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Protein, enzymatic and mineral indicators of clinical and subclinical pregnancy toxemia during the transitional period in dairy goats

Indicadores proteicos, enzimáticos e minerais da toxemia da prenhez clínica e subclínica durante o período de transição de cabras leiteiras

Rodolfo José Cavalcanti Souto[1*], Gliere Silmara Leite Soares[1], Alexandre Tadeu Mota Macedo[1], Jobson Filipe de Paula Cajueiro[1], Udhanysson Felipe dos Santos[1], Pierre Castro Soares[1], José Augusto Bastos Afonso[1], Carla Lopes de Mendonça[1]

[1]Universidade Federal Rural de Pernambuco (UFRPE), Clínica de bovinos de Garanhuns, Pernambuco, Brazil.
[*]Corresponding author: rodolfojcsouto@gmail.com

Abstract
The purpose of this study was to evaluate the influence of clinical and subclinical pregnancy toxemia (PT) forms on the dynamics of blood metabolites, composing the protein, enzymatic and mineral profiles of dairy goats during the transitional period. 111 multiparous dairy goats were used in this research. The animals were raised under an intensive system. Experimental groups (n=3) were created using the βHB blood concentrations as a cut-off point. The G1 or control group (n = 40), G2 or subclinical PT group (n = 39) were established when least one of the experimental assessment times presented βHB values between 0.8 mmol/L and 1.6 mmol/L; whereas G3 or clinical PT group (n = 32), which at any of the experimental assessment times of βHB values were higher than 1.6 mmol / L when verified and also presented clinical signs of PT. The animals were evaluated on the 30th , 20th and 10th day *ante-partum* (dap), at parturition and on the 10th, 20th and 30th day *postpartum* (dpp). Total proteins (TP), albumin, globulin, urea, creatinine, aspartate aminotransferase (AST), Gamma glutamiltransferase (GGT), creatine kinase (CK), amylase, phosphorus, chloride and calcium, sodium and potassium ions were measured. The analysis of variance (F Test) was performed in order to investigate the effects and interactions between group and assessment times. Clinical and/or subclinical disease during the transitional period resulted in an increase of phosphorus and potassium concentrations (P<0.05), while a decrease in total protein, albumin, globulin, and ionized calcium values was observed (P<0.05). No statistical effect of PT on urea, creatinine, AST, GGT, CK, amylase and sodium was observed (P>0.05). Except the ionized calcium and phosphorus, the levels of all the metabolites were influenced (P<0.05) by late pregnancy, parturition and lactation. The occurrence of the subclinical form was higher than the clinical form during the transitional period. Among the all variables studied, mineral profile were those that suffered alteration resulting from PT, highlighting the ionized calcium. Attention is drawn to the magnitude of the impact of disease on these components interfering in animal health.
Keywords: goats; ketosis; late pregnancy; lactation; blood metabolites

Resumo
O objetivo deste estudo foi avaliar a influência das formas clínica e subclínica da toxemia da prenhez (TP) na dinâmica dos metabólitos sanguíneos, compondo os perfis proteico, enzimático e mineral das cabras leiteiras durante o período de transição. Foram utilizadas 111 cabras multíparas que eram criadas em sistema intensivo. Grupos experimentais (n=3) foram criados utilizando diferentes concentrações séricas de βHB como ponto de corte, o G1 grupo controle (n=40), G2 grupo subclínico da TP (n=39) estabelecido quando pelo menos um dos momentos experimentais apresentou valores de βHB entre 0,8 mmol/L e 1,6 mmol/L; e o grupo G3, com manifestação clínica da TP (n=32), que em qualquer um dos momentos os valores de βHB foram superiores a 1,6 mmol/L. Os animais foram avaliados aos 30º, 20º e 10º dias antes do parto (dap), no parto e no 10º, 20º e 30º dia pós-parto (dpp). Proteínas totais (PT), albumina, globulina, ureia, creatinina, aspartato aminotransferase (AST), gama glutamiltransferase (GGT), creatina quinase (CK), amilase, fósforo, cloreto e cálcio, sódio e potássio foram mensurados. A análise de variância (Teste F) foi realizada com o objetivo de investigar os efeitos e interações entre os tempos dos grupos. A doença clínica e/ou subclínica resultou num aumento das concentrações de fósforo e potássio (P<0,05), foi observada uma diminuição nos valores de proteína total, albumina, globulina e cálcio ionizado (P<0,05). Não houve efeito estatístico da TP sobre a uréia, creatinina, AST, GGT, CK, amilase e sódio (P>0,05). A ocorrência da forma subclínica foi maior que a clínica durante o período de transição. As variáveis que compõem o perfil mineral foram as que apresentaram alterações decorrentes da TP, destacando-se, o cálcio ionizado. Chama-se a atenção o impacto que a doença reflete nesses componentes e na saúde do animal.
Palavras-chave: caprinos; cetose; final da gestação; lactação; metabólitos sanguíneos.

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1. Introduction

With the greater dissemination of technologies in the production of milk from goats and the adoption of management practices such as feedlotting, which require greater productivity and individual efficiency from the animals, the risk of occurrence of metabolic diseases increases, especially during the transition period[1,2]. This period is considered the most critical for the female, as she goes from a pregnant and non-lactating state to a non-pregnant and lactating state, which leads to stress caused by large and abrupt changes in metabolism, anatomy, and physiology[3].

Metabolic disorders arise in this period as a result of the challenges and lack of planning in food management, which increase the likelihood of their emergence, with emphasis on pregnancy toxemia (PT)[4]. Pregnancy toxemia is the most important multifactorial disorder of energy metabolism in small ruminants, usually occurring at different stages of the pregnancy-lactation cycle[5,6]. The disease can be classified based on its degree of severity, according to the biochemical profile of β-hydroxybutyrate (βHB) and clinical signs[7]. However, PT can also occur in the subclinical form, which is defined as a preclinical stage of the disease, characterized by an increase in the levels of ketone bodies in the blood without the clinical manifestations of the disease[8].

Due to the economic and social importance of goat milk farming in the semi-arid region of northeastern Brazil and the impact of PT on this production, it is necessary to understand the aspects of this metabolic disorder in clinical and subclinical forms on blood metabolites during the transition period. The current study aimed to evaluate protein, enzymatic, and mineral blood alterations in dairy goats with PT in the clinical and subclinical forms before, during, and after parturition.

2. Material and methods

The study was carried out in eight rural properties characterized as performing dairy activities, located in the semi-arid region of the state of Pernambuco, Brazil. Laboratory analyses were carried out at the Bovine Clinic of Garanhuns (CBG/UFRPE). The research obtained a favorable opinion from the Commission for Ethics in the Use of Animals (CEUA) of the Federal Rural University of Pernambuco (approval no. 070/2016 CEPE/UFRPE), complying with the regulations of the National Council for the Control of Animal Experimentation (CONCEA) and the Guide for Care and Use of Laboratory Animals of the National Institute of Health.

In total, 111 multiparous dairy goats were clinically monitored on all properties during the transition period, and the vast majority were twin pregnancies. The goats were crossbred Saanen, Alpine Brown, American Alpine, and Toggenburg breeds. Only goats with pregnancy toxemia or ketosis due to metabolic/nutritional disease excluding intercurrent diseases were used. Body condition score (BCS) was evaluated on a scale of 1-5[9]. These animals were raised in an intensive regime in which they received a diet composed of fresh sugar cane (*Saccharum sp*), sugarcane bagasse, palm (*Opuntia tuna* (*L.*) *Mill*), corn, wheat, cotton, and soybean meal. Nutritional management was similar on all properties, with the diet being prepared to meet nutritional needs during pregnancy and lactation periods. Mineral salt and water were provided *ad libitum*. The animals were systematically vaccinated and dewormed according to the protocol adopted on each farm.

2.1 Experimental design

The following experimental time points were established: 30, 20, and 10 days before kidding (dap); time of kidding; and 10, 20, and 30 days postpartum (dpp). The goats underwent ultrasonographic examination (Ultrasson GE - Logiq 100 Pro 2, Milwaukee, USA) for diagnosis and determination of the gestation period.

The goats were distributed into three experimental groups based on the result of the serum concentration of βHB[10,11] as well as whether or not clinical signs of PT were present. Goats that showed βHB values < 0.8 mmol/L at all evaluation time points comprised the G1 or control group (n=40). Those which exhibited βHB values between 0.8 and 1.6 mmol/L at least one of the experimental time points composed the G2 or subclinical group (n=39), and those which displayed βHB values greater than 1.6 mmol/L at any of the experimental time points, including the presence of clinical signs, made up the G3 or clinical group (n=32). It is worth mentioning that the animals in G1 and G2 did not show a clinical framework of PT at any of the evaluation time points, while the goats in G3 demonstrated a clinical framework of variable intensity, such as lack of appetite, apathy, edema of the limbs, nervous symptoms, and a drop in milk production. In these cases, a therapeutic intervention was recommended when necessary, involving oral propylene glycol administration, calcium borogluconate intravenously and subcutaneously, as well as 5% glucose solution also intravenously[12,13].

2.2 Collection, storage, and laboratory processing of samples

Blood samples were collected by jugular venipuncture with a 25x8 mm needle in tubes without anticoagulant (serum collection) for biochemical analysis. Subsequently, the samples were centrifuged

(Centrifuga Fanem Ltda, Baby I, Mod. 206, Brazil) at 3600 rpm/10 min. Serum aliquots were placed in polyethylene Eppendorf tubes and stored in an ultrafreezer at -80 °C (Ultralow freezer NuAire Inc., 2100, USA).

To evaluate the protein, enzymatic, and mineral metabolites, the following variables were used: total protein (TP) by the biuret method, albumin by the bromocresol green method, globulin by the difference between TP and albumin, urea (CE Urea), creatinine, aspartate aminotransferase (AST - Liquiform), gamma glutamyltransferase (GGT - Liquiform), creatine kinase (CK - NAC Liquiform), amylase (CNPG - Liquiform), phosphorus (P, UV - Liquiform), and chlorides (Chlorides Liquiform). All variables were determined by routine analyses using a semi-automatic biochemical analyzer (Bioplus 2000, Bioplus Produtos de Laboratório Ltd., Brazil) at 37 °C, using commercial kits (Labtest Diagnóstica S.A, Brazil). The ionizable calcium (Ca^{2+}), potassium (K^{+}), and sodium (Na^{+}) ions were determined using an electrolyte analyzer (Electrolyte Analyzer, Roche 9180 - Mannheim/Germany).

2.3 Statistical analysis

Data are described using means and standard deviations. The parameters were initially tested for their normal distribution, using the Kolmogorov-Smirnov test. Those which did not meet the normality assumptions underwent transformation with a logarithmic base (LogX+1) or by the square root [SR (X+1/2)]. The data that met the assumptions of normality or were transformed were later subjected to analysis of variance (F test) as repeated measures over time points. This analysis effectively partitioned the sources of variation, considering the effects of three distinct groups (G1, G2, and G3) and their interactions with time points spanning the pre-kidding, kidding, and post-partum periods, as this is a split-plot experiment with completely randomized groupings that aimed to investigate the effects and interactions between them. When significance was detected by the F test, the means were compared by the least significant difference (LSD) of the Student-Newman-Keuls test. Data were analyzed using the Statistical Analysis System [14] computer program.

3. Results

3.1 Clinical observations

The goats in the G3 group showed a clinical framework in the prepartum period that lasted an average of 10 days, displaying signs such as lack of appetite, apathy, dehydration, mucous congestion, prolonged recumbency, edema of the limbs, and nervous symptoms such as opisthotonos and teeth grinding. During the lactation period, the clinical manifestations were less intense than those found during the gestation period, which can be observed through the behavior of the serum concentration of βHB between groups and time points (Table 1). Thus, when any clinical sign was present, all the goats in G3 were medicated according to the duration of the symptoms. Among these, 20 were medicated during pregnancy and the remainder during lactation. No change was observed in BCS over the evaluation time points, with values remaining between 3.0 and 3.5, regardless of the group.

Table 1. Effect of treatments (G1, G2, and G3), collection time points, and the effects of βHB in healthy goats (G1), goats with subclinical PT (G2), and goats with clinical PT (G3) during late pregnancy and early lactation (mean ± standard deviation).

Energy profile	Group	Evaluation time point							OM	Source of variation		
		30 dap	20 dap	10 dap	P	10 dpp	20 dpp	30 dpp		Groups	Time points	G x T
βHB (mmol/L)	G1	0.4±0.13^{Ba}	0.4±0.13^{Ba}	0.4±0.10^{Ba}	0.4±0.14^{Ba}	0.4±0.14^{Ca}	0.4±0.13^{Ca}	0.4±0.11^{Ba}	-	<0.0001	0.0064	<0.0001
	G2	0.5±0.18^{ABb}	0.6±0.35^{Bab}	0.6±0.34^{Bab}	0.7±0.34^{Ba}	0.7±0.29^{Ba}	0.7±0.26^{Ba}	0.7±0.30^{Aa}				
	G3	0.5±0.28^{Ab}	1.6±1.62^{Aa}	1.6±1.73^{Aa}	1.6±2.27^{Aa}	1.3±1.28^{Aab}	1.0±0.98^{Aab}	0.7±0.47^{Aab}				

βHB: beta-hydroxybutyrate; dap: days *antepartum*; dpp: days *postpartum*; GM: overall mean; a,b: Different lowercase letters in the same row differ from each other (P<0.05), characterizing a time point effect. A,B: Different uppercase letters in the same column differ statistically from each other (P<0.05), characterizing a group effect.

3.2 Protein profile

Lower TP, albumin, and globulin concentrations were observed in groups G2 and G3, when compared to G1 (P<.0001). As for the behavior of these variables during the transition period, there was an increase in the concentration of both (P<.0001) during the lactation period (Table 2). During the transition period, there was a decrease in albumin values close to parturition (P<.0001), with a gradual increase during lactation (Table 2).

There was no significant difference in urea and creatinine values between the three analyzed groups (P>0.05). As for the time point effect, there was a slight increase in urea concentration (P=0.0006) and a decrease in creatinine (P<.0001) in the postpartum period (Table 2).

Table 2. Mean and standard deviation values (x±s) of the blood serum protein profile variables of healthy goats (G1), goats with subclinical pregnancy toxemia (G2), and goats with clinical pregnancy toxemia (G3) monitored during the transition period.

Protein profile	Group	Evaluation time point							OM	Source of variation (Pr>F)		
		30 dap	20 dap	10 dap	P	10 dpp	20 dpp	30 dpp		Groups	Time points	G x T
TP(g/dL)	G1	7.28±0.71	7.21±0.71	7.18±0.95	7.22±0.91	7.75±0.69	7.95±0.63	8.08±0.65	7.51^{A}			
	G2	6.98±1.00	6.89±1.05	6.63±0.97	6.86±0.82	7.31±0.72	7.64±1.00	7.96±0.97	7.18^{B}	<.0001	<.0001	0.9383
	G3	6.72±1.02	6.48±0.95	6.59±0.88	6.67±0.73	7.39±0.85	7.56±0.97	7.70±0.79	7.03^{B}			
	OM	7.05^{c}	6.91^{c}	6.82^{c}	6.93^{c}	7.49^{b}	7.73^{a}	7.92^{a}				
Albumin (g/dL)	G1	2.53±0.32	2.47±0.25	2.40±0.33	2.42±0.33	2.47±0.35	2.56±0.36	2.58±0.35	2.49^{A}			
	G2	2.49±0.42	2.37±0.44	2.26±0.40	2.30±0.42	2.35±0.39	2.44±0.44	2.47±0.48	2.38^{B}	<.0001	<.0001	0.9798
	G3	2.53±0.24	2.25±0.29	2.21±0.29	2.20±0.34	2.29±0.26	2.35±0.35	2.41±0.36	2.32^{B}			
	OM	2.51^{a}	2.38^{ab}	2.3^{b}	2.31^{b}	2.37^{ab}	2.46^{a}	2.5^{a}				
Globulin (g/dL)	G1	4.76±0.68	4.74±0.70	4.79±0.88	4.80±0.93	5.28±0.69	5.39±0.69	5.50±0.70	5.02^{A}			
	G2	4.49±0.96	4.52±0.96	4.36±0.82	4.56±0.77	4.90±0.59	5.21±0.88	5.45±0.98	4.79^{B}	<.0001	<.0001	0.8828
	G3	4.19±1.04	4.23±0.85	4.38±0.73	4.46±0.58	5.10±0.83	5.21±0.92	5.28±0.81	4.71^{B}			
	OM	4.53^{c}	4.53^{c}	4.52^{c}	4.62^{c}	5.09^{b}	5.27^{ab}	5.42^{a}				
Urea (mg/dL)	G1	51.53±17.65	50.99±17.39	49.15±15.41	50.98±17.29	54.21±17.69	51.97±14.98	58.08±27.17	52.32^{A}			
	G2	49.63±17.37	43.96±13.77	46.60±12.59	50.22±21.43	57.37±20.68	55.36±18.40	59.68±26.58	51.96^{A}	0.7289	0.0006	0.7449
	G3	57.30±25.15	45.52±24.52	46.21±21.46	49.50±22.37	62.58±19.64	56.75±21.53	58.41±23.51	53.69^{A}			
	OM	52.23^{abc}	47.13^{c}	47.44^{bc}	50.28^{abc}	57.69^{a}	54.57^{ab}	58.75^{ab}				
Creatinine (mg/dL)	G1	0.67±0.12	0.72±0.13	0.74±0.13	0.76±0.17	0.61±0.12	0.63±0.11	0.64±0.13	0.68^{A}			
	G2	0.79±0.17	0.73±0.13	0.77±0.14	0.72±0.14	0.64±0.14	0.65±0.13	0.64±0.12	0.70^{A}	0.1913	<.0001	0.2572
	G3	0.77±0.19	0.72±0.15	0.74±0.19	0.71±0.17	0.64±0.18	0.60±0.12	0.62±0.17	0.68^{A}			
	OM	0.74^{a}	0.72^{a}	0.75^{a}	0.73^{a}	0.63^{b}	0.63^{b}	0.63^{b}				

Distinct lowercase letters in the same row differ statistically from each other (P<0.05) characterizing a time point effect. Different uppercase letters in the same column differ statistically from each other (P<0.05).

3.3 Enzyme profile

Regarding the group effect, no statistically significant differences (P>0.05) were observed in the mean values of serum AST, GGT, CK, and amylase activity between the studied groups. However, when considering the time point effect, AST and GGT exhibited a similar pattern, with a significant increase in serum enzyme activity observed during lactation (P<0.05) (Table 3). With regard to CK, there was an increase in concentration at parturition, which remained stable throughout the lactation period (P<0.0001). Meanwhile, amylase displayed an initial slight increase at parturition, remaining high at the beginning of lactation (P=0.0002) (Table 3).

Table 3. Mean values and standard deviation (x±s) of the enzymatic profile variables in the blood serum of healthy goats (G1), goats with subclinical pregnancy (G2), and goats with clinical pregnancy toxemia (G3) monitored during the transition period.

Enzyme profile	Group	Evaluation time point							OM	Source of variation (Pr>F)		
		30 dap	20 dap	10 dap	p	10 dpp	20 dpp	30 dpp		Groups	Time points	G x T
AST(U/L)	G1	79.12±18.12	81.05±40.55	101.94±102.41	101.69±90.79	108.84±57.89	108.15±59.57	91.82±35.88	95,67^{A}			
	G2	84.14±28.16	85.07±29.21	80.13±23.81	86.04±25.50	112.21±63.19	105.36±54.90	104.91±71.19	94,35^{A}			
	G3	80.24±26.48	81.19±21.94	84.53±24.76	100.64±43.50	132.07±100.20	95.41±30.19	84.56±23.20	94,62^{A}	0,9399	<.0001	0,7022
	OM	81.1^{c}	82.46^{c}	89.45^{c}	95.94^{bc}	116.47^{a}	103.47^{ab}	94.45^{bc}				
GGT (U/L)	G1	55.85±44.78	50.13±36.26	53.16±50.42	59.40±82.89	57.80±44.44	62.10±41.98	59.18±23.93	56,61^{A}			
	G2	50.59±15.56	51.00±13.88	49.41±20.20	49.38±13.12	58.99±20.28	64.92±22.83	68.41±28.38	56,25^{A}			
	G3	46.59±23.11	40.58±10.68	43.53±9.61	46.21±8.92	60.11±22.11	62.29±18.59	61.75±17.42	51,99^{A}	0,0617	<.0001	0,6961
	OM	51.89^{b}	48.1^{b}	49.18^{b}	52.03^{b}	58.88^{a}	63.17^{a}	63.25^{a}				
CK(U/L)	G1	167.18±88.87	154.37±74.89	178.71±105.64	187.85±97.93	189.56±127.43	173.57±72.03	189.29±69.15	176,75^{A}			
	G2	135.32±58.23	152.87±89.67	161.44±77.25	205.32±177.95	183.49±97.41	206.77±89.21	208.17±88.41	179,50^{A}			
	G3	140.19±94.19	138.50±85.43	149.89±73.46	209.57±161.96	213.36±144.27	214.23±110.81	184.74±93.79	179,98^{A}	0,8282	<.0001	0,6118
	OM	150.02^{c}	149.61^{c}	164.67^{bc}	200.22^{ab}	193.83^{ab}	197.29^{a}	194.79^{a}				
Amylase(U/L)	G1	132.95±52.62	121.18±49.56	131.92±51.98	137.55±53.18	158.90±66.50	156.90±81.63	152.98±73.00	141,18^{A}			
	G2	124.71±59.88	120.19±50.63	126.85±45.09	137.70±54.18	156.73±63.42	138.86±41.13	136.00±50.05	134,81^{A}	0,7027	0,0002	0,9997
	G3	121.03±45.66	116.28±40.57	128.77±54.63	149.02±74.09	153.20±70.33	145.90±55.36	151.35±68.30	138,55^{A}			
	OM	127.36^{bc}	119.53^{c}	129.26^{bc}	140.98^{ab}	156.52^{a}	147.5^{ab}	146.33^{ab}				

Distinct lowercase letters in the same row differ statistically from each other (P<0.05), characterizing a time point effect. Different uppercase letters in the same column differ statistically from each other (P<0.05), characterizing a group effect.

3.4 Mineral profile

With respect to Ca^{2+}, there was a significant difference (P<.0001) between groups G3 (0.66 mmol/L) and G2 (0.84 mmol/L) in relation to G1 (1.12 mmol/L), with G3 showing the lowest value. During the evaluation period, no significant difference was observed between the time points (P=0.4997), (Table 4; Figure 1).

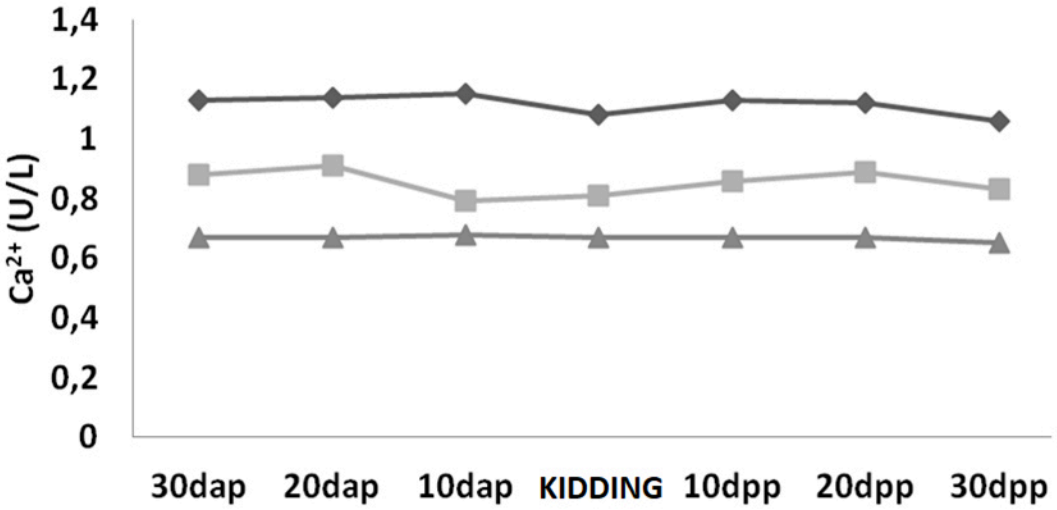

Figure 1. Mean values of ionized calcium (mmol/L) in the blood serum of healthy dairy goats (G1 n=40), goats with subclinical pregnancy toxemia (G2 n=39), and goats with clinical pregnancy toxemia (G3 n=32) monitored before, during, and after kidding.

Phosphorus concentrations were higher in goats in G3 compared to G1 (P=0.0422). Group 2 showed intermediate values between G3 and G1, with no statistical difference from either. With regard to the time points, there was no significant difference between them (P=0.1630) (Table 4; Figure 2).

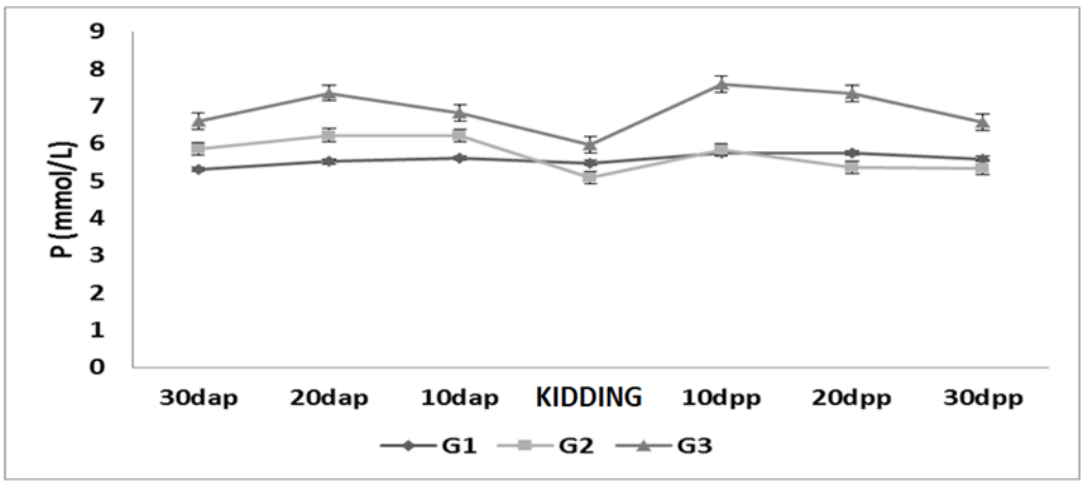

Figure 2. Mean values of phosphorus (mmol/L) in the blood serum of healthy dairy goats (G1 n=40), goats with subclinical pregnancy toxemia (G2 n=39), and goats with clinical pregnancy toxemia (G3 n=32) monitored before, during, and after kidding.

Serum Cl^- levels were significantly lower in G3 compared to G2 and G1 (P=0.0095). As for the time point effect, a significant decrease (P<.0001) was observed in the mean values in the postpartum period (Table 4, figure 3).

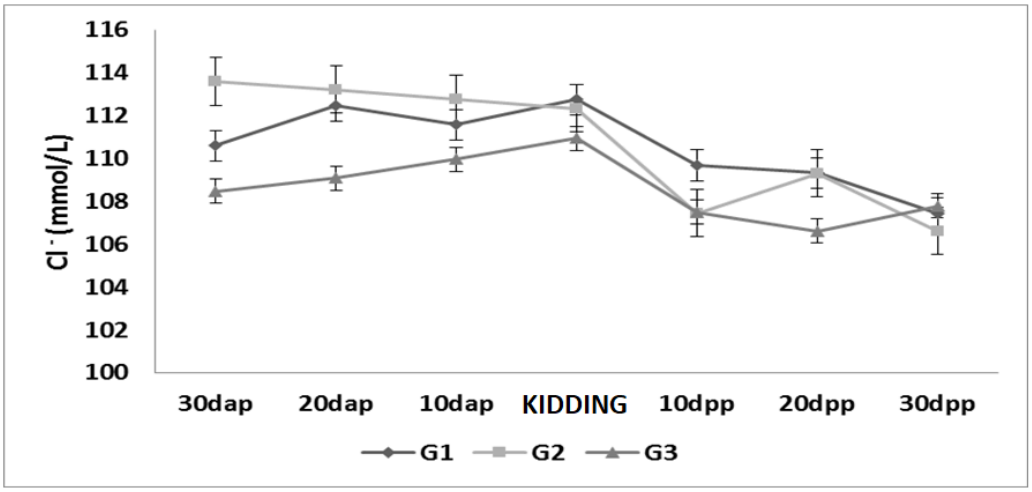

Figure 3. Mean values of chloride (mmol/L) in the blood serum of healthy dairy goats (G1 n=40), goats with subclinical pregnancy toxemia (G2 n=39), and goats with clinical pregnancy toxemia (G3 n=32) monitored before, during, and after kidding.

Table 4. Mean values and standard deviation (x±s) of the mineral profile variables in the blood serum of healthy goats (G1), goats with subclinical pregnancy toxemia (G2), and goats with clinical pregnancy toxemia (G3) monitored during the transition period.

Mineral profile	Group	Evaluation time point							OM	Source of variation (Pr>F)		
		30 dap	20 dap	10 dap	p	10 dpp	20 dpp	30 dpp		Groups	Time points	G x T
Ca^{2+} (mmol/L)	G1	1.13±0.10	1.14±0.09	1.15±0.10	1.08±0.09	1.13±0.13	1.12±0.11	1.06±0.12	1.12^{A}			
	G2	0.88±0.18	0.91±0.07	0.79±0.15	0.81±0.16	0.86±0.08	0.89±0.07	0.83±0.14	0.84^{B}	<.0001	0.4997	0.4023
	G3	0.67±0.16	0.64±0.17	0.68±0.13	0.67±0.12	0.67±0.12	0.67±0.12	0.65±0.17	0.66^{C}			
	OM	0.87^{a}	0.85^{a}	0.89^{a}	0.82^{a}	0.85^{a}	0.84^{a}	0.81^{a}				
P (mmol/L)	G1	2.03±0.76	2.08±0.54	2.07±0.55	1.90±0.70	1.83±0.55	1.81±0.58	1.87±0.68	1.95^{B}			
	G2	2.23±0.58	2.34±0.56	2.28±0.55	1.96±0.66	2.21±0.62	2.06±0.70	2.00±0.60	2.16^{AB}	0.0422	0.163	0.1845
	G3	2.21±0.50	2.44±0.68	2.26±0.55	1.99±0.69	2.50±0.71	2.38±0.97	2.17±0.52	2.72^{A}			
	OM	2.14^{a}	3.13^{a}	2.20^{a}	1.95^{a}	2.16^{a}	2.10^{a}	2.00^{a}				
Cl^- (mmol/L)	G1	110.60±4.65	112.46±8.41	111.58±5.21	112.76±7.38	109.69±7.18	109.34±8.67	107.45±8.55	110.60^{A}			
	G2	113.60±9.93	113.23±9.63	112.78±7.94	112.33±7.71	107.44±7.27	109.32±12.02	106.63±5.87	110.67^{A}	0.0095	<.0001	0.6233
	G3	108.48±6.04	109.08±7.40	109.96±4.85	110.94±5.77	107.50±8.37	106.62±4.64	107.80±7.54	108.63^{B}			
	OM	111.12^{a}	111.82^{a}	111.56^{a}	112.07^{a}	108.25^{b}	108.55^{b}	107.25^{b}				
Na^+ (mmol/L)	G1	143.95±8.85	146.55±4.09	146.03±4.71	144.44±11.2	144.50±5.67	143.08±9.03	144.23±7.23	144.72^{A}			
	G2	146.58±3.96	146.59±4.38	147.27±7.43	147.25±6.59	143.11±8.73	142.80±5.15	143.31±5.91	145.21^{A}	0.4727	<.0001	0.7588
	G3	145.09±5.78	146.65±8.21	147.38±8.91	147.68±5.64	145.26±3.79	143.39±5.66	143.00±5.61	145.51^{A}			
	OM	145.1^{ab}	146.59^{a}	146.84^{a}	146.36^{a}	144.18^{a}	143.07^{b}	143.55^{b}				
K (mmol/L)	G1	4.37±0.42	4.48±0.55	4.43±0.31	4.38±0.54	4.29±0.72	4.26±0.69	4.08±0.49	4.33^{B}			
	G2	4.52±0.27	4.70±0.34	4.60±0.38	4.47±0.43	4.34±0.60	4.32±0.58	4.24±0.44	4.45^{A}	0.0029	<.0001	0.8309
	G3	4.58±0.24	4.73±0.47	4.44±0.45	4.43±0.53	4.44±0.36	4.35±0.42	4.40±0.55	4.48^{A}			
	OM	4.47^{ab}	4.62^{a}	4.49^{ab}	4.42^{b}	4.35^{bc}	4.31^{bc}	4.23^{c}				

Distinct lowercase letters in the same row differ statistically from each other (P<0.05), characterizing a time point effect. Different uppercase letters in the same column differ statistically from each other (P<0.05), characterizing a group effect.

There was no statistically significant difference between groups regarding Na^+ concentrations (P=0.4727). However, mean values during lactation were lower (P<.0001) in relation to kidding and pre-kidding (Table 4).

In relation to K^+, higher and significant values were found in groups G3 and G2 compared to G1 (P=0.0029). It was found that the levels decreased in the postpartum period, highlighting the significant difference found at 30 dpp (P<.0001) (Table 4; Figure 4).

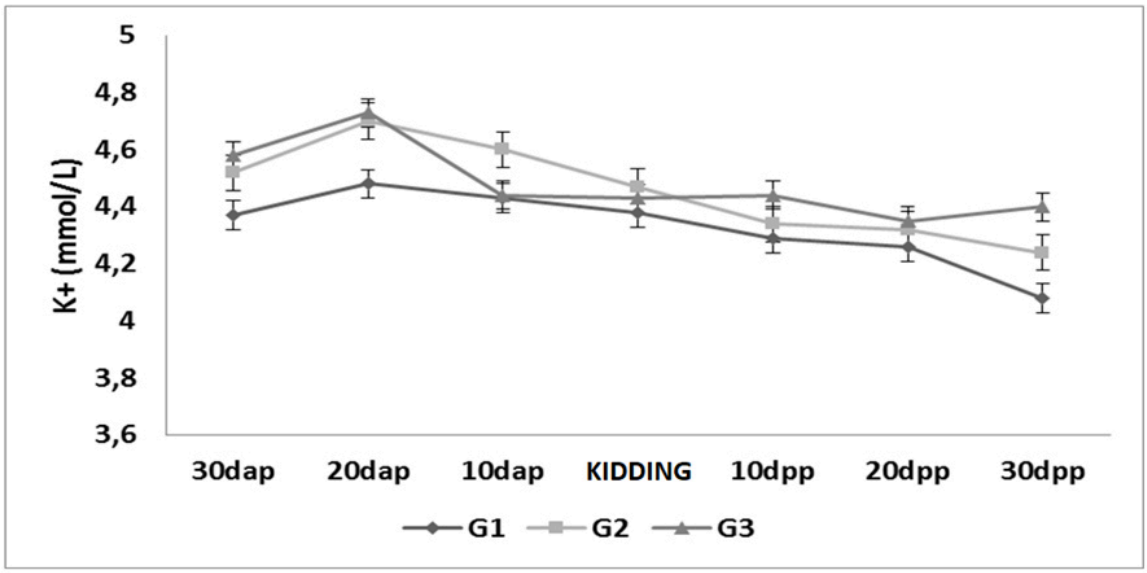

Figure 4. Mean values of potassium (mmol/L) in the blood serum of healthy dairy goats (G1 n=40), goats with subclinical pregnancy toxemia (G2 n=39), and goats with clinical pregnancy toxemia (G3 n=32) monitored before, during, and after kidding.

4. Discussion

4.1 Clinical observations

The clinical signs found in the animals of the G3 group are compatible with the findings of Hefnawy et al.[15], Souto et al.[16], and Vasava et al[17]. The greater intensity of these symptoms may be related, among other factors, to the higher concentration of βHB during the gestation period in relation to lactation. Therefore, βHB values are essential for the diagnosis of clinical and subclinical PT in dairy goats, especially in view of the characteristic of nonspecific symptoms[18].

4.2 Protein profile

The lower values of TP, albumin, and globulin in animals with clinical and subclinical PT, when compared to the control group, demonstrated the influence of the metabolic disorder on these variables. However, the absence of changes in the concentration of TP and albumin has been reported in the clinical form[16, 19] and in the subclinical form of PT[8].

The behavior of the protein fractions, which were similar throughout the transition period, is comparable to the results of Bockor[20] and Santos et al.[21], who stated that the reduction in TP before kidding is due to the transfer of immunoglobulins to the colostrum, which are recovered after kidding. Karapehlivan et al.[22] also found higher concentrations of TP, albumin, and globulin during the lactation period in ewes compared to the dry period.

The absence of statistical differences between the three groups demonstrates that urea is not a good biomarker for monitoring subclinical and clinical PT in herds of dairy goats. According to Souto et al.[16], this is due to a high degree of recycling of this metabolite between the blood and the rumen. This finding, particularly in clinical cases, may be in response to the therapeutic intervention performed in view of the observed symptoms.

However, Ismail et al.[11] found significantly higher urea concentrations in goats with subclinical PT when compared with animals in the control group, due to the mobilization of proteins that occurs at the end of pregnancy. The findings of Barakat et al.[23] and Souto et al.[16] support this observation, as they attributed the increase in serum urea levels in clinical PT in goats to fatty infiltration in the tubular epithelium of the kidneys. This infiltration leads to azotemia, which is associated with a decrease in glomerular filtration rate and a consequent reduction in the urinary excretion of this compound.

Regarding the higher postpartum urea

concentration, despite not being very significant, it is likely due to the reestablishment of the clinical condition of most animals, with consequent improvement in appetite and greater production of ammonia in the ruminal environment. In addition, according to Pichler et al.[24], the energy needs increase considerably with the onset of lactation. As mentioned by Kozloski[25], in this condition, the rate of protein synthesis in the muscle is lower than the rate of degradation, and amino acids are oxidized and, mainly, released into the bloodstream.

The absence of statistical differences for creatinine between the groups demonstrates that, under the conditions of this study, this variable was not a good parameter in the determination of clinical and subclinical PT, since despite being an indicator of renal failure, this variable only increases when 75% of nephrons are compromised[26]. On the other hand, studies have found an increase in creatinine concentration in the clinical form of PT in goats[16,17], in ewes[27, 28], and in the subclinical form of ketosis in cows[29], which is related to the decrease in the glomerular filtration rate and consequent reduction in the urinary excretion of this compound.

Other authors working with goats[30], cows[31], and ewes[21] have similarly reported higher creatinine values during the final period of pregnancy in comparison to the beginning of lactation. This increase has been linked to maternal protein mobilization, which serves both muscle development and the elimination of fetal waste.

4.3 Enzyme profile

With regard to AST, these findings are consistent with those found by Ismail et al.[11] who reported that the serum activity of this enzyme in goats with subclinical PT showed no difference in relation to the control group. The authors related this result to lower ketonemia intensity and to the fact that there may be variations in fat metabolism and liver susceptibility to insults between different ruminant species. This interpretation is also reported by Morali[32], who stated that alterations in the serum activity of liver enzymes are generally not useful for the diagnosis of PT, since they are often within the normal range. Contrasting results were found by Albay et al.[33] and Vasava et al.[17], in which serum AST activity was increased in goats with subclinical and clinical PT. The authors attributed this finding to fatty infiltration of the liver as a function of negative energy balance (NEB).

The increase in the activity of this enzyme after parturition has no clinical significance, since the average values remained within the normal range for the species[34]. This dynamic is compatible with the findings of Gupta et al.[35] and Lima et al.[36] in small ruminants, who attributed this increase to the metabolic failure of the liver in a period susceptible to metabolic disturbances, such as the peripartum period. In contrast, Mundim et al.[37] observed mean values of AST lower than those described for the species in lactating goats. However, greater activity of this enzyme was observed in second-lactation goats, due to the greater mobilization of fat and increase in ketone bodies, which cause damage to hepatocytes and consequent leakage of this enzyme into the circulating blood.

Regarding GGT, Hallford & Sanson[38] and de Van Saun[39] also reported a lack of effect between groups in ewes showing clinical signs of PT. Different results were reported by Yarim & Ciftci[27] and Santos et al.[28], who found an increase in serum GGT activity in ewes with clinical PT, as a result of liver damage. The same finding was observed in the subclinical form of the disease, as reported by Feijó et al.[8] and Marutsova[40], as a result of hepatic impairment. The increase in GGT observed in the postpartum period of the goats was also reported by Lima et al.[36] in ewes. However, Cajueiro et al.[41] did not observe significant differences in the value of this variable between the pre and postpartum periods in dairy goats.

The absence of statistical difference between groups for CK was also reported by Marutsova[40] and Marutsova[42] in ewes and cows with PT and ketosis respectively, both in clinical and subclinical form. This behavior of CK, concomitant with AST, demonstrates that PT in its clinical or subclinical form did not trigger muscle damage in the goats used in this study, especially due to the milder manifestation of the clinical form, as indicated by the mean ßHB values. However, in a study carried out by Barakat et al.[23] and Santos[28], CK was significantly higher in goats and ewes with clinical PT.

As for the time point effect, the increase observed during parturition was also reported in ewes by Lima et al.[36], which was associated with contractions of the uterus that occur at the time of kidding. The increase that lasts during lactation could be explained by the intensification of management during this period since this enzyme is highly sensitive and specific. Therefore, sudden increases in its activity may occur as a result of greater muscle activity, mechanical trauma, application of intramuscular injection, or even prolonged decubitus[41].

The lack of an effect on serum amylase activity between the studied groups demonstrates good functioning of the exocrine pancreas[43]. The increase in the activity of this enzyme during lactation occurs due to the increase in the concentration of carbohydrates provided in the diet. Souto et al.[16] and Cajueiro[44] also observed this dynamic in goats with clinical PT and hypocalcemia.

4.4 Mineral profile

The observed difference between the G2 and G3 groups for Ca^{2+} shows that the degree of hypocalcemia increases with the severity of the metabolic disorder. Hypocalcemia in goats with clinical and subclinical PT was also reported by Albay et al.[33] due to reduced feed

intake and intestinal absorption of calcium. It is noteworthy that hypocalcemia, when associated with hyperketonemia, contributes to the development of PT by exerting important depressive effects on glucose homeostasis[45]. That said, Cajueiro et al.[41] demonstrated that approximately 50% of goats with subclinical hypocalcemia also displayed subclinical PT during the transition period.

Calcium levels were uniform throughout the transition period, with no influence of the physiological phases (late pregnancy and early lactation), which is in agreement with Gupta et al.[35], who did not find significant differences between calcium concentrations in pre- and postpartum goats. However, Moghaddam & Hassanpour[46] identified significantly higher concentrations of calcium in the prepartum period than in the postpartum period, associating it with the secretion of calcium in the milk in this period in healthy ewes and in ewes with subclinical PT.

The higher serum concentrations of P found in G3 compared to G1 may be the result of drug intervention with the use of solutions containing Ca and P, since P, unlike Ca, does not undergo endocrine regulation. Thus, its blood level may vary largely, which is physiologically well tolerated[47]. This finding differs from the results of Hallford & Sanson[38] and de Vasava et al.[17], who did not show a significant difference in the P concentrations of healthy ewes and goats compared to those with clinical PT. They also differ from the findings of Hefnawy et al.[15] and dos de Souto et al.[16], who identified a drop in the concentration of this mineral in goats clinically affected by the disease.

Regarding the absence of a time point effect, the results are in line with those of Gupta et al.[35], who did not observe significant variation in serum P levels before and after parturition in goats with PD and subclinical ketosis. Similarly, Mundim et al.[37] found no variations in P concentration during lactation in Saanen goats. In contrast, Feijó et al.[8] observed a decrease in the serum concentration of this mineral in ewes subjected to subclinical PT induction due to a decrease in dry matter intake.

Although there was a more significant reduction of Cl^- in G3 compared to the other groups, its concentrations are within the normal range described by Kaneko et al.[48]. Lima et al.[49] and Souto et al.[16] found no change in this element in dairy goats with clinical PT. It is noteworthy that the decrease observed in G3 may be related to the reduction in feed intake, concomitant with mineral salt. However, Lima et al.[50] observed an increase in Cl^- concentrations in goats with PT compared to healthy goats during pregnancy and lactation. With regard to time points, the significant decrease during the lactation period is similar to the results of Antunovic et al.[51] in ewes and Skrzypczak et al.[52] in cows, being related to the secretion of this variable in milk. However, Cajueiro et al.[41] found Cl^- values within the normal range during the transition period in dairy goats.

Stable Na^+ concentrations between groups are in line with the findings of Gupta et al.[35], who did not observe significant variations in Na^+ levels in goats with clinical and subclinical PT. Similarly, Albay et al.[33] did not observe a significant difference between Na^+ levels in healthy goats and those with the subclinical form of PT. However, regarding the clinical form, the values were lower and considered a good parameter that is indicative of the disease. Likewise, Van Saun[39] and Hefnawy et al.[15] found a significant reduction in Na^+ in ewes and goats with clinical PT. Lima et al.[49] also found low concentrations of this element in goats with the disease, attributing it to renal failure and dehydration. No such alterations were observed in our study, likely due to the therapeutic intervention performed in animals with a clinical framework of PT. According to Souto et al.[16], the serum decrease found in goats with clinical PT is related to the decrease in appetite and in the motility of the gastrointestinal tract since sodium fundamentally enters the rumen with saliva and not with food, and is actively absorbed according to the concentration of the electrochemical gradient across the ruminal wall and along the gut.

The behavior shown by Na^+ in the transition period is similar to the results of Krajnicakova et al.[53] in goats, of Elnageeb & Adelatif[54] in ewes, and of Silva Filho et al.[55] in cows. These authors described a gradual decrease in this element with the advancement of lactation, which increases the demand for this element in milk, although ruminants have a great capacity to maintain adequate levels of this mineral in tissues and fluids.

Regarding the higher K^+ values shown by G2 and G3, this outcome might be related to the lower concentration of insulin observed in goats with PT[5,56], because, according to Conrado[57], endogenous insulin secretion and stimulation of β2-adrenergic receptors by adrenaline promote cellular influx of potassium in the liver and muscle through increased activity of membrane Na^+, K^+-ATPase, with insulin being the most important hormone in shifting K^+ into cells after feed ingestion. However, this finding differs from most results by other authors, such as Souto et al.[16] and Gupta et al.[35], who did not show significant variations in the K^+ levels of goats with PT in relation to healthy goats. It also disagrees with Albay et al.[33], who found lower concentrations of K^+ in goats with clinical PT compared to the subclinical form and healthy animals. These authors attributed this finding to the marked loss of K^+ in the urine observed in patients with ketoacidosis and ketonuria, in addition to the fact that the animals were not eating due to the disease. Therefore, the K^+ input from the diet ends up being reduced. This condition was reported by Hefnawy et al.[15] and Lima et al.[50], who found a reduction in serum K^+

levels in goats with clinical PT associated with inadequate feed intake and incomplete renal tubular absorption of the element.

The K^+ results seen at the studied time points are in line with the findings of Gupta et al.[35], who also observed a slight variation in serum levels during the pregnancy and lactation periods in goats. However, they differ from the results of Silva et al.[58] and de Silva Filho et al.[55], who did not observe variations in K^+ values in ewes and cows in the peripartum period.

In short, the different results observed regarding the statistical group effect of the variables are likely related to the intensity of the disease, whose evolution was minimized due to the drug intervention performed in animals with clinical signs, which explains the reduced magnitude of clinical cases of PT and biochemical alterations.

5. Conclusion

Mineral metabolites, phosphorus, chlorides, potassium, and ionized calcium were altered due to pregnancy toxemia, with particular emphasis on potassium and calcium in the subclinical form of the disease. Attention is drawn to the presence of hypocalcemia observed in both subclinical and clinical manifestations of the condition, with a more pronounced effect seen in the latter. Further research is imperative to further investigate the biochemical intricacies of pregnancy toxemia and its repercussions on livestock production, especially in the context of its subclinical manifestation.

Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Author Contributions

Conceptualization: R.J.C. Souto. *Data curation:* R.J.C. Souto. *Methodology*: G.S.L. Soares. A.T.M. Macedo and J.F.P. Cajueiro. *Writing (proofreading and editing)*: U.F. Santos. *Formal analysis*: P.C. Soares. *Project administration*: J.A.B. Afonso and C.L. Mendonça.

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