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Influence of management and lactation order in the serum concentrations of proteins, metabolites, minerals, and enzymes of Bonsmara breed cows

Influência da gestação e ordem de lactação nas concentrações séricas de proteínas, metabólitos, minerais e enzimas de vacas da raça Bonsmara

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Abstract
The aim of this study was to evaluate the influence of pregnancy and lactation order on the serum concentrations of proteins, metabolites, minerals and enzymes of first, second and third lactation order Bonsmara cows. The Bonsmara breed, originated in South Africa, stands out for its tolerance to heat, and productive characteristics, such as meat quality, high fertility, sexual precocity, ease of calving and good maternal ability. These characteristics make the breed a genetic alternative for crossbreeding with zebu breeds. Blood samples were collected from 93 cows, from which 34, 29 and 30 were first, second and third lactation order cows, respectively. Samples were processed in a multichannel automated analyzer using Labtest Diagnostics® kits. To compare the values between lactation orders, the Kruskall-Wallis test was chosen. To compare between pregnant and nonpregnant cows within lactation orders, the Mann-Whitney test was used. Amongst the constituents analyzed, the order of lactation significantly influenced only the serum albumin concentration (ALB) ($p<0.0001$). Pregnancy significantly influenced the A:G ratio ($p=0.034$) in third lactation cows; cholesterol (Chol) ($p=0.004$), triglycerides (TRI) ($p<0.0001$), inorganic phosphorus (iP) ($p=0.033$), iron (Fe) ($p=0.001$), aspartate aminotransferase (AST) ($p=0.018$) and alkaline phosphatase (ALP) ($p=0.039$) in second lactation order cows and the value of the overall group. Creatinine (Crea) ($p<0.0001$) values were influenced by pregnancy only in the overall group. It was concluded that pregnancy and lactation order significantly influenced the concentration of several serum biochemical constituents of Bonsmara cows, especially in second order lactation cows.
Key words: Beef cattle; Phases of production; Serum biochemical; Metabolic profile

Resumo
Objetivou-se avaliar a influência da gestação e ordem de lactação nas concentrações séricas de proteínas, metabólitos, minerais e enzimas de vacas da raça Bonsmara de primeira, segunda e terceira ordem de lactação. A raça Bonsmara, originada na África do Sul, se destaca por sua tolerância ao calor, e caracteristicas produtivas, como a qualidade da carne, a alta fertilidade, precocidade sexual, facilidade ao parto e boa habilidade materna. Essas características tornam a raça uma alternativa genética para realização de cruzamentos com raças zebuínas. Foram colhidas amostras de sangue de 93 vacas, sendo 34 de primeira ordem de lactação, 29 de segunda ordem de lactação e 30 de terceira ordem de lactação. As amostras foram processadas em analisador automático multicanal, utilizando kits da Labtest Diagnóstica®. Para confrontar os valores entre as ordens de lactação optou-se pelo teste Kruskall-Wallis. Para comparar vacas gestantes e não gestantes dentro das ordens de lactação, foi utilizado o teste de Mann-Whitney. Dos constituintes analisados, a ordem de lactação influenciou significativamente apenas a concentração sérica de albumina (ALB) ($p<0,0001$). A gestação influenciou significativamente na relação A:G ($p=0,034$), nas vacas de terceira lactação, no colesterol (COL) ($p=0,004$), triglicérides (TRI) ($p<0,0001$), fósforo inorgânico (Pi) ($p=0,033$), ferro (Fe) ($p=0,001$), aspartato aminotransferase (AST) ($p=0,018$) e fosfatase alcalina (FAL) ($p=0,039$) nas de segunda ordem e no valor do grupo geral. A creatinina (Crea) ($p<0,0001$) foi influenciada somente no grupo geral. Conclui-se que a gestação e ordem de lactação influenciou significativamente na concentração de vários constituintes bioquímicos séricos de vacas da raça Bonsmara, em especial nas de segunda ordem de lactação.
Palavras-chave: Bovino de corte; Fases de produção; Bioquímica sérica; Perfil metabólico.

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Introduction

The serum biochemical profile provides information on the normal values of a breed and can be used as an indicator of the organism's adaptive processes to nutritional and physiological challenges, specific metabolic and imbalances, and imbalances in energy,

protein, and mineral metabolism.[1, 2] The interpretation of the serum biochemical profile is complex due to the mechanisms that control the blood concentrations of various metabolites and their high variation according to several factors, such as breed, age, stress, diet, management, climate, and physiological state (gestation and lactation).[1, 3]

The Bonsmara breed, originated in South Africa from the genetic combination of 5/8 Afrikaner, 3/16 Shorthorn, and 3/16 Hereford by researcher Prof. Jan Bonsma, was introduced in Brazil in 1997.[4] The productive characteristics, including its meat quality, are more similar to *Bos taurus* than to zebu breeds.[5] These characteristics make the Bonsmara breed a genetic alternative for crossbreeding with Zebu breeds.

Physiological changes occur during gestation and lactation, increasing nutritional needs to support fetal growth and development, as well as maternal metabolism and the development of specific tissues for reproduction.[6, 7] A higher development of placental, fetal, glandular, and mammary tissues occurs in the final third of gestation, contributing to increased energy demand.[7] Lactation is a physiological state in which adaptations in metabolism occur. Early lactation imposes severe metabolic changes, which challenge the organism to maintain a homeostatic balance to compensate for the expenditure of nutrients that lactogenesis requires.[8] Assessment of the metabolic profile is more relevant during the lactation period, when the animals are more susceptible to metabolic changes, such as at the beginning of lactation, considering herd characteristics, geographic location, and physiological state of the animals.[8, 9]

Importantly, age, breed, physiological state, stage and order of lactation, individual productivity, climate, and diet reflect changes in the metabolic profile pattern of producing cows.[10] Researchers have highlighted the importance of knowing the physiological changes that occur at these stages to avoid misdiagnosis of metabolic, nutritional, and infectious diseases.[11, 2] Thus, considering the importance of knowing the serum biochemistry as a diagnostic tool and the lack of information in the literature regarding the variations in serum biochemical constituents of lactating, pregnant, and non-pregnant Bonsmara cows, this study aimed to evaluate the influence of gestation and lactation order on serum concentrations of proteins, metabolites, minerals, and enzymes in first-, second-, and third-lactation order Bonsmara cows.

Material and methods

he experiment was conducted from November 2018 to March 2019 on a property located in the city of Uberlândia, MG, Brazil, under the coordinates 18°55′0.7″S and 48°16′38″W. Ninety-three cows were divided into three groups according to the lactation order, 34 of which were first-lactation (10 pregnant and 24 non-pregnant), 29 of second-lactation (13 pregnant and 16 non-pregnant), and 30 of third-lactation order (16 pregnant and 14 non-pregnant). Only animals in good nutritional status and considered healthy, which did not present clinical or pathological signs, and a body score between 3 and 4, were included in the study, being monitored by a veterinarian, responsible for the sanitary, zootechnical, and reproductive management of the herd. The animals were maintained in pastures of *Brachiaria brizantha* cv. Marandu and BRS Piatã, *B. ruziziensis*, and *B. decumbens*, with water and mineralized salt ad libitum. They were vaccinated according to the regional sanitary calendar, and the control of ecto-and endoparasites was established according to the monitoring of infestations.

An aliquot of 10 mL of blood was collected from each animal by venipuncture of the middle coccygeal vein, using 25×8 mm needles attached to sterile tubes dried with clot activator (Vacutainer®), always in the morning. After collection, the blood samples were packed in isothermal boxes and transported to the Veterinary Clinical Laboratory of the Federal University of Uberlândia. The samples were immediately centrifuged at 720g for 10 minutes, the obtained serum was transferred in 1.0-mL aliquots to microtubes (Eppendorf®), and frozen at −20 °C for a maximum period of 48 hours until analyses. The samples were processed in a ChemWell™ automated multichannel analyzer previously calibrated (Calibra H®) and measured with universal control serum (Qualitrol®), using commercial kits from Labtest Diagnóstica®. The concentrations of total protein (TP) (biuret method), albumin (ALB) (bromocresol green), creatinine (Crea) (alkaline picrate), urea (UV enzymatic kinetic method), cholesterol (Chol) and triglycerides (TRI) (Trinder enzymatic method), calcium (Ca^{+}) (cresolphthalein complexone – CPC method), inorganic phosphorus (iP) (UV kinetic method), magnesium (Mg) (Magon sulphonate method), iron (Fe) (modified Goodwin method), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (UV kinetic method – IFCC), and gamma-glutamyl transferase (GGT) (modified Szasz method). The globulin (Glob = TP − ALB), albumin to globulin ratio (A:G), and calcium to phosphorus ratio (Ca^{+}:iP) values were calculated.

The data were analyzed by descriptive statistics and subjected to the Levene test to verify homoscedasticity and the Shapiro-Wilk test to verify normality. The medians and non-parametric Kruskal-Wallis test were used to compare the values between lactation orders as the data did not meet these assumptions. Mann-Whitney test at a 5% significance level was used to compare pregnant and non-pregnant cows within lactation orders.

The experiment followed the ethical principles of

animal experimentation with approval from the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Uberlândia, according to protocol 053/2018.

Results

The comparison of the serum concentrations of proteins, metabolites, minerals, and enzymes between lactation orders showed that ALB in second-lactation cows was significantly higher than that of first-lactation cows and similar to third-lactation cows. The other evaluated serum constituents did not differ statistically (Table 1).

Table 1. Median (MD), standard error (SE), mean (ME), and standard deviation (SD) of serum concentrations of proteins, metabolites, minerals, and enzymes of first-, second-, and third-lactation order Bonsmara cows, Uberlândia, MG, Brazil

Element	Median / Mean	1st lactation order (34)	2nd lactation order (29)	3rd lactation order (30)	General group (93)
TP	MD±SE	6.85 ± 0.13	6.98 ± 0.17	7.21 ± 0.12	6.98 ± 0.08
(g/dL)	ME±SD	6.98 ± 0.77	7.22 ± 0.83	7.11 ± 0.65	7.09 ± 0.74
ALB	MD±SE	2.76 ± 0.08^{b}	3.13 ± 0.07^{a}	3.03 ± 0.06^{ab}	2.98 ± 0.04
(g/dL)	ME±SD	2.85 ± 0.47	3.18 ± 0.31	3.09 ± 0.31	3.02± 0.41
Glob	MD±SE	4.05 ± 0.12	3.86 ± 0.13	4.15 ± 0.11	4.12 ± 0.07
(g/dL)	ME±SD	4.14 ± 0.68	4.04 ± 0.63	4.02 ± 0.59	4.07 ± 0.63
A:G	MD±SE	0.67 ± 0.03	0.78 ± 0.02	0.75 ± 0.04	0.75 ± 0.02
ratio	ME±SD	0.71 ± 0.18	0.80 ± 0.12	0.79 ± 0.22	0.76 ± 0.19
Crea	MD±SE	1.20 ± 0.04	1.15 ± 0.05	1.50 ± 0.05	1.23 ± 0.03
(mg/dL)	ME±SD	1.23 ± 0.23	1.20 ± 0.26	1.40 ± 0.29	1.28 ± 0.27
Urea	MD±SE	23.10 ± 1.67	30.20 ± 2.35	21.40 ± 1.70	23.60 ± 1.09
(mg/dL)	ME±SD	23.92 ± 9.69	29.44 ± 11.26	24.40 ± 9.27	25.55 ± 10.15
Chol	MD±SE	150.95 ± 6.10	104.00 ± 8.51	153.75 ± 7.34	144.90 ± 4.28
(mg/dL)	ME±SD	141.59 ± 35.52	117.35 ± 40.82	146.06 ± 40.22	136.73 ± 39.95
TRI	MD±SE	16.10 ± 2.20	33.90 ± 3.04	33.60 ± 2.90	21.40 ± 1.55
(mg/dL)	ME±SD	22.05 ± 12.83	26.43 ± 14.58	27.10 ± 15.88	24.95 ± 14.43
Ca^{+}	MD±SE	8.99 ± 0.21	9.17 ± 0.18	8.87 ± 0.19	9.00 ± 0.13
(mg/dL)	ME±SD	8.75 ± 1.23	8.91 ± 0.84	8.77 ± 1.04	8.87 ± 1.17
iP	MD±SE	5.50 ± 0.19	5.20 ± 0.27	5.05 ± 0.19	5.40 ± 0.13
(mg/dL)	ME±SD	5.36 ± 1.12	5.69 ± 1.30	5.03 ± 1.05	5.30 ± 1.25
Ca^{+}:iP	MD±SE	1.65 ± 0.06	1.63 ± 0.08	1.81 ± 0.06	1.72 ± 0.04
ratio	ME±SD	1.69 ± 0.36	1.64 ± 0.39	1.81 ± 0.34	1.75 ± 0.37
Mg	MD±SE	1.90 ±0.10	1.90 ±0.11	2.20 ± 0.08	2.00 ± 0.06
(mg/dL)	ME±SD	1.94 ±0.59	1.99 ±0.51	2.24 ± 0.43	2.05 ± 0.53
Fe	MD±SE	85.00 ± 6.01	90.00 ± 8.84	104.00 ± 6.47	91.00 ± 4.01
(µg/dL)	ME±SD	88.62 ± 35.05	97.52 ± 42.39	103.93 ± 35.46	96.25 ± 37.40
AST	MD±SE	84.00 ± 4.82	64.20 ± 4.87	90.50 ± 4.67	83.00 ± 2.89
(U/L)	ME±SD	85.78 ± 28.11	69.50 ± 23.37	88.27 ± 25.59	82.34 ± 26.92
ALT	MD±SE	46.00 ± 2.38	46.00 ± 4.41	52.50 ± 3.19	49.00 ± 1.86
(U/L)	ME±SD	46.53 ± 13.85	51.00 ± 21.31	53.50 ± 17.45	50.11 ± 17.31
ALP	MD±SE	122.95 ± 9.75	82.20 ± 13.50	108.20 ± 9.18	108.10 ± 6.05
(U/L)	ME±SD	117.31 ± 56.87	107.74 ± 64.74	116.37 ± 50.28	114.46 ± 56.41
GGT	MD±SE	16.80 ± 1.00	13.00 ± 0.77	15.85 ± 1.28	15.40 ± 0.63
(U/L)	ME±SD	16.03 ± 5.80	13.97 ± 3.67	16.76 ± 7.01	15.73 ± 5.74

Different lowercase letters in the rows represent significantly different values ($p<0.05$) between lactation orders.

The comparison of serum values of proteins, metabolites, minerals, and enzymes in pregnant and non-pregnant cows within each lactation order showed a higher value of the A:G ratio in pregnant cows in the third lactation. Serum concentrations of Chol, Fe, AST, and ALP in second-lactation pregnant cows were higher than those in non-pregnant ones. TRI and iP values were statistically higher in non-pregnant cows of the same group (Table 2). In the general group, Crea, Chol, Fe, and ALP showed significantly higher values in pregnant cows. TRI and iP showed higher values in non-pregnant cows (Table 2).

Table 2. Median and standard error (MD±SE) of serum concentrations of proteins, metabolites, minerals, and enzymes of pregnant and non-pregnant first-, second-, and third-lactation order Bonsmara cows, Uberlândia, MG, Brazil.

Element	P	1st lactation order	2nd lactation order	3rd lactation order	General order
	NP	(P = 10) (NP = 24)	(P = 13) (NP = 16)	(P = 16) (NP = 14)	(P = 39) (NP = 54)
TP	P	6.98 ± 0.30	7.50 ± 0.43	7.14 ± 0.20	7.02 ± 0.16
(g/dL)	NP	6.80 ± 0.14	6.98 ± 0.16	7.21 ± 0.13	6.96 ± 0.08
ALB	P	2.79 ± 0.19	3.35 ± 0.16	3.15 ± 0.08	3.10 ± 0.08
(g/dL)	NP	2.76 ± 0.08	3.10 ± 0.06	2.98 ± 0.09	2.96 ± 0.05
Glob	P	4.08 ± 0.22	4.15 ± 0.29	4.16 ± 0.14	4.12 ± 0.11
(g/dL)	NP	4.05 ± 0.14	3.86 ± 0.14	4.21 ± 0.18	4.11 ± 0.09
A:G	P	0.75 ± 0.05	0.78 ± 0.03	0.80 ± 0.02[a]	0.78 ± 0.02
ratio	NP	0.64 ± 0.04	0.79 ± 0.03	0.71 ± 0.09[b]	0.71 ± 0.03
Crea	P	1.33 ± 0.08	1.17 ± 0.14	1.54 ± 0.07	1.45 ± 0.05[a]
(mg/dL)	NP	1.17 ± 0.05	1.10 ± 0.04	1.29 ± 0.07	1.13 ± 0.03[b]
Urea	P	24.15 ± 1.91	34.80 ± 4.67	21.70 ± 2.27	24.20 ± 1.68
(mg/dL)	NP	21.60 ± 2.23	28.05 ± 2.58	21.40 ± 2.47	21.95 ± 1.40
Chol	P	150.95 ± 6.32	150.80 ± 7.06[a]	154.75 ± 9.74	151.90 ± 5.21[a]
(mg/dL)	NP	149.75 ± 8.27	94.60 ± 9.74[b]	151.00 ± 11.25	119.15 ± 5.89[b]
TRI	P	14.30 ± 3.87	8.50 ± 1.54[b]	23.85 ± 4.08	14.20 ± 2.52[b]
(mg/dL)	NP	19.20 ± 2.61	37.40± 2.58[a]	37.25 ± 4.15	33.90 ± 1.82[a]
Ca^{+}	P	8.77 ± 0.50	9.26 ± 0.50	8.81 ± 0.33	8.80 ± 0.24
(mg/dL)	NP	9.12 ± 0.22	9.15 ± 0.14	8.97 ± 0.16	9.05 ± 0.11
iP	P	5.35 ± 0.39	4.80 ± 0.11[b]	4.85 ± 0.29	4.90 ± 0.18[b]
(mg/dL)	NP	5.70 ± 0.22	6.00 ± 0.36[a]	5.30 ± 0.24	5.55 ± 0.16[a]
Ca^{+}:iP	P	1.69 ± 0.11	1.83 ± 0.11	1.83 ± 0.09	1.80 ± 0.06
ratio	NP	1.65 ± 0.07	1.58 ± 0.11	1.78 ± 0.08	1.65 ± 0.05
Mg	P	1.75 ± 0.18	1.90 ± 0.10	2.40 ± 0.14	2.00 ± 0.09
(mg/dL)	NP	1.90 ± 0.13	2.00 ± 0.13	2.20 ± 0.07	2.00 ± 0.07
Fe	P	106.50 ± 12.85	130.00 ± 8.97[a]	104.00 ± 10.99	114.00 ± 7.10[a]
(µg/dL)	NP	82.00 ± 6.54	78.50 ± 8.89[b]	94.50 ± 4.70	83.00 ± 4.09[b]
AST	P	86.50 ± 4.23	88.00 ± 6.02[a]	99.50 ± 7.12	88.00 ± 3.81
(U/L)	NP	73.50 ± 6.65	59.10 ± 5.89[b]	93.50 ± 6.06	71.25 ± 4.02
ALT	P	43.00 ± 5.35	83.00 ± 10.96	56.50 ± 5.47	53.00 ± 3.98
(U/L)	NP	46.50 ± 2.61	45.00 ± 2.60	53.50 ± 3.00	47.00 ± 1.65
ALP	P	132.45 ± 18.91	141.00 ± 28.96[a]	125.45 ± 15.51	126.60 ± 11.06[a]
(U/L)	NP	108.30 ± 11.00	78.45 ± 12.25[b]	108.60 ± 8.36	104.25 ± 6.47[b]
GGT	P	12.90 ± 1.47	13.00 ± 1.28	13.60 ± 1.62	13.00 ± 0.94
(U/L)	NP	17.05 ± 1.21	13.65 ± 1.81	17.80 ± 1.99	16.40 ± 0.81

Different lowercase letters in the columns represent significantly different values ($p<0.05$) for pregnant and non-pregnant cows within each lactation order. P = pregnant, NP = non-pregnant.

Discussion

The mean values of most of the analyzed constituents for the 93 cows (general group), regardless of being pregnant or not, were within or close to the intervals proposed by Kaneko et al.,[12] except for Glob, Chol, TRI, and ALT, which were above the maximum reference values. Ca^{+} and iP were slightly below the minimum reference value (Table 1). As the authors did not specify animal age, breed, reproductive status, or management conditions and methodologies used, these variables become imprecise and of little application to diagnose metabolic disorders. The means of TP, Crea, urea, Chol, TRI, Ca^{+}, iP, ALP, and GGT remained within and the means of ALB and AST were slightly above the ranges found by Conceição et al.[13] for non-pregnant and non-lactating Nelore and Girolando cows from 24 to 36 months of age raised in the State of Maranhão, Brazil.

The lower ALB value in first lactation cows ($p<0.0001$) can be explained by the high demand for amino acids necessary for the synthesis of milk proteins[2, 7] and the high nutritional demand due to the growth phase. According to Rossato et al.,[8] the number of lactations can influence the serum values of the metabolic profile of cows. However, the serum albumin concentration can be influenced by the protein level in the diet and the reduced synthesis capacity in the liver due to fat accumulation.[1] The results obtained for ALB and urea show that protein was not a limiting factor, as a protein-

deficient diet is characterized by serum albumin levels below 3.0 g/dL and urea levels below 15 mg/dL.[1] Liver function enzymes are within the physiological ranges for the species, which shows the non-impairment of the liver in these animals. These results corroborate with Alvarenga et al.,[14] who found low albumin levels in Holstein and Jersey cows, with no hepatic alterations.

The serum values of Chol and TRI were significantly affected by the physiological state of the animals, with values above those proposed by Kaneko et al.[12] This result may be related to the high demand for regulatory mechanisms involved in all processes of milk synthesis and gestation,[7, 15] leading to changes in lipolysis and lipogenesis.[16] Lipid mobilization from stores in the adipose tissue involves the release of free fatty acids such as triglycerides into the bloodstream.[16] Despite metabolic variations, serum concentrations of AST and GGT enzymes remained within the ranges considered physiological, possibly characterizing the non-impairment of the liver function. Freitas Júnior et al.[10] and Alvarenga et al.[14] also observed this condition.

Serum concentrations of Ca^{+} and iP close to the lower limit established by Kaneko et al.[12] are possibly due to the fact that the cows in the present study were lactating and 39 (42%) were pregnant, showing a higher requirement for these minerals for milk production and fetal development. Grünwaldt et al.[17] also reported lower values of serum calcium in lactating Aberdeen Angus and Argentine Criollo cows. These authors observed serum iP values of 4.34 mg/dL, a value below the physiological range reported by Kaneko et al.,[12] which were attributed to the iP levels in the diet. According to Piccione et al.,[7] animals need minerals such as calcium, magnesium, and phosphorus for growth, reproduction, and lactation, also functioning as catalytic components of enzymes or regulating various mechanisms involved in gestation and lactation.

The serum activity of AST, ALP, and GGT enzymes within the physiological ranges for the species is consistent with the findings of Otto et al.[3] in lactating African Angoni cows. Serum ALT values in Bonsmara cows above the physiological limits for the species are related to their greater muscle mass although ALT is primarily used as a biomarker of liver damage.[15] Unlike AST, equine, swine, and ruminant hepatocytes do not present a high ALT activity and, therefore, the increase in the serum activity of the enzyme during liver injury is insignificant even in necrosis.[18] Therefore, the muscle should be considered as a potential source for increasing serum activity of the enzyme since the total muscle mass is much higher than liver mass.

The higher value of the A:G ratio in third-lactation pregnant cows is due to the higher ALB value although it did not differ statistically from non-pregnant ones. The A:G ratio increased with an increase in albumin.

The Crea value in pregnant cows in the general group was higher than in non-pregnant cows ($p<0.0001$) due to the higher serum concentration in third-lactation pregnant cows, not differing statistically from non-pregnant ones. Importantly, most of the energy required by the metabolism and growth of the fetus in the pregnant cow is supplied by glucose and amino acids, which can lead to glucose reduction and consequent muscle protein catabolism.[9, 11] During gestation, the cow assumes the load of organic residues from the fetus through the fetal maternal circulation. Thus, an increase in serum creatinine in pregnant cows of the general group can also be attributed to the development of fetal muscles.[7] This result differs from that found by Otto et al.,[3] who observed similar values of the metabolite in pregnant and non-pregnant Angoni cows.

The probable reason for the higher value of serum Chol in second-lactation pregnant cows and the general group ($p=0.004$) is the higher mobilization of previously stored body fat and the release of fatty acids and glycerol into the bloodstream.[15, 16] Pregnant females aim to save the consumption of glucose and amino acids to meet the requirements of the fetus,[3] as lipids participate in a small proportion in the direct supply of energy to the fetus.[9] Lactating cows may present physiological hypercholesterolemia due to the lipid mobilization caused by lactation and the increase in lipoprotein synthesis.[19] In addition, cholesterol levels during gestation reach maximum values because of the synthesis of gonadal steroids. Pogliani et al.[6] observed similar values for serum Chol in pregnant and non-pregnant Holstein heifers, showing that gestation did not influence the serum levels of the metabolite. The discrepancy between Chol values in cows of the present study and those of the literature is attributed to the use of pluriparous lactating cows in the present study to evaluate the possible influence of lactation order on the evaluated serum constituents.

The higher serum TRI concentration in second-lactation non-pregnant cows and the general group ($p<0.0001$) differs from the findings of Pogliani et al.,[6] who observed no statistically significant differences in the serum TRI concentrations in pregnant and non-pregnant cows. The difference between the findings in this study and those in the literature is attributed to the fact that the non-pregnant cows were in the first months of lactation, a period of higher milk production and, therefore, with higher demand for nutrients. However, pregnant cows were at the end of lactation, a period of lower milk production and, consequently, lower nutrient demand. Also, as lactation progresses, and with changes in the endocrine profiles during gestation, lipolysis decreases and lipogenesis replenishes the stores of triglycerides in the adipose tissue, which will later be used after delivery and the beginning of lactation.[19]

The lower serum iP value in second-lactation pregnant cows (p=0.033) and the general group can be attributed to the passage of iP through the placenta to meet the needs of fetal development and the negative effect of parathyroid hormone (PTH), increasing urinary iP elimination.[20]

The higher serum Fe activity in second-lactation pregnant cows and the general group is attributed to an increase in Fe demand for fetal hematopoiesis and the fact that they were at the final third of lactation, a phase of lower milk production and, consequently, less mineral excretion in the milk.

The higher serum AST concentration (p=0.018) in second-lactation order pregnant cows can be attributed to the state of lipomobilization in the pregnant, which is a mechanism of physiological adaptation.[16] It results in increased hepatocyte membrane permeability, causing varying degrees of elevation in serum concentrations of the enzyme.[15] On the other hand, cholestasis was not evidenced since the serum GGT values were similar for pregnant and non-pregnant cows, remaining within the reference range proposed by Kaneko et al.[12] The increase in protein catabolism in the muscle tissue to supply the organism energy demand via gluconeogenesis must be considered.[2, 11]

The higher serum ALP concentration (p=0.039) in second-lactation pregnant cows and the general group may be related to the fetal growth and the release of bone enzymes and isoenzymes of placental origin. Similar results were observed by Yokus and Cakir[20] in pregnant cows and Brscic et al.[9] in pregnant heifers and primiparous and multiparous cows.

The predominance of differences between second-lactation pregnant and non-pregnant cows is possibly due to the higher loss of body reserves during the first lactation. The transition from gestation to lactation generates major endocrine and metabolic changes due to childbirth and higher demand for nutrients for milk production. According to Rossato et al.,[8] first- and second-calving cows undergo higher loss of body reserves during lactation than cows with three or more lactations.

It is worth mentioning that this is one of the first studies on the serum biochemical profile of Bonsmara cows in Brazil and we expect that it could be a stimulus for further studies on the subject.

Conclusion

Gestation and lactation order are factors with significant influence on the concentration of several biochemical serum constituents of Bonsmara cows, especially in the second-lactation order. Therefore, these production phases are factors of variability that must be considered for the correct interpretation of the serum biochemical profile.

Conflict of interests

The authors declare no conflict of interest.

Author Contributions

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