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Influence of age and sex on blood biochemical profile of Bonsmara cattle breed up to two years

Influência da idade e sexo no perfil bioquímico sanguíneo de bovinos da raça Bonsmara até dois anos

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Abstract
This study aimed to evaluate the effects of animal age and sex on serum concentrations of proteins, metabolites, minerals, and enzymes in Bonsmara cattle up to two years of age. Blood samples were collected from 179 animals (92 males and 87 females), aged between 15 days and 24 months. The animals were divided into four age groups: G1: 15 days ┤ 2 months, G2: 2 ┤ 6 months, G3: 6 ┤ 12 months, and G4: 12 ┤ 24 months of age. The samples were processed in an automatic multichannel analyzer using Labtest Diagnóstica™ kits. Animal age had a significant influence on most of the serum biochemical constituents except for magnesium (Mg) and the Ca^{+}:iP ratio. As age increased, G3 showed the highest concentrations of total proteins (TP), globulins (Glob), urea, and cholesterol (Chol); while G4 had a gradual increase and higher values of creatinine (Crea), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Conversely, albumin:globulin (A:G) ratio, calcium (Ca^{+}), inorganic phosphorus (iP), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) decreased from G1 to G4. Moreover, animal sex influenced serum values of albumin (ALB), Glob, A:G ratio, Crea, urea, triglycerides (TRI), Ca^{+}, iP, Ca^{+}:iP ratio and Mg. In conclusion, the factors of age group and sex significantly influenced the concentrations of serum biochemical constituents in Bonsmara cattle in the growth phase.
Keywords: beef cattle; growth phase; sexual factor; serum biochemical profile

Resumo
Objetivou-se avaliar a influência da idade e sexo nas concentrações séricas de proteínas, metabólitos, minerais e enzimas em bovinos da raça Bonsmara com até dois anos de idade. Foram colhidas amostras de sangue de 179 animais (92 machos e 87 fêmeas), de 15 dias a 24 meses de idade, distribuídos em quatro grupos etários: G1: 15 dias ┤ 2 meses, G2: 2 ┤ 6 meses, G3: 6 ┤ 12 meses e G4: 12 ┤ 24 meses de idade. As amostras foram processadas em analisador automático multicanal, utilizando kits da Labtest Diagnóstica®. A variável idade dos animais influenciou significativamente nos valores da maioria dos constituintes bioquímicos séricos avaliados, exceto no magnésio (Mg) e na relação Ca^{+}:Pi. O aumento da idade culminou com a maior concentração das proteínas totais (PT), globulinas (Glob), ureia e colesterol (COL) no G3; aumento gradual e maior valor de creatinina (Crea), aspartato aminotransferase (AST) e alanina aminotransferase (ALT) no G4. Em contrapartida, houve diminuição da relação albumina:globulina (A:G), cálcio (Ca^{+}), fósforo inorgânico (Pi), fosfatase alcalina (FAL) e gama glutamiltransferase (GGT) do G1 ao G4. O fator sexo influenciou nos valores séricos da albumina (ALB), Glob, relação A:G, Crea, ureia, triglicérides (TRI), Ca^{+}, Pi, relação Ca^{+}:Pi e Mg. Conclui-se que o fator idade e sexo influenciaram significativamente nos valores dos constituintes bioquímicos séricos analisados de bovinos da raça Bonsmara em fase de crescimento.
Palavras-chave: bovino de corte; fase de crescimento; fator sexual; perfil bioquímico sérico

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Introduction

Bonsmara is a bovine breed introduced in Brazil in 1997. It originated in South Africa, from the genetic combination of 5/8 Afrikaner, 3/16 Shorthorn, and 3/16 Hereford, by researcher Prof Jan Bonsma. The breed was developed due to the need to generate an animal fully adapted to the South African climate, with good productivity rates[1]. Its productive characteristics, including meat quality, are more similar to *Bos taurus* than to zebu breeds[1]. These characteristics make Bonsmara a genetic alternative for crossbreeding with zebu breeds.

Serum biochemical profile can be used as an indicator of adaptive processes of organisms to nutritional and physiological challenges, and specific imbalances in energy, protein, and mineral metabolism[2]. Interpretation of serum biochemical profile is complex both in herds and in individuals, at all stages of life, from birth to adulthood. Due to mechanisms that control blood concentrations of various metabolites and, also, great variation in these depending on factors such as race, age, stress, diet, management, climate, and physiological state[3].

The correct interpretation of laboratory findings depends on knowledge of the basic mechanisms of each lab test, recognition of disease effects on physiological processes, and their results[4]. Therefore, reference values for the study region, whether individual or for a particular population, are essential[5]. Blood biochemical values obtained in other countries may not apply to our conditions due to differences in breed, climate, management type, and analytical methods used by researchers[4,5].

Given the importance of serum biochemistry as a diagnostic tool, serum biochemical profile should be known for each breed, age group, and sex of animals to identify metabolic disorders and low productivity in farm animals. In Brazil, there are more than 30 Bonsmara breeders distributed in different states; however, studies on blood biochemistry for this breed are scarce. Allied to this, only one study has been carried out on the serum biochemical profile of pregnant and non-pregnant, lactating, and non-lactating cows[2]. Thus, our goal was to evaluate the influence of age and sex on serum concentrations of proteins, metabolites, minerals, and enzymes in Bonsmara cattle from 15 days to 24 months of age.

Material and methods

The experiment was carried out on a farm located in the city of Uberlândia, Minas Gerais State, Brazil (18° 55' 0.7" S and 48° 16' 38" W). A total of 179 animals (males and females) were used, divided into four groups according to age, namely: G1: 46 animals (26 males and 20 females) of 15 days ┤2 months of age; G2: 38 animals (19 males and 19 females) of 2 ┤6 months of age; G3: 39 animals (19 males and 20 females) of 6 ┤12 months of age; G4: 56 animals (28 males and 28 females) of 12−24 months of age.

As they did not show any clinical or pathological signs, only animals in good nutritional status and considered healthy were included and followed by a veterinarian, which was responsible for the sanitary, zootechnical, and reproductive management of the herd. At birth, calves were breastfed, weighed, identified, and had their navel healed. Regarding sanitary management, animals (males and females) were vaccinated at two, three, and seven months of age for clostridiosis, and only females at the seventh month for brucellosis. Thereafter, they were vaccinated according to the regional health calendar. As for parasite control, animals were dewormed at seven, twelve, and eighteen months of age. Lastly, ectoparasites were controlled according to infestations.

Aliquots of 10 mL blood were sampled from each animal by jugular venipuncture, using 25x0.8-mm needles attached to dry, sterile tubes with a clot activator (Vacutainer™). These samples were always collected in the morning. After collection, the blood samples were packed in isothermal boxes and transported to the Veterinary Clinical Laboratory of the Veterinary Hospital of the Federal University of Uberlândia. As soon as they arrived, the samples were centrifuged at 720g for 10 minutes, and the serum obtained was transferred in 1.0 mL aliquots to microtubes (Eppendorf™) and frozen at -20 °C for a maximum of 48 hours until analysis. The samples were processed in an automated multichannel ChemWell™ analyzer, previously calibrated (Calibra H™), and measured with universal control serum (Qualitrol™), using Labtest Diagnóstica™ commercial kits.

The analyzed parameters and respective determination methods were: total protein (TP; Biuret reagent), albumin (ALB; Bromocresol Green), creatinine (Crea; Alkaline picrate), urea (U; kinetic UV enzyme), cholesterol and triglycerides (Chol and TRI; Trinder enzyme), calcium (Ca^{+}; Cresolftalein Complexone), inorganic phosphorus (iP; UV kinetics), magnesium (Mg; sulfonated Magon), aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (AST, ALT, and ALP; UV kinetic IFCC), and gamma-glutamyltransferase (GGT; modified Szasz). Globulins (Glob = TP-ALB), albumin/globulins ratio (A: G), and calcium/phosphorus ratio were calculated (Ca^{+}:iP).

Data were subjected to descriptive statistical

analysis for means, medians, standard deviation, and standard error. The data were also subjected to Levene's homoscedasticity test and Shapiro-Wilk normality test. As they did not meet the assumptions, to compare means, we chose to use the medians and the non-parametric Kruskal-Wallis test for age groups, and the Mann-Whitney test for sex groups within each age group, both at a 5% significance level. Our study followed the ethical standards for animal experimentation and was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Uberlândia (protocol n.° 053/2018).

Results

Tables 1, 2, 3, and 4 display the medians, standard errors, means, standard deviations, and statistical analysis of the serum biochemical constituents analyzed in Bonsmara cattle from 15 days to 24 months of age.

Table 1. Medians (Med), standard errors (SE), means (Me), and standard deviations (SD) of serum proteins in Bonsmara cattle, males (M), females (F), and males/females (M/F) in the different age groups and the general group, Uberlândia, Minas Gerais, Brazil

Constituent	Sex	Med/ Me	G1 (15 days ┤ 2 months) (M = 26 F = 20)	G2 (2 ┤ 6 months) (M = 19 F = 19)	G3 (6 ┤ 12 months) (M = 19 F = 20)	G4 (12 ┤ 24 months) (M = 28 F = 28)	General Group (15 days ┤ 24 months) (M = 92 F = 87)	Reference value
TP (g/dL)	M	Med	5.80 ± 0.18	6.62 ± 0.28	6.76 ± 0.34	5.96 ± 0.23	6.34 ± 0.14	
	F	Med	5.73 ± 0.21	6.29 ± 0.15	7.51 ± 0.50	6.04 ± 0.15	6.32 ± 0.18	
	M/F	Med	5.79 ± 0.14c	6.37 ± 0.16b	7.20 ± 0.32a	6.00 ± 0.14bc	6.33 ± 0.16	
		Me	(5.78 ± 0.93)	(6.65 ± 1.02)	(7.95 ± 1.97)	(6.09 ± 1.02)	(6.53 ±1.50)	6.7 - 7.4*
ALB (g/dL)	M	Med	2.81 ± 0.08	2.89 ± 0.08A	2.99 ± 0.06	2.26 ± 0.06B	2.72 ± 0.04	
	F	Med	2.58 ± 0.14	2.69 ± 0.07B	2.88 ± 0.07	2.71 ± 0.08A	2.73 ± 0.05	
	M/F	Med	2.79 ± 0.08bc	2.74 ± 0.06ab	2.90 ± 0.04a	2.57 ± 0.06c	2.73 ± 0.05	
		Me	(2.61 ± 0.52)	(2.78 ± 0.34)	(2.90 ± 0.28)	(2.45 ± 0.41)	(2.66 ± 0.44)	3.0 - 3.6*
Glob (g/dL)	M	Med	3.11 ± 0.18	3.89 ± 0.30	4.00 ± 0.34	3.80 ± 0.21A	3.66 ± 0.13	
	F	Med	3.11 ± 0.17	3.44 ± 0.15	4.51 ± 0.52	3.31 ± 0.09B	3.60 ± 0.17	
	M/F	Med	3.11 ± 0.12c	3.80 ± 0.17ba	4.20 ± 0.33a	3.51 ± 0.12bc	3.63 ± 0.15	
		Me	(3.17 ± 0.83)	(3.87 ± 1.03)	(5.05 ± 2.03)	(3.64 ± 0.89)	(3.87 ± 1.40)	3.0 - 3.5*
A:G ratio	M	Med	0.86 ± 0.06	0.70 ± 0.09	0.65 ± 0.08	0.65 ± 0.03B	0.69 ± 0.03	
	F	Med	0.78 ± 0.07	0.72 ± 0.04	0.60 ± 0.06	0.83 ± 0.02A	0.75 ± 0.03	
	M/F	Med	0.84 ± 0.04a	0.71 ± 0.05ab	0.65 ± 0.05b	0.71 ± 0.02b	0.72 ± 0.03	
		Me	(0.88 ± 0.30)	(0.78 ± 0.30)	(0.68 ± 0.32)	(0.70 ± 0.16)	(0.76 ± 0.28)	0.84 - 0.94*

Different uppercase letters in the columns represent significantly different values ($p<0.05$) for males and females within each age group. Different lowercase letters in the rows represent significantly different values ($p<0.05$) between age groups, regardless of sex. *Kaneko, Harvey, and Bruss[6].

Table 2. Medians (Med), standard errors (SE), means (M), and standard deviations (SD) of serum metabolites in Bonsmara cattle, males (M), females (F), and males/females (M/F) in different age groups and the general group, Uberlândia, Minas Gerais, Brazil

Constituent	Sex	Med / Me	G1 (15 days ┤ 2 months) (M = 26 F = 20)	G2 (2 ┤ 6 months) (M = 19 F = 19)	G3 (6 ┤ 12 months) (M = 19 F = 20)	G4 (12 ┤ 24 months) (M = 28 F = 28)	General Group (15 days ┤ 24 months) (M = 92 F = 87)	Reference value
Crea (mg/dL)	M	Med	1.05 ± 0.06	1.23 ± 0.07	1.23 ± 0.09	1.51 ± 0.05A	1.23 ± 0.04	
	F	Med	0.95 ± 0.08	1.39 ± 0.08	1.21 ± 0.09	1.38 ± 0.04B	1.22 ± 0.04	
	M/F	Med	1.03 ± 0.05c	1.28 ± 0.05ab	1.22 ± 0.06b	1.45 ± 0.03a	1.23 ± 0.04	
		Me	(0.95 ± 0.32)	(1.29 ± 0.33)	(1.24 ± 0.40)	(1.41 ± 0.23)	(1.23 ± 0.36)	1.0 - 2.0*
Urea (mg/L)	M	Med	27.55 ± 3.22A	23.60 ± 3.05	24.40 ± 2.37	16.40 ± 1.22	22.65 ± 1.33	
	F	Med	17.05 ± 2.42B	21.40 ± 1.35	26.30 ± 1.19	19.55 ± 1.09	21.80 ± 0.82	
	M/F	Med	20.75 ± 2.17a	22.60 ± 1.73ab	25.40 ± 1.29a	17.75 ± 0.81b	22.23 ± 1.08	
		Me	(26.35 ± 14.72)	(24.13 ± 10.72)	(26.20 ± 8.06)	(18.78 ± 6.06)	(23.48 ± 10.71)	23 - 58*
Chol (mg/dL)	M	Med	114.55 ± 5.88	137.10 ± 10.84	170.40 ± 8.71	86.05 ± 2.91	110.25 ± 4.51	
	F	Med	109.50 ± 7.17	154.00 ± 6.26	177.40 ± 10.21	87.50 ± 5.00	118.00 ± 5.15	
	M/F	Med	110.50 ± 4.53b	147.90 ± 6.21a	176.60 ± 6.84a	86.05 ± 2.91c	114.13 ± 4.83	
		Me	(113.42 ± 30.74)	(146.13 ± 38.30)	(171.21 ± 42.71)	(88.92 ± 21.80)	(125.29 ± 45.69)	80 - 120*
TRI (mg/dL)	M	Med	32.60 ± 2.97	29.30 ± 2.93B	25.70 ± 1.91	39.85 ± 1.41A	33.10 ± 1.28	
	F	Med	24.45 ± 3.54	40.40 ± 3.11A	24.45 ± 2.92	29.35 ± 1.00B	29.90 ± 1.37	
	M/F	Med	29.25 ± 2.29ab	33.70 ± 2.31ab	25.70 ± 1.75b	32.80 ± 1.16a	31.50 ± 1.33	
		Me	(31.39 ±15.58)	(34.28 ± 14.26)	(28.34 ± 10.90)	(34.03 ± 8.67)	(32.17 ± 12.53)	0 - 14*

Different uppercase letters in the columns represent significantly different values ($p<0.05$) for males and females within each age group. Different lowercase letters in the rows represent significantly different values ($p<0.05$) between age groups, regardless of sex. *Kaneko, Harvey, and Bruss[6].

Table 3. Medians (Med), standard errors (SD), means (Me), and standard deviations (SD) of serum minerals in Bonsmara cattle, males (M), females (F), and males/females (M/F) in the different age groups and the general group, Uberlândia, Minas Gerais, Brazil

Constituent	Sex	Med/ Me	G1 (15 days ┤ 2 months) (M = 26 F = 20)	G2 (2 ┤ 6 months) (M = 19 F = 19)	G3 (6 ┤ 12 months) (M = 19 F = 20)	G4 (12 ┤ 24 months) (M = 28 F = 28)	General Group (15 days ┤ 24 months) (M = 92 F = 87)	Reference value
Ca^{+} (mg/dL)	M	Med	10.29 ± 0.48	10.24 ± 0.20B	9.96 ± 0.31	9.12 ± 0.13A	9.77 ± 0.18	
	F	Med	10.12 ± 0.74	10.92 ± 0.20A	9.64 ± 0.28	8.64 ± 0.12B	9.55 ± 0.22	
	M/F	Med	10.25 ± 0.41a	10.46 ± 0.15a	9.69 ± 0.21a	8.73 ± 0.09b	9.66 ± 0.20	
		Me	(11.32 ± 2.81)	(10.51 ± 0.95)	(10.05 ± 1.28)	(8.87 ± 0.69)	(10.10 ± 1.89)	9.7 - 12.4*
iP (mg/dL)	M	Med	9.80 ± 0.30A	7.00 ± 0.67	7.70 ± 0.32	5.70 ± 0.13B	7.10 ± 0.24	
	F	Med	8.70 ± 0.55B	7.00 ± 0.25	8.35 ± 0.53	7.65 ± 0.23A	7.70 ± 0.21	
	M/F	Med	9.50 ± 0.31a	7.00 ± 0.38ab	7.90 ± 0.32a	6.50 ± 0.18b	7.10 ± 0.23	
		Me	(8.89 ± 2.09)	(7.89 ± 2.32)	(8.41 ± 2.01)	(6.59 ± 1.32)	(7.85 ± 2.15)	5.6 - 6.5*
Ca^{+}:iP ratio	M	Med	1.07 ± 0.08B	1.48 ± 0.11	1.25 ± 0.08	1.62 ± 0.05A	1.37 ± 0.04	
	F	Med	1.52 ± 0.08A	1.50 ± 0.05	1.16 ± 0.09	1.11 ± 0.05B	1.27 ± 0.04	
	M/F	Med	1.18 ± 0.06	1.50 ± 0.06	1.23 ± 0.06	1.33 ± 0.04	1.32 ± 0.04	
		Me	(1.33 ± 0.40)	(1.43 ± 0.38)	(1.27 ± 0.38)	(1.41 ± 0.33)	(1.36 ± 0.37)	1:1 - 2:1**
Mg (mg/dL)	M	Med	2.40 ± 0.13	2.10 ± 0.28	2.10 ± 0.17	2.80 ± 0.14A	2.50 ± 0.09A	
	F	Med	2.35 ± 0.19	2.40 ± 0.11	2.40 ± 0.14	2.00 ± 0.06B	2.10 ± 0.06B	
	M/F	Med	2.40 ± 0.11	2.30 ± 0.15	2.20 ± 0.11	2.35 ± 0.10	2.30 ± 0.08	
		Me	(2.44 ± 0.73)	(2.43 ± 0.93)	(2.44 ± 0.68)	(2.49 ± 0.72)	(2.45 ± 0.76)	1.8 - 2.3*

Different uppercase letters in the columns represent significantly different values ($p<0.05$) for males and females within each age group. Different lowercase letters in the rows represent significantly different values ($p<0.05$) between age groups, regardless of sex. *Kaneko, Harvey, and Bruss(6), ** McDowell[7].

Table 4. Medians (Med), standard errors (SE), means (Me), and standard deviations (SD) of serum enzymes in Bonsmara cattle, males (M), females (F), and males/females (M/F) in different age groups and the General group, Uberlândia, Minas Gerais, Brazil

Constituent	Sex	Med/ Me	G1 (15 days ┤ 2 months) (M = 26 F = 20)	G2 (2 ┤ 6 months) (M = 19 F = 19)	G3 (6 ┤ 12 months) (M = 19 F = 20)	G4 (12 ┤ 24 months) (M = 28 F = 28)	General Group (15 days ┤ 24 months) (M = 92 F = 87)	Reference value
AST (U/L)	M	Med	53.50 ± 4.05	63.00 ± 4.14	66.00 ± 5.95	71.50 ± 5.39	63.00 ± 2.70	
	F	Med	50.00 ± 4.74	64.00 ± 2.29	70.00 ± 5.73	88.00 ± 5.04	69.00 ± 2.87	
	M/F	Med	52.00 ± 3.08c	64.00 ± 2.34b	68.00 ± 4.08b	81.50 ± 3.68a	66.00 ± 2.79	
		Me	(51.63 ± 20.91)	(65.26 ± 14.40)	(64.83 ± 25.51)	(86.04 ± 32.54)	(67.84 ± 26.29)	78 - 132*
ALT (U/L)	M	Med	26.50 ± 1.78	29.00 ± 3.44	30.00 ± 3.97	46.75 ± 1.81	36.00 ± 1.60	
	F	Med	19.50 ± 2.60	20.00 ± 2.56	30.00 ± 5.19	48.00 ± 1.91	34.00 ± 1.91	
	M/F	Med	23.00 ± 1.52c	27.50 ± 2.17bc	30.00 ± 3.27b	48.00 ± 1.31a	34.00 ± 1.24	
		Me	(24.85 ± 10.28)	(27.66 ± 13.40)	(35.69 ± 20.45)	(47.99 ± 9.77)	(35.84 ± 14.95)	11 - 40*
ALP (U/L)	M	Med	225.00 ± 9.48	221.10 ± 14.99	119.90 ± 26.75	107.85 ± 7.10	193.65 ± 8.99	
	F	Med	223.90 ± 11.02	229.80 ± 16.01	131.00 ± 21.10	99.05 ± 4.87	166.30 ± 9.02	
	M/F	Med	233.10 ± 7.11a	228.50 ± 10.82a	122.60 ± 16.72b	105.35 ± 4.32c	168.90 ± 6.78	
		Me	(234.15 ± 48.22)	(245.23 ± 66.68)	(177.17 ± 104.39)	(112.36 ± 32.33)	(215.48 ± 92.62)	0 - 488*
GGT (U/L)	M	Med	32.85 ± 12.51	19.70 ± 2.18	16.40 ± 2.40	13.65 ± 1.37	18.40 ± 3.93	
	F	Med	21.00 ± 20.78	16.90 ± 2.24	15.20 ± 8.03	14.85 ± 0.97	15.90 ± 5.21	
	M/F	Med	22.25 ± 11.35a	18.05 ± 1.59ab	15.50 ± 4.28ab	14.25 ± 0.83b	17.15 ± 4.57	
		Me	(48.88 ± 77.00)	(19.16 ± 9.82)	(24.10 ± 26.73)	(14.94 ± 6.23)	(26.55 ± 43.22)	6.1 - 17.4*

Different uppercase letters in the columns represent significantly different values ($p<0.05$) for males and females within each age group. Different lowercase letters in the rows represent significantly different values ($p<0.05$) between age groups, regardless of sex. *Kaneko, Harvey, and Bruss[6].

When comparing the protein metabolic profile constituents between age groups, a gradual increase in TP from G1 to G3 was observed, with G3 statistically differing from all other groups. ALB and Glob concentrations in G3 animals were higher than those of G1 and G4 but similar to those of G2. Glob showed a gradual increase from G1 to G3 and a subsequent reduction in G4. Higher A:G ratio was observed in G1 animals, which was statistically superior to those of G3 and G4 (Table 1).

As for the other metabolites, the serum concentration of Crea in G4 animals was significantly higher than those of G1 and G3. Urea concentration in G3 was higher than that of G4 but similar to those of G1 and G2. Chol showed a gradual increase from G1 to G3 and subsequent reduction in G4, with G2 and G3 statistically differing from G1 and G4. TRI content in G3 animals was significantly lower than in G4 and similar to the other groups (Table 2).

Regarding mineral profile, serum Ca^{+} concentration in G4 animals was statistically lower than those of the other groups, and phosphorus in G4 was lower than in G1 and G3. The Ca^{+}:iP ratio and magnesium content did not differ in the four age groups studied (Table 3). AST enzyme activity increased gradually with animal age, with G4 animals showing concentrations statistically superior to the other groups. Similar behavior was observed for ALT. ALP serum activity showed a significant reduction from 6 months of age onwards. GGT in G1 animals showed a significantly higher serum concentration than in G4 (Table 4).

When confronting serum proteins and metabolites for males and females within each age group (Tables 1 and 2), ALB content in G2 was higher in males than females, and in G4 in females than males. For Glob, G4 males had higher values than females, and for the A:G ratio, G4 females had higher values than males. Serum concentrations of Crea in G4 males and urea in G1 males were higher than in females. For TRI, G2 females and G4 males were statistically higher than the others.

As for minerals (Table 3), significant differences ($p<0.05$) between males and females within each age group were observed for Ca^{+} in G2, with females showing a higher value than males; in G4, males had higher values than females. For iP, G1 males had a higher value than females; however, G4 females had a higher value than males. The Ca^{+}:iP ratio was higher in G1 females and G4 males. For Mg, a higher value was observed for G4 males.

Comparing the medians of all serum biochemical constituents analyzed found for males and females, of the

179 animals (General group from 15 days to 24 months of age), regardless of age group, only Mg differed statistically, with a higher serum concentration in males.

Discussion

Of the 16 biochemical constituents analyzed for the general group (92 males and 87 females), the means of TP, ALB, A:G ratio and AST were below the ranges proposed by Kaneko, Harvey, and Bruss[6] and McDowell[7]. GLOB, Chol, TRI, Mg, and GGT remained above the other constituents within the reference intervals proposed by the researchers (Tables 1, 2, 3, and 4). Differences between our findings and the literature may be due to disparities in age group and sex, handling, environmental conditions, food, breed, and protocols used by researchers.

Our results demonstrate that animal age significantly influenced ($p<0.05$) most serum biochemical constituents evaluated, except for Mg and Ca^{+}:iP ratio (Tables 1, 2, 3, and 4). Variations in serum concentrations of biochemical constituents are justified, as serum concentrations of blood metabolites change from birth to adulthood, i.e., at maximum body development[4,8]. When comparing age groups regardless of the sex, protein profile showed a significant relationship with the age factor (Table 1), which corroborates Mohri, Sharifi, and Eidi[4] for Holstein cattle and Borges et al.[9] for Pantaneira breed.

Higher TP content in G3 can be attributed to increased serum concentrations of Glob and ALB in this group. Serum protein concentrations vary as a function of physiological variations in globulins and albumin. The higher serum ALB in G3 animals partially reflects hepatic synthesis and, possibly, protein content in the diet, as the same group also showed higher TP and urea contents (Tables 1 and 2). This was a period in which animals showed greater body development and gain, as mentioned by Delfino et al.[10].

The significant increase in Glob in G2 and G3 animals compared to G1 is due to the synthesis of their immunoglobulins in the face of antigenic stimuli from environmental pathogens, and mainly vaccinations. The higher A:G ratio observed in G1 is due to the lower Glob content in this group of animals.

Serum Crea concentrations showed an increasing trend with age, with the lowest value in animals up to 60 days of age (G1) and the highest in G4 animals, but still within the physiological limit for the species. These results are consistent with those obtained in previous studies[9]. Crea concentrations in G4 animals corroborate the results obtained by Gregory et al.[11], who found statistically higher values in Jersey cattle above 12 months of age. The highest serum Crea content in G4 is a result of muscle mass gains, which is a peculiar characteristic of the Bonsmara breed[1]. Creatinine is a product of creatine metabolism found in muscle tissues[8, 12] and its serum concentration is directly related to muscle mass[12], thus justifying increases in animals from 12 to 24 months of age (G4), due to greater muscle mass compared to the younger ones.

Serum urea concentrations in animals from 12 to 24 months of age (G4) were significantly lower than in G1 and G3, due to lower protein metabolism. Moreover, G4 also showed a lower serum ALB and significantly reduced serum TP content (Tables 1 and 2). As researchers have claimed, urea is synthesized in the liver in amounts proportional to ammonia concentrations produced in the rumen. Its serum concentration is directly related to protein levels in the diet and the energy/ protein ratio in the diet[13].

The significantly higher Chol serum concentrations in G2 and G3 can be attributed to a peculiarity of Bonsmara. The breed has a high-fat content interspersed in muscle tissue, increasing Chol levels in the blood. Moreover, animals up to 210 days of age (G3) are infants, so milk still represents an exogenous source of Chol[14], thus animals up to 12 months of age must have higher serum Chol concentrations. The lower Chol value in G4 animals is due to the peripubertal period, in which cholesterol is the precursor of steroid hormones, responsible for forming several hormones including cortisol, aldosterone in the adrenal glands, sex hormones such as progesterone, the various estrogens, testosterone and derivatives[6]. The serum Chol concentration in G3 animals was similar to that obtained in 3 to 11-month-old Pantaneira animals by Borges et al.[9]. However, ours were higher than those obtained by Pogliani and Birgel Júnior[15] for Holstein animals. These differences might be due to breed, farming type (milk or meat), feeding management, and animal weight gain.

Our results for TRI showed a significant relationship with the age group. G3 animals had lower TRI contents than those of G4 and were similar to those of G1 and G2. This difference can be considered as a physiological variation related to animal energy demand at the full growth phase. Animals make use of energy from triglycerides for muscle deposition, which may reflect low TRI blood concentrations. This result can still be attributed to animal weaning at the seventh month of age. After all, a milk-based diet is gradually replaced with volatile fatty acids absorbed in the rumen, derived from the ingested forage, and concentrate. These findings are similar to those obtained by Pogliani and Birgel Junior[15] for Holstein animals.

Calcium, together with phosphorus, is the most abundant mineral in the animal organism and is responsible for bone matrix formation and its mineralization, mainly in the growth phase[3, 16]. Serum Ca^{+} and iP concentrations had a similar trend in the

animals studied and were significantly higher in those up to 12 months of age, followed by a significant reduction in those between 12 to 24 months of age. This finding is consistent with that of Doornenbal et al.[8], who reported reductions both in calcium and phosphorus with age from 12 months onwards. It can be attributed to higher serum Ca^{+} concentrations in animals up to 12 months of age (G1, G2, and G3), and due to being infants up to the seventh month of age. It should be considered that in addition to milk being rich in Ca^{+}, young animals have a greater capacity to absorb and use calcium. As it is known, this mineral is highly required due to its need for bone growth[3, 16], results that corroborate those of other researchers[17].

Older animals (G4) had lower serum iP values than younger ones (G1 to G3). This inversely proportional behavior with age can be due to the greater availability of iP in milk[18], in addition to the high activity of growth hormone in younger animals. These factors increase intestinal absorption and renal reabsorption of phosphate as a result of an increased bone development rate[6]. Such findings are similar to those of other studies which claim that serum iP concentrations are inversely proportional to increasing age[17]. Nonetheless, other researchers disagree with this assertion and state that iP concentration is directly related to age[19]. Serum concentrations of Ca^{+} and iP had a similar trend, thus the Ca^{+}:iP ratio did not differ significantly among age groups, remaining between 1:1 to 2:1. This range is ideal for bone formation and growth according to McDowell[7]. Therefore, animals in our study have proper proportions of these minerals for the age groups.

Increases in serum AST and ALT values with age can be attributed to an age-proportional increase in muscle mass. According to some authors, higher serum AST concentration in older animals may be related to greater muscle mass in these animals, or as a consequence of endogenous production[20]. This high concentration of AST is due to an enzyme used as a biomarker of liver and/or muscle damage, which is present in large amounts in these tissues[3]. A gradual and significant increase in serum AST concentrations with age was also observed in Holstein animals by Benesi et al.[21] and by Mohri, Sharifi, and Eidi[4], and in the Pantaneira breed by Borges et al.[9]. Although ALT is mostly used as a biomarker of liver damage, unlike AST, equine, swine, and ruminant hepatocytes do not have high ALT activity. Therefore, muscles should be considered as a potential source of elevation in serum enzyme activity, since total muscle mass is much greater than liver mass[21].

The highest serum ALP values in animals up to six months of age (G1 and G2), accompanied by a significant gradual reduction, are correlated with the release of large amounts of bone isoenzymes into the bloodstream, due to intense osteoblastic activity in animals during active bone growth[3, 6]. Our results are similar to other studies with young, growing cattle[9, 19, 22]. According to the literature, serum concentration of ALP is two to three times higher in young animals due to intense osteoblastic activity in animals in the growth phase[23].

Significant reduction of GGT from G1 to G4 is due to the remaining enzyme ingested in colostrum, as there were 15-day-old calves in G1. Studying Senepol calves, Delfino et al.[10] found a higher value of GGT in young animals up to 15 days of age and attributed it to a greater absorption via colostrum, that is, a high concentration of enzyme. These findings are similar to those of several authors with different breeds of cattle[9, 19, 21]. Conversely, Coppo et al.[22] did not find such a significant difference in GGT between age groups.

When males and females were within each age group, G2 males had higher ALB concentrations. This difference may be related to a normal physiological variation, as there were no significant differences between sexes neither for TP and Glob nor for the A: G ratio in G2. In G4, ALB was statistically superior in females, while Glob was higher in males. The latter is attributed to anabolic hormones such as testosterone and diethylstilbestrol (DES) in males. These hormones are responsible for the reduction in albumin and increase in globulin[6]. In addition, higher serum ALB concentration in females determined their higher A: G ratio than in males, since ALB concentration increases as the A:G ratio increases. Borges et al.[9] evaluated Pantaneira breed cattle and reported no effect of sex on protein profile.

The superior serum Crea concentrations in males of G4 are related to their greater muscle mass compared to females. For Knowles et al.[12], serum Crea concentrations are directly related to muscle mass. However, other authors, such as Borges et al.[9] and Gregory et al.[11], observed no influence of the sex factor on serum Crea concentrations in cattle.

As for urea, the highest concentration in G1 males may be related to increased protein metabolism, as males have growth and weight gain higher than females. It is worth mentioning that male Bonsmara calves in this study had higher birth weights and at 60 days of age compared to female calves, unlike Borges et al.[9] and Gregory et al.[11], who observed higher urea values in females compared to males.

The lower serum concentration of TRI in G2 males may be related to higher energy demand by males. In this age group, males show greater weight gains than females, as they grow faster. The same condition was observed in G4, where serum TRI value in females was lower than in males, even though females grow more than males in this age group. Pogliani and Birgel Júnior[15] also observed the influence of the sex factor on serum levels of TRI in Holstein cattle.

Regarding the mineral profile, higher serum Ca^+ values in G2 females may be due to males having faster growth and greater requirement of this mineral for activation of metabolism and bone growth. These factors were also determinants for lower serum concentrations of Ca^+ in females of G4 because within this age group males have slower growth. Given the static nature of the Ca^+:iP ratio in bones, Ca^+ metabolism may affect blood iP concentrations. This nature also determined the higher iP values in G1 males and G4 females. Significant differences between males and females in the Ca^+:iP ratio are due to differences in serum iP values between G1 and G4 and Ca^+ between G2 and G4. The higher Mg concentrations in G4 males may be following the behavior of Ca^+ in the same group, which determined a higher value of Mg in males in the general group (15 days to 24 months), which can be considered a normal physiological variation.

Studies about the influence of sex factors on serum enzyme activity are scarce, but our findings are consistent with those of Coppo, Coppo, and Lazarte[14]; Fagliari et al.[19]; and Coppo et al.[22], who also noted significant differences in serum concentrations of AST and GGT between males and females. It should be noted that ours is one of the first studies in Brazil on the serum biochemical profile of Bonsmara cattle in the growth phase, and may serve as a stimulus for further studies on the subject of this breed.

Conclusion

The factors of age group and sex significantly influenced the concentrations of serum biochemical constituents in Bonsmara cattle in the growth phase.

Conflict of Interests

The authors declare no conflict of interest.

Author contributions

Conceptualization: F. C. Barbosa, W. J. Oliveira, J. G. K. Faria, D. S. Vieira, E. C. Guimarães, A. V. Mundim; *Data curation*: F. C. Barbosa, W. J. Oliveira; *Formal Analysis*: F. C. Barbosa, E. C. Guimarães, A. V. Mundim; *Investigation*: F. C. Barbosa, W. J. Oliveira, J. G. K. Faria, D. S. Vieira; *Methodology*: F. C. Barbosa; A. V. Mundim; *Project administration*: F. C. Barbosa, W. J. Oliveira, J. G. K. Faria, D. S. Vieira; *Resources*: F. C. Barbosa; A. V. Mundim; *Supervision*: F. C. Barbosa, A. V. Mundim; *Validation*: F. C. Barbosa, E. C. Guimarães, A. V. Mundim; *Visualization*: F. C. Barbosa, A. V. Mundim; *Writing – original draft*: F. C. Barbosa; A. V. Mundim; *Writing – review & editing*: F. C. Barbosa; A. V. Mundim

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