum magnesium did not show differences in the comparison among the physiological periods. However, in all reproductive stages the serum concentrations exceeded the reference values recommended for the species (Kaneko et al., 2008). This observation may be a risk factor for cows, which can trigger hypermagnesemia associated with lower serum calcium concentrations in the late pregnancy period, acting to competitively inhibit the entry of calcium into the motor neuron, decreasing uterine muscle contractions, contributing to the occurrence of problems especially at parturition (Silva Filho et al., 2015). The increase or decrease of magnesium at parturition involves the balance between the use of this mineral for the production of colostrum and its replacement through the diet, in parallel with the bone absorption and the decrease of renal excretion by the action of parathyroid hormone (Alvarenga et al., 2015). Considering that maintaining adequate levels of magnesium contributes to calcium homeostasis, since bone resorption of calcium is dependent on magnesium, and parathyroid hormone to activate adenyl cyclase must bind to receptors present in bones and kidneys or then activate phospholipase C. The adenyl cyclase and phospholipase C have a binding site with the $Mg2^{+}$ ion, which must be filled in order to respond to the hormone (Goff, 2008).

According the protein profile of cows it was verified that the total protein ($p > 0.05$), albumin ($p > 0.05$) and creatinine ($p > 0.05$) did not differ between reproductive stages, however, the serum urea concentrations were altered ($p < 0.05$) (Table 3).

The serum variation of albumin is very controversial in the scientific community, and there is no agreement among the researchers about the behavior of this metabolic component. Similar results were evidenced by Garcia, Cardoso, Campos, Thedy, & González (2011) and Alvarenga et al. (2015) from parturition until the puerperium. Alterations in the values of the albumin concentrations may be related to the distribution of the protein to the mammary gland and preparation of the lactation, as well as to the hepatic synthesis (Park et al., 2010). It is also emphasized that, albumin reflects the long-term protein status.

Table 3. Protein profile of Nellore cows at different reproductive periods.

Reproductive stage	Protein Metabolites			
	Total Proteins (g dL^{-1})	Albumin (g dL^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Non-pregnant	7.08[a]	3.17[a]	27.78[e]	0.82[a]
Initial pregnancy	7.12[a]	3.15[a]	32.19[de]	0.95[a]
Middle pregnancy	7.13[a]	3.21[a]	40.26[bcd]	0.87[a]
Late pregnancy	7.11[a]	3.25[a]	41.84[bc]	0.86[a]
Parturition	7.21[a]	3.25[a]	51.30[a]	0.92[a]
1 day postpartum	7.15[a]	3.43[a]	47.05[ab]	0.78[a]
30 days postpartum	7.22[a]	3.35[a]	41.71[bc]	0.80[a]
60 days postpartum	7.22[a]	3.20[a]	33.34[cde]	0.80[a]
Reference[1]	6.74-7.46	3.03-3.55	42.8-64.2	1-2
P-value	0.7030	0.1439	<0.0001	0.0560
SEM[2]	0.0240	0.0274	0.9534	0.0148

*Means followed by different letters in the same column were statistically different ($p < 0.05$) by the Tukey test; [1]Reference range for adult cattle (Kaneko et al., 2008); [2]SEM= standard error of mean.

Serum urea was below the lower limit for the species (Kaneko et al., 2008) during all phases that preceded the parturition, however, with increasing levels. At parturition and one day postpartum physiological values were observed as established by Kaneko et al. (2008). Unlike albumin, urea represents short-term protein status, showing momentary variation of the metabolite in the body.

The supplementation that was given to the cows had great importance at the different stages of the gestational period and in the postpartum period, since the response emitted was satisfactory from the evaluation of the protein metabolism, as shown in Table 3, noting variation only for serum urea concentrations, leading to the assertion that throughout the experimental phases, the cows received a complete diet that supply the nutritional requirements in proteins.

Most of the amino acids absorbed by ruminants are derived from the microbial protein synthesized in the rumen and absorbed through the small intestine. The microbial protein can supply 50 to 100% of the metabolizable protein required for beef cattle, being considered a source of good quality in relation to its intestinal digestibility (about 80%) and its amino acid profile.

According to Valadares Filho, Marcondes, Chizzotti, and Paulino (2010) guarantee the adequate protein supply to animals means to provide them with an essential nutrient for maintenance of homeostasis, allowing its production efficiently. Ruminants have particularities in their protein nutrition, however, their protein demands are supplied through amino acids absorbed in the small intestine, as in any other animal, although much of the absorbable protein (50 to 80%) is derived from the synthesized microbial protein in the rumen (Bach, Calsamiglia, & Stern, 2005).

Ammonia in excess synthesized in the rumen goes into the bloodstream, being carried to the liver and bio transformed in urea, can be used as a source of nitrogen or remain circulating in the blood or be excreted in the urine (Oliveira et al., 2014). Urea is a component synthesized in the liver in amounts proportional to the ammonia concentration produced rumen and its concentration is directly related to the protein levels of the diet and to the relation between energy and protein of the diet.

One explanation for the elevation of serum urea concentrations in parturition is the advent of recycling urea through saliva which is synthesized through animal metabolism, where excess ammonia resulting from degradation of dietary protein or ingested urea are absorbed by the ruminal epithelium and transported to the liver to be converted to urea in the endogenous form.

Concentrations of triglycerides ($p < 0.05$) and glucose ($p < 0.05$) were influenced by periods of pregnancy ($p < 0.05$) as shown in Table 4, whereas serum cholesterol concentrations ($p > 0.05$) remained unchanged. The serum triglycerides concentrations increased at parturition and 1 day postpartum, exceeding the reference values established by Kaneko et al. (2008). Cholesterol concentrations in the evaluated periods remained within the normal range for the species (Kaneko et al., 2008), except for the late pregnancy, which presented concentrations above the reference range.

This increase in serum triglycerides evidenced at parturition and in the first day postpartum, probably associated with low food intake in these periods, due to the stress caused by the physiological events resulting from the hormonal changes that occur a few days before the parturition, with mobilization of lipids to supply the demand. According to Contreras and Sordilho (2011) the lipid mobilization is based on a physiological adaptation used in the moments in which the reduction in nutrient intake is defined, being defined by the imbalance between lipogenesis and lipolysis in adipose tissue.

Another explanation would be the inability of the liver to oxidize all the free fatty acids from the great mobilization of body fat during the peripartum, since the excess of free fatty acids contributes to the increase of the serum concentrations of triglycerides. Although no dosages of pancreatic hormones have been performed, reductions in post-partum concentrations may be linked to increased endocrine secretions of insulin, increasing the uptake of circulating triglycerides by muscle and adipose cells (Balikci, Yildiz, & Gurdogan, 2007).

Table 4. Energy profile in the different reproductive physiological periods of Nellore cows.

Reproductive stage	Energetic metabolites			
	Triglycerides (mg dL^{-1})	Cholesterol (mg dL^{-q})	Glucose (mg dL^{-1})	Beta hydroxybutyrate (mmol L^{-1})
Non-pregnant	12.81[b]	117.64[a]	72.88[a]	0.2249[a]
Initial pregnancy	13.51[b]	119.94[a]	72.09[a]	0.2250[a]
Middle pregnancy	14.21[b]	117.10[a]	72.08[a]	0.2709[a]
Late pregnancy	13.65[b]	121.83[a]	49.44[c]	0.3760[b]
Parturition	15.98[a]	117.10[a]	70.50[ab]	0.4354[b]
1 day postpartum	16.11[a]	114.31[a]	57.74[bc]	0.2765[a]
30 days postpartum	14.66[b]	115.57[a]	58.10[bc]	0.2800[a]
60 days postpartum	11.13[b]	117.21[a]	73.90[a]	0.4622[b]
[1]Reference	0-14	80-120	45-75	0.4-1.0
P-value	<0.0001	0.1201	<0.0001	<0.0001
[2]SEM	1.6614	2.5112	1.2720	0.01298

*Means followed by different letters in the same column were statistically different ($p < 0.05$) by the Tukey test; [1]Reference range for adult cattle (Kaneko et al., 2008); [2]SEM= standard error of mean.

Blood glucose decreased until the middle pregnancy, followed by elevation in the following stages. The reduction in serum glucose concentrations in the immediate pre-partum may be related to the progress of gestational development, since at the end of gestation, the energetic requirements are increased, caused by the exponential growth of the foetus up to 80% of its size and final weight (Rodrigues, Rodrigues, Branco, Queiroz, & Araújo, 2006) and by the needs for the development of the udder as well as for the synthesis of lactose. The results found in the present study for glucose during pregnancy disagree with Oliveira et al. (2014) who found increasing values of glucose until the parturition and corroborating with the findings by Janovick et al. (2011) who found higher concentrations of glucose at parturition. The elevation that occurs for glycemia is most likely due to the release of endogenous glucocorticoids in response to the stress caused by the moment of parturition, since cortisol is a stimulator of the gluconeogenic pathway, making glucose more bioavailable. According to Rastani, Del Rio, Gressley, Dahl, and Grummer (2007) another plausible explanation would be the interference of glucagon in gluconeogenic metabolism, since it has a peak on the day of parturition, stimulating lipomobilization and the use of hepatic glycogen, triggering a circulating glucose peak, which is reduced shortly thereafter.

There was variation ($p < 0.05$) in the concentration of beta hydroxybutyrate (BHB), with increase in the late pregnancy and at parturition, allowing to infer that there was deficit in energy availability in this period, since the increase of BHB is directly associated with lipolysis, where the increased amount of BHB in the bloodstream becomes indicative of the increase in energy demand (Frigotto, Ollhoff, Filho, & Almeida, 2009; Ospina, Mcart, Overton, Stokol, & Nydam, 2013). This increase, although it did not occur drastically in the concentrations of BHB in the late pregnancy, is probably due to the small lipid mobilization of the body reserves in the final gestation period and the beginning of lactation, where nutritional needs intensify, since the mobilization of adipose tissue from the body reserves promotes the release of free fatty acids and the accumulation of these in the liver (Smith, Hippen, Beitz, & Young, 1997).

The accumulation of triglycerides from lipomobilization in the liver is often physiological. However, when there is drastic lipid mobilization, there is an overload of hepatic metabolism during the oxidation process of free fatty acids, accumulating large amounts of intermediate metabolites of NEFA oxidation, in the form of ketone bodies mainly BHB (Li et al., 2012). According to Vap and Weisser (2007), the closer to parturition, there was an increase in BHB concentrations. Concentration above 1.4 mmol L^{-1} is indicative of negative energy balance and predisposes animals to the risk of metabolic diseases such as subclinical ketosis, abomasal displacement and placental retention. Concentrations between 0.6 and 1.0 mmol L^{-1} are suggestive of mobilization of body reserves and dependent on the performance of the adaptive mechanisms to the negative energy balance (NEB). However, values lower than 0.6 mmol L^{-1} are indicative that the animal is not losing body reserve (Alvarenga et al., 2015).

In the present study values below 0.4 mmol L^{-1} showed that throughout the experimental phases the cows remained with the same body condition score. This provides sufficient basis to affirm that in Nellore cows during all gestational stages, they are submitted to only a small lipomobilization in the periods of higher metabolic requirements, confirmed by the serum triglycerides values not characterizing NEB.

This was probably due to mechanisms of nutrient partitioning different from that occurring in milk cows, since beef cattle during a gestation period have as their main priority their body reserves. however, in the periods of higher metabolic requirements corresponding to the final stage of gestation and subsequent lactation, it guarantees sufficient nutritional intake for maximum foetal development and to meet the small requirement caused by low milk production.

The enzymatic profile was influenced by periods of pregnancy (Table 5), but values for alkaline phosphatase (AF) remained within the normal limits for the species (Kaneko et al., 2008). Aspartate aminotransferase (AST) varied among the analysed periods with values above the reference for species.

Table 5. Enzymatic serum profile of Nellore cows at different reproductive stages.

Reproductive stage	Enzymatic metabolites (UI L^{-1})	
	Alkaline phosphatase	Aspartate aminotransferase
Non-pregnant	356.63^{ab}	36.16^{ab}
Initial pregnancy	361.97^{a}	34.86^{b}
Middle pregnancy	344.51^{b}	34.95^{b}
Late pregnancy	346.44^{b}	43.46^{a}
Parturition	364.34^{a}	39.23^{ab}
1 day postpartum	365.98^{a}	31.80^{bc}
30 days postpartum	352.66^{ab}	25.97^{c}
60 days postpartum	346.62^{b}	35.49^{b}
Reference[1]	0-488	20-34
P-value	<0.0001	<0.0001
SEM[2]	1.3902	0.7622

*Means followed by different letters in the same column were statistically different ($p < 0.05$) by the Tukey test; [1]Reference range for adult cattle (Kaneko et al., 2008); [2]SEM= standard error of mean.

There was a significant difference ($p < 0.05$) in Alkaline phosphatase values over the periods studied, with differences between the initial third of gestation in relation to the gestational thirds, at the end and the second puerperal month, with the highest values of AF at parturition and one day after parturition. Alkaline phosphatase is considered of little importance in the evaluation of hepatic diseases in ruminants, due to its great range of physiological variation.

High concentrations of aspartate aminotransferase were observed in non-pregnant cows during pregnancy (initial, middle and late) and parturition, which reduced at postpartum, but within the reference range for the species recommended by Kaneko et al. (2008) until the second month of puerperium. The stage of higher concentration of aspartate aminotransferase was during the late pregnancy, probably influenced by the great mobilization of nutrients to the foetus, since at that stage the foetal growth is accentuated (Gravena et al., 2010). Another possible explanation would be the intense muscular effort that occurs at parturition causing lysis of muscle fibers and increasing serum concentrations in the early phase of the puerperium, but because the half-life of this enzyme to be short, was accelerated, justifying the low concentration in the periods of one day postpartum and in the 30 days postpartum (Fagliari et al., 1998; Souza, Collona, Garcia, Birgel, & Birgel Junior, 2008; Oliveira et al., 2014).

Conclusion

The reproductive stages of the major changes in metabolic biochemistry of Nellore cows were the late pregnancy and parturition. During the early stages of pregnancy, the cows prioritized the maintenance. However, in the Late pregnancy and parturition (periods of high energy demand), occurred discrete mobilization and subclinical hypocalcaemia occurred in the postpartum period.

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