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Influence of clinical and subclinical pregnancy toxemia on the energy and hormonal profiles of dairy goats during the transitional period

Influência da toxemia da prenhez clínica e subclinica sobre o perfil energético e hormonal de cabras leiteiras durante o período de transição

Influencia de la toxemia de la gestación clínica o subclinical en los perfiles energético y hormonal de cabras lecheras durante el período de transición

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Abstract
The purpose of this study was to evaluate the influence of clinical and subclinical pregnancy toxemia (PT) on blood metabolites of dairy goats in the peripartum period. 111 multiparous goats were divided into three groups on the basis of cut-off blood concentrations of β-hydroxybutyric acid and clinical symptoms of PT. The control group (n=40, βHB ≤ 0.8 mmol/L), whereas the subclinical group (n=39, βHB between 0.8 and 1.6 mmol/L) and the clinical group (n=32, βHB ≥ 1.6 mmol/L). The evaluations were performed on the -30, -20, -10, 0, +10, +20, and +30 day in relation to parturition. βHB, non-esterified fatty acid, glucose, fructosamine, cholesterol, triglycerides, insulin, cortisol, and free T3 and free T4 were measured. The ANOVA was performed to investigate the effects and interactions between groups and assessment times using the SAS. A higher occurrence of clinical PT was observed during late pregnancy, whereas subclinical PT was more frequently in early lactation. Clinical and subclinical PT during the transitional period resulted in raising the βHB, NEFA, and cortisol ($P<0.05$). Meanwhile, a decrease was observed in fructosamine, triglycerides and insulin ($P<0.05$). The occurrence of subclinical PT was higher than the clinical form during peripartum. The βHB validated the possibility of detecting goats that are at a high risk of developing PT approximately three weeks before parturition. Clinical and subclinical PT triggered marked changes in serum concentrations of βHB, NEFA, insulin, and cortisol, being those biomarkers in potential of PT.
Keywords: Goat; Ketosis; Metabolic disease; Metabolism; Negative energy balance.

Resumo

O objetivo deste estudo foi avaliar a influência da toxemia da prenhez (TP) clínica e subclínica sobre os metabólitos sanguíneos de cabras leiteiras no período periparto. 111 cabras multíparas foram distribuídas em três grupos com base nas concentrações sanguíneas de ácido β-hidroxibutírico e sintomas clínicos de TP: o grupo controle (n=40, βHB ≤ 0,8 mmol/L), o grupo subclínico (n= 39, βHB entre 0,8 e 1,6 mmol/L) e o grupo clínico (n=32, βHB ≥ 1,6 mmol/L). As avaliações foram realizadas nos dias -30, -20, -10, 0, +10, +20 e +30 em relação ao parto. A concentração de βHB, ácidos graxos não esterificados, glicose, frutosamina, colesterol, triglicerídeos, insulina, cortisol, T3 livre e T4 livre foram medidos. ANOVA foi realizada para investigar efeitos e interações entre grupos e tempos de teste usando o SAS. Observou-se maior incidência de TP clínica no final da gestação, enquanto a TP subclínica foi mais frequente no início da lactação. A TP clínica e subclínica durante o período de transição resultou em aumento de βHB, NEFA e cortisol (P<0,05). Por outro lado, houve diminuição de frutosamina, triglicerídeos e insulina (P<0,05). A ocorrência de TP subclínica foi maior do que a forma clínica durante o periparto. O βHB validou a possibilidade de detectar cabras com alto risco de desenvolver TP aproximadamente três semanas antes do parto. As TP clínicas e subclínicas resultaram em alterações marcantes nas concentrações séricas de βHB, NEFA, insulina e cortisol, que são potenciais biomarcadores de TP.

Palavras-chave: Balanço energético negativo; Cabra; Cetose; Doença metabólica; Metabolismo.

Resumen

El propósito de este estudio fue evaluar la influencia de la toxemia de la gestación clínica y subclínica (TG) en los metabolitos sanguíneos de cabras lecheras en el período periparto. 111 cabras multíparas fueron distribuidas en tres grupos en base a las concentraciones sanguíneas de ácido β-hidroxibutírico y los síntomas clínicos de TG: el grupo control (n=40, βHB ≤ 0,8 mmol/L), el grupo subclínico (n=39, βHB entre 0,8 y 1,6 mmol/L) y el grupo clínico (n=32, βHB ≥ 1,6 mmol/L). Las evaluaciones se realizaron en los días -30, -20, -10, 0, +10, +20 y +30 en relación al parto. Se midió la concentración de βHB, ácidos grasos no esterificados, glucosa, fructosamina, colesterol, triglicéridos, insulina, cortisol, T3 libre y T4 libre. El ANOVA se realizó para investigar los efectos y las interacciones entre los grupos y los tiempos de evaluación utilizando el SAS. Se observó una mayor incidencia de TG clínica al final del embarazo, mientras que la TG subclínica fue más frecuente al comienzo de la lactancia. TG clínica y subclínica durante el período de transición resultó en aumento de βHB, NEFA y cortisol (P<0,05). Por su parte, se observó una disminución de fructosamina, triglicéridos e insulina (P<0,05). La ocurrencia de la TG subclínica fue mayor que la forma clínica durante el periparto. El βHB validó la posibilidad de detectar cabras con alto riesgo de desarrollar TG aproximadamente tres semanas antes del parto. TG clínica y subclínica resultaron en marcados cambios en las concentraciones séricas de βHB, NEFA, insulina y cortisol, siendo estos biomarcadores en potencial de TG.

Palabras clave: Balance energético negativo; Cabra; Cetosis; Enfermedad metabólica; Metabolismo.

1. Introduction

Better access to milk yielding technologies along with incorrect feed management and high requirements of productivity and animal efficiency, especially during the transitional period, have led to a higher occurrence of metabolic diseases, such as pregnancy toxemia (PT) (Brozos et al., 2011). Pregnancy toxemia is characterized by disorders of the energy, protein, and hormonal profiles. As per clinical findings, it is expressed in the form of death of offspring and the dams themselves (Afonso, 2011).

In general, it affects females with multiple fetuses and occurs in the final third part of pregnancy in small ruminants due to the inability of the diet to supply the energy demand in this period. Conditions that increase the energy requirements or reduce energy intake may also predispose animals to this disease, even during the lactation period (Baumgartner, 2013; Campos et al., 2010; Rook, 2000; Sadjadian et al., 2013). The PT may also occur in the subclinical form, being defined as a preclinical stage of the disease, characterized by elevated concentrations of ketone bodies in the blood without the clinical manifestations of the disease (Binev et al., 2014; Feijó et al., 2016).

Because of the economic and social importance of goat MY and of a strong negative effect of PT on this yield, it is necessary to understand clinical and subclinical aspects of this metabolic disorder in order to minimize possible damages. Therefore, the purpose of this study was to evaluate the influence of clinical and subclinical PT on the energy and hormonal profiles of dairy goats during the transitional period.

2. Methodology

Animals and procedures: The present study was carried out in eight dairy goat farms, located in the semi-arid region of the State of Pernambuco. The analyses were performed at the laboratory of Garanhuns Cattle Clinic and at the Research Support Center, both of which are affiliated with the at Universidade Federal Rural de Pernambuco (UFRPE). During the transitional period, 111 crossbred multiparous pregnant dairy goats were monitored clinically, including evaluation for the body condition score (BCS) on a scale of 1-5. A large majority of animals considered in this investigation were pregnant with twins (Silva-Filho, 2016).

All the goats were raised under an intensive system. The diet was prepared to cover nutritional requirements throughout the experimental period. They were submitted to a diet that comprised of sugarcane bagasse (Saccharum sp.) with forage cactus (Opuntia tuna (L.) Mill) and was concentrated with wheaten, corn, soybean, and cotton. The nutritional, hygiene, sanitary management schemes adopted were similar in all the farms. Freshwater and mineral salt were available ad libitum. All the animals were vaccinated systematically and dewormed according to the protocol conventionally adopted in each farm.

Experimental protocol: Concerning clinical and laboratory analyses, all the animals were evaluated on the 30th, 20th, and 10th day antepartum (dap), at the time of parturition, and on the 10th, 20th, and 30th day postpartum (dpp). Ultrasonography was performed to diagnose and monitor gestation and was used as a selection criterion for goats in the last month of gestation (Ultrasson GE - Logiq 100 Pro, Milwaukee, USA). The animals were raised under an intensive system and were divided into three experimental groups (control, subclinical, and clinical groups), using the cut-off concentrations of β-hydroxybutyric acid blood concentrations (βHB) and clinical diagnoses of PT as reference. The control group (G1, n=40) presented βHB concentrations of ≤ 0.8 mmol/L, whereas subclinical group (G2, n=39) presented βHB between 0.8 and 1.6 mmol/L, and the clinical group (G3, n=32) had βHB ≥ 1.6 mmol/L during at least one of the assessment times. The clinical behavior of PT was only observed in G3 goats, and therapeutic intervention was recommended as necessary.

Sampling and measurement: Blood samples were collected by jugular venipuncture, using 25 × 8 mm needles; samples were collected in sterile vacuum tubes without any anticoagulant (to obtain serum) to perform biochemical and hormonal analyses. For glycemia determination, samples were taken in tubes containing sodium fluoride and K3EDTA as anticoagulants (Vacuette® FC Mix tube, Greiner Bio-One, São Paulo, Brazil). Subsequently, the samples were centrifuged (Centrifuga Fanem Ltda, Baby I, Mod. 206, Brazil) at 3600 rpm for 10 min. The plasma and serum thus obtained were transferred to an Eppendorf and stored in an ultra-low temperature freezer (Ultralow freezer NuAire Inc., USA) at −80°C until further analysis.

To evaluate the energy status, β-hydroxybutyric acid (Tris buffer 100 nmol pH 8.5 method, βHB/Rambut RANDOX Laboratories Ltd., UK), non-esterified fatty acids (NEFA colorimetric method - RANDOX Laboratories Ltd.), and glucose (GOD-Trinder method, Glicose Liquiform, Labtest Diagnóstica S.A., Brazil) were analyzed in serum and plasma specimens. Serum concentrations of fructosamine were determined by nitro blue tetrazolium (NBT) reduction method (Frutosamina, Labtest Diagnóstica S.A, Brazil), while cholesterol and triglycerides were analyzed using Trinder reaction method (Colesterol; Triglicérides liquiform - Labtest Diagnóstica S.A., Lagoa Santa, Brazil). All this experimentation was performed as traditional analyses at 37°C with commercial kits and a semi-automatic biochemical analyzer (Labquest - Labtest Diagnóstica S.A., Lagoa Santa, Brazil). The hormonal determinations of cortisol (Access Immunoassay Systems Cortisol - Beckman Counter®, Alphaville, Brazil), insulin (Access Immunoassay Systems Ultrasensitive Insulin - Beckman Counter®, Alphaville, Brazil), free T3 (Triiodothyronine, Access Immunoassay Systems Free T3 - Beckman Counter®, Alphaville, Brazil), and free T4 (Thyroxine, Access Immunoassay Systems Free T4 - Beckman Counter®, Alphaville, Brazil) were performed by chemiluminescent immunoassays (Chemiluminescent Beckman Counter, Inc.).

The intra-assay coefficients of variation (CV) were calculated using an in-house control serum assayed 10 times: βHB

(at 0.4 mmol/L, CV=10.0%), NEFA (at 0.2 mmol/L, CV=5.7%), glucose (at 2.9 mmol/L, CV=5.5%), fructosamine (at 227.9 µmol/L, CV=7.7%), triglycerides (at 0.3 mmol/L, CV=2.2%), cholesterol (at 2.4 mmol/L, CV=16.6%), cortisol (at 36.62 nmol/L, CV=0.97%) , insulin (at 26.50 pmol/L, CV=1.14%), free T3 (at 5.19 pmol/L, CV=0.96%) and free T4 (at 6.78 pmol/L, CV=0.84%).

Statistical analysis: The data were described by means of least square means and standard deviations. The parameters were initially tested for their normal distribution using the Kolmogorov-Smirnov test. Those that did not satisfy the normality assumptions were submitted to transformation with logarithmic basis (LogX + 1) or by the square root [RQ (X + 1/2)]. The data subjected to normality or transformed assumptions were subsequently subjected to analysis of variance (F-Test) as time-repeated measures, which separated, as causes of variation, the effects of the groups (G1, G2, and G3) and assessment times (prepartum, parturition, and postpartum periods). This protocol was performed in sub-divided plots with completely random clusters. It aimed to ascertain the effects and interactions between them as well. When significance was found via the F-test, the means were compared with the least significant difference of the Student-Newman-Keuls test. Data were analyzed using the Statistical Analysis System program (SAS, 2009).

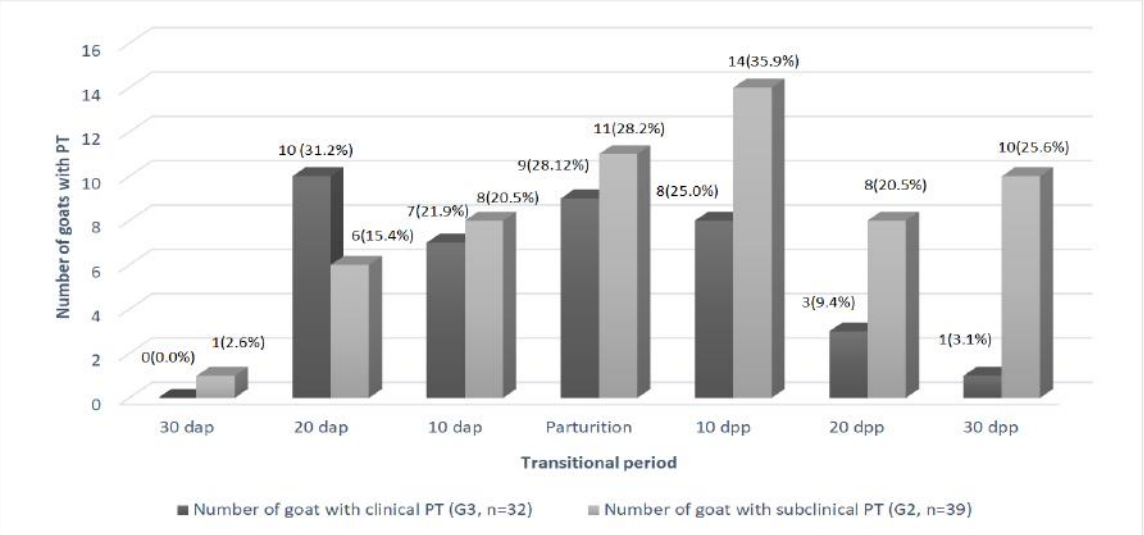
Ethical committee: The research was approved by the Animal Ethics Committee (CEUA - Comissão de Ética no Uso de Animais) of the Universidade Federal Rural de Pernambuco under license nº 070/2016 CEPE/UFRPE, the Brazilian School for Animal Experimentation (COBEA – Colégio Brasileiro de experimentação animal), and the National Institute of Health Guide for the Care and Use of Laboratory Animals.

3. Results

Clinical observations

The goats showed a milk yield (MY) of more than 3 kg/day/goat. No change was observable in the BCS throughout the assessment times, and it remained between 3.0 and 3.5 independently of the group. Another characteristic was the prolificacy that was on average 2 kids/goat. The occurrence of the subclinical and clinical forms of PT during the different assessment times is shown in Figure 1.

Figure 1 - Clinical and subclinical PT (%) of dairy goats during the transitional period.



Source: Authors.

The goats, with a clinical diagnosis *prepartum*, presented signs, such as lack of appetite, apathy, dehydration, mucosal congestion, prolonged decubitus, edema of limbs, and symptoms of nervous system disorder, including opisthotonus and grinding of teeth. During the lactation period, clinical manifestations, such as apathy, dehydration, reduction of feed intake, and a decrease in MY, were generally less intense than those found during pregnancy; they were also non-specific and could be confused with symptoms of a digestive disorder.

Energy metabolism indicators

Concerning βHB blood concentrations, a correlation between its values for the group and the assessment time was observed (P<.0001). The G3 clinical group, particularly, showed higher blood concentrations of βHB at all assessment times than concentrations in the other groups (Table 1).

Table 1 - Effect of treatment (G1, G2 and G3), sampling time and their interaction of metabolites of the energy profile on healthy goats (G1) and goat with subclinical (G2) and clinical (G3) PT monitored during the late pregnancy and early lactation (LS means ± standard error).

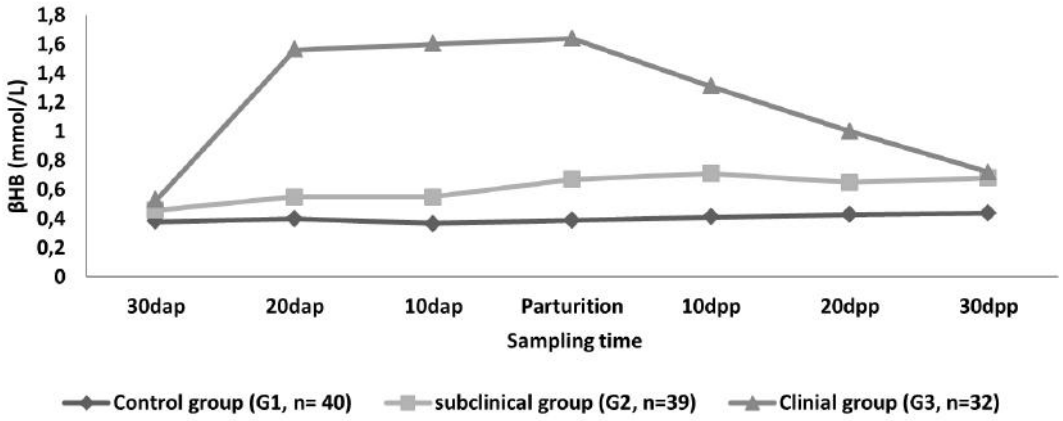
Energy profile	Group	Sampling time							GM	Variation factor		
		30th dap	20th dap	10th dap	Parturition	10th dpp	20th dpp	30th dpp		Treatment	time	Interaction (Txt)
βHB (mmol/L)	G1	0.4±0.13^{Ba}	0.4±0.13^{Ba}	0.4±0.10^{Ba}	0.4±0.14^{Ba}	0.4±0.14^{Ca}	0.4±0.13^{Ca}	0.4±0.11^{Ba}		<0.0001	0.0064	<0.0001
	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}				
NEFA (mmol/L)	G1	0.2±0.15	0.2±0.14	0.2±0.13	0.5±0.43	0.3±0.18	0.3±0.34	0.2±0.12	**0.3^{C}**	<0.0001	<0.0001	0.2834
	G2	0.3±0.25	0.3±0.18	0.5±0.44	0.6±0.35	0.4±0.28	0.4±0.33	0.3±0.25	**0.4^{B}**			
	G3	0.3±0.23	0.5±0.35	0.6±0.37	0.7±0.51	0.5±0.44	0.4±0.39	0.3±0.42	**0.5^{A}**			
	GM	**0.3^{d}**	**0.3^{bcd}**	**0.4^{b}**	**0.6^{a}**	**0.4^{b}**	**0.4^{bc}**	**0.3^{cd}**				
Glucose (mmol/L)	G1	2.8±0.38	2.6±0.41	2.7±0.53	7.7±19.06	3.1±0.46	3.0±0.34	3.1±0.36	**3.5^{A}**	0.2373	0.0005	0.2334
	G2	2.7±0.53	2.9±1.13	2.7±0.51	3.5±1.83	2.9±0.43	3.0±0.44	2.9±0.46	**2.9^{A}**			
	G3	2.5±0.49	2.5±0.62	2.6±0.56	4.1±2.65	2.6±0.58	3.0±0.85	2.9±0.33	**2.9^{A}**			
	GM	**2.7^{b}**	**2.7^{b}**	**2.7^{b}**	**5.2^{a}**	**2.9^{b}**	**3.0^{b}**	**3.0^{b}**				
Fructosamine (µmol/L)	G1	219.3±19.89	215.6±26.28	209.3±30.25	225.9±23.92	235.3±26.88	232.4±33.32	237.5±30.47	**224.5^{A}**	0.0187	<0.0001	0.9097
	G2	212.8±21.20	206.0±35.13	196.8±37.37	224.5±43.85	227.4±24.34	222.3±41.41	230.1±24.83	**217.2^{B}**			
	G3	214.2±23.97	198.4±34.40	200.7±27.47	228.6±29.55	225.3±19.66	231.1±18.80	236.4±28.93	**219.5^{AB}**			
	GM	**215.9^{b}**	**207.7^{bc}**	**202.5^{c}**	**226.2^{a}**	**229.6^{a}**	**228.4^{a}**	**234.5^{a}**				
Cholesterol (mmol/L)	G1	2.3±0.67	2.2±0.67	2.3±0.60	2.2±0.66	2.2±0.59	2.3±0.81	2.4±0.71	**2.3^{A}**	0.0841	0.0410	0.8689
	G2	2.3±0.72	2.3±0.63	1.9±0.48	1.9±0.55	2.1±0.61	2.2±0.63	2.4±0.81	**2.1^{A}**			
	G3	2.5±0.66	2.3±0.71	2.2±0.65	2.1±0.64	2.2±0.58	2.1±0.51	2.3±0.72	**2.2^{A}**			
	GM	**2.4^{a}**	**2.3^{ab}**	**2.1^{ab}**	**2.0^{b}**	**2.2^{ab}**	**2.2^{ab}**	**2.3^{ab}**				
Triglycerides (mmol/L)	G1	0.3±0.13	0.3±0.19	0.3±0.11	0.2±0.14	0.1±0.05	0.2±0.16	0.2±0.11	**0.3^{A}**	0.0026	<0.0001	0.9086
	G2	0.3±0.08	0.3±0.11	0.3±0.13	0.2±0.09	0.1±0.06	0.2±0.06	0.2±0.06	**0.2^{B}**			
	G3	0.3±0.11	0.3±0.17	0.3±0.19	0.3±0.25	0.2±0.05	0.2±0.04	0.2±0.16	**0.2^{A}**			
	GM	**0.3^{a}**	**0.3^{a}**	**0.3^{a}**	**0.2^{b}**	**0.2^{c}**	**0.2^{bc}**	**0.2^{b}**				

βHB: beta-hydroxybutyrate; NEFA: non-esterified fatty acids; dap: day *ante-partum*; dpp: day *postpartum*; GM: General mean; a,b: Different letters on the same line represent significant difference amongst the sampling times (P<0.05). A,B: Different letters indicate a significant difference amongst the treatments.

Source: Authors.

This variable in G3, increased considerably by 20th dap (P=0.0064), reaching a peak at parturition, which was followed by a decrease during the first month of lactation. Lower concentrations of βHB were observed in G2, when compared to concentrations in G3 at all the assessment times, whereas the concentration rose significantly at parturition and remained stable during lactation. It is noteworthy that this increase in G3 values occurred earlier (20th dap) as compared to in G2. As for the control group, no effect of assessment time was observed (P>0.05), although the highest mean concentrations were observed at parturition and lactation (Table 1; Figure 2).

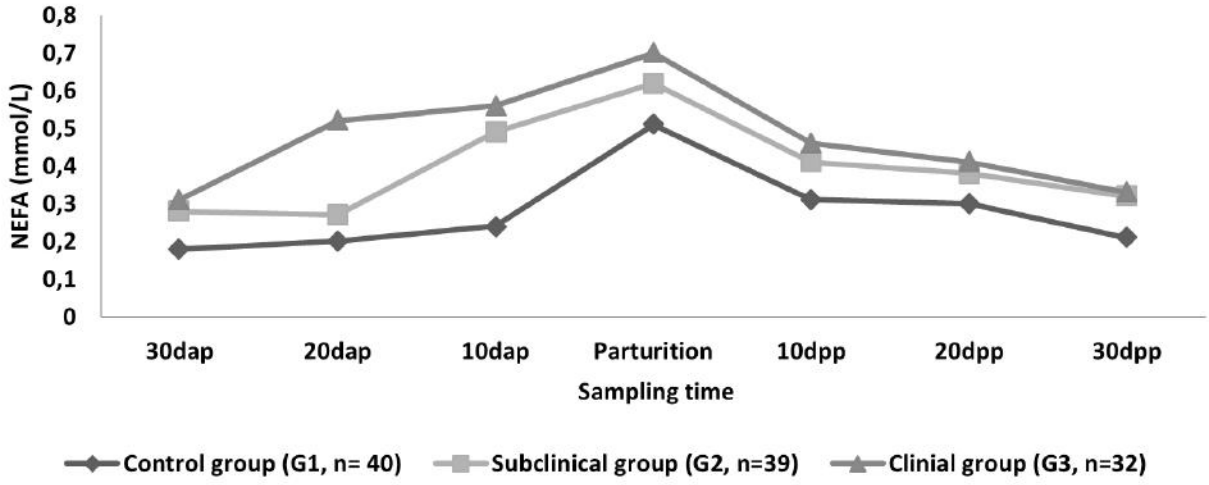
Figure 2 - Blood β-hydroxybutyrate concentrations (βHB, mmol/L) in blood serum of control group (G1, control, n=40), subclinical group (G2, n=39) and clinical group (G3, n=32) during the transitional period.



Source: Authors.

Higher concentrations of NEFA were observed in G3, followed by G2 and G1 control groups (P <0.0001). Higher NEFA concentration was observed on the day of parturition as compared to concentrations in *pre* and *postpartum* periods (Table 1; Figure 3). It is worth noting that the NEFA values in G3 crossed the normal values for goats from the 20th dap to the 10th dpp.

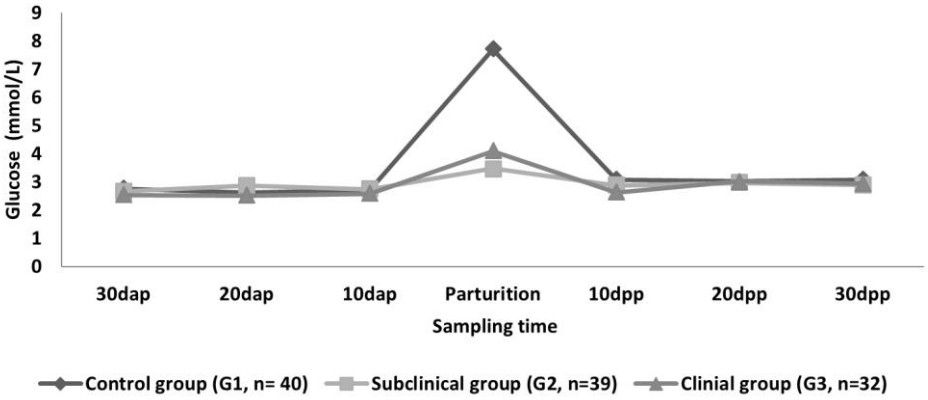
Figure 3 - Blood non-esterified fatty acid concentrations (NEFA, mmol/L) in dairy goats of control group (G1, n=40), subclinical group (G2, n=39) and clinical group (G3, n=32) during the transitional period.



Source: Authors.

In case of glucose concentration, no statistical difference was observed between the three groups (P=0.2373). However, there was a significant increase (P=0.0005) in the mean values of blood glucose at parturition (5.18 mmol/L) as compared to values at other periods in time (Table 1; Figure 4). Higher values of fructosamine were observed in the control group (P<0.0187), when compared to its value in other groups. A significant increase was observed at the time of parturition (P<.0001), which remained stable during the first month of lactation (Table 1).

Figure 4 - Blood glucose concentrations (mmol/L) in dairy goats of control group (G1,n=40), subclinical group (G2, n=39) and clinical group (G3, n=32) during the transitional period.



Source: Authors.

There was no statistically significant difference between groups as per their serum cholesterol concentrations (P=0.0841). A gradual decrease was observed in the mean values of this variable (P=0.0410), however, they shifted back to the *prepartum* values (Table 1). The lowest concentration of triglycerides was observed in G2 (P=0.0026) as compared to concentrations in other groups. During the assessment times, this metabolite decreased at parturition, while remaining lower than its value observed *prepartum* (P<.0001) (Table 1).

Hormonal profile

In case of insulin, lower concentrations were observed in groups, G2 and G3, as compared to concentrations in G1 (P<.0001). There was a reduction in insulin values from the 20th dap, and they decreased significantly until parturition, after which the lowest mean values were observed. The values remained low during lactation as compared to the values *prepartum* (P<.0001, Table 2; Figure 5). There were higher concentrations of cortisol in the G2 and G3 groups (P=0.0396) as compared to concentrations in the control group. There was also a significant increase of cortisol at parturition as compared to the other assessment times (P<.0001; Table 2, Figure 6).

Table 2 - Effect of treatment (G1, G2 and G3), sampling time and their interaction of metabolites of the hormonal profile on healthy goats (G1) and goat with subclinical (G2) and clinical (G3) PT monitored during the late pregnancy and early lactation

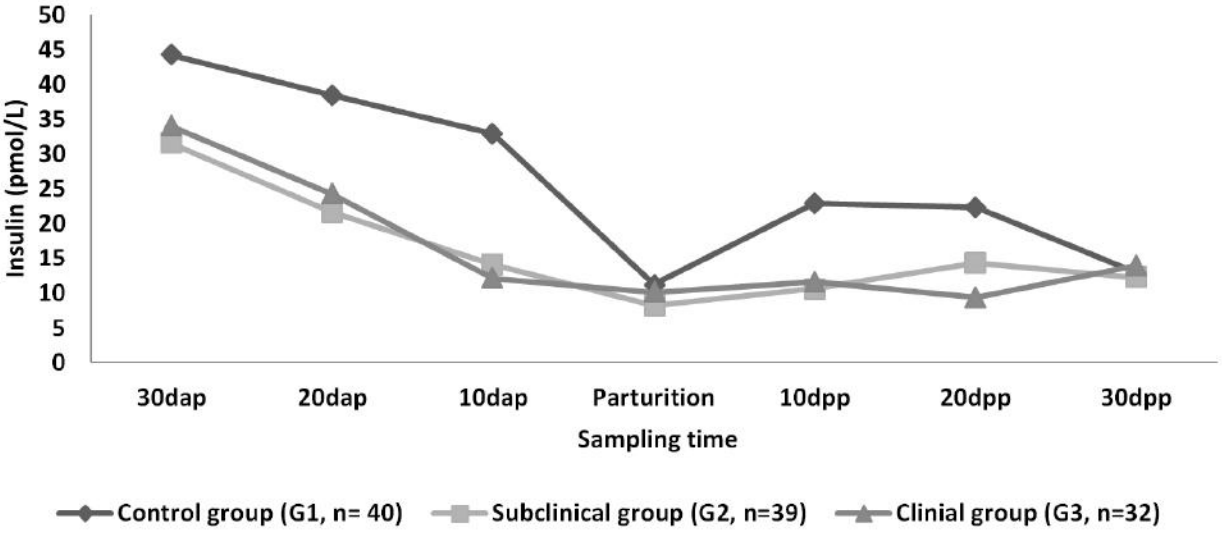
Hormonal profile	Group	Sampling time							GM	Variation factor		
		30th dap	20th dap	10th dap	Parturition	10th dpp	20th dpp	30th dpp		Treatment	time	Interaction (Txt)
Insulina (pmol/L)	G1	44.2±48.96	38.5±35.79	32.9±48.61	11.2±13.43	22.9±24.52	22.4±27.91	12.9±10.90	27.0^{A}	<0.0001	<0.0001	0.5832
	G2	48.9±66.92	24.4±31.56	17.4±20.77	8.8±11.46	10.0±13.00	14.9±16.79	12.9±9.52	18.8^{B}			
	G3	30.3±37.34	19.7±25.98	12.5±14.52	10.6±14.42	11.7±11.75	10.6±9.64	13.6±10.41	14.8^{B}			
	GM	41.6^{a}	26.9^{b}	20.0^{c}	10.0^{d}	14.0^{cd}	15.1^{cd}	13.2^{cd}				
Cortisol (nmol/L)	G1	41.8±31.97	29.2±28.28	33.5±17.55	68.7±39.42	34.4±28.86	26.2±18.40	22.9±21.64	36.8^{B}	0.0396	<0.0001	0.9713
	G2	66.2±34.18	39.8±18.86	35.9±19.62	76.1±80.26	36.1±24.81	39.8±25.19	35.4±40.86	46.4^{A}			
	G3	57.4±19.12	32.1±26.58	36.2±30.34	79.0±78.12	31.7±23.43	30.4±29.94	24.4±17.03	41.4^{AB}			
	GM	55.4^{b}	34.1^{c}	35.3^{c}	75.2^{a}	34.2^{c}	32.8^{c}	28.1^{c}				
Free T3 (pmol/L)	G1	5.0±0.90	5.1±1.15	5.3±1.77	5.1±0.91	5.3±1.52	5.7±1.19	5.5±1.13	5.3^{A}	0.9432	0.0001	0.3879
	G2	4.9±1.15	5.1±1.21	5.4±1.16	5.7±1.19	5.3±0.96	5.5±1.01	5.6±1.06	5.3^{A}			
	G3	47±1.50	4.4±1.7	5.3±1.73	5.0±1.64	5.8±1.49	5.8±1.77	6.0±1.36	5.3^{A}			
	GM	4.9^{b}	4.9^{b}	5.4^{ab}	5.3^{ab}	5.5^{ab}	5.7^{a}	5.7^{a}				
Free T4 (pmol/L)	G1	7.4±1.55	7.3±1.61	7.3±1.88	6.4±2.00	6.4±1.62	6.5±1.23	6.8±1.08	6.9^{A}	0.0641	0.0033	0.4223
	G2	6.9±1.42	7.3±1.79	7.4±1.66	7.1±1.53	6.3±0.93	6.5±1.15	6.5±1.03	6.9^{A}			
	G3	7.1±2.48	7.2±2.20	7.8±1.90	6.5±1.80	7.3±1.41	7.2±1.44	7.4±1.62	7.2^{A}			
	GM	7.1^{ab}	7.3^{ab}	7.5^{a}	6.7^{b}	6.6^{b}	6.7^{b}	6.9^{ab}				

Free T3: triiodothyronine; Free T4: thyroxine; dap: day *ante-partum*; dpp: day *postpartum*; GM: General mean; a,b: Different letters on the same line represent significant difference amongst the sampling times (P<0.05). A,B: Different letters indicate a significant difference amongst the treatments.

(LS means ± standard error).

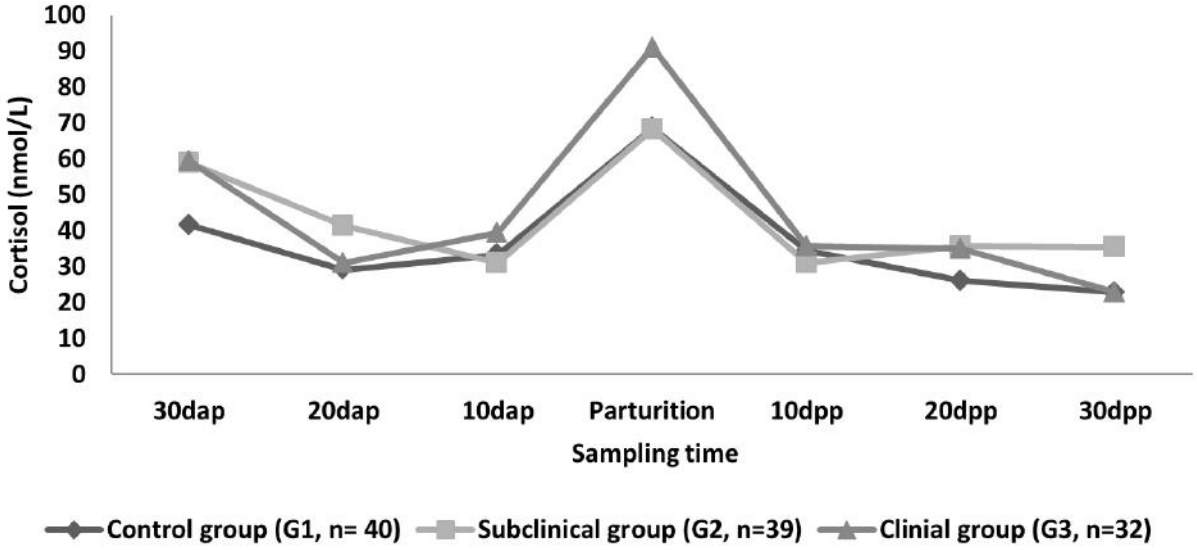
Source: Authors.

Figure 5 - Blood insulin concentrations (pmol/L) in dairy goats of control group (G1, n=40), subclinical group (G2, n=39) and clinical group (G3, n=32) during the transitional period.



Source: Authors.

Figure 6 - Blood cortisol concentrations (nmol/L) in dairy goats of control group (G1, n=40), subclinical group (G2, n=39) and clinical group (G3, n=32) during the transitional period.



Source: Authors.

As for free T3 and T4, no significant difference between the groups was observed (P>0.05). However, during the assessment times, there were distinctly different behaviors between free T3 and T4. Free T3 concentrations were higher during lactation, while free T4 was higher *prepartum* (P=.0001 and P=0.0033, respectively; Table 2).

4. Discussion

The highest occurrence rate of clinical PT that affects goats *prepartum* is expressed in the magnitude of the serum βHB concentrations. This is attributable to the fact that there is a greater energy demand in this period due to the fetal growth, a lower intake of dry matter, and the preparation of mammary glands for production of milk (Mesquita, 2014; Silva, 2010). These results are corroborated by findings of Bani-Ismail et al. (2015) and Amirul et al. (2016), who showed that clinical PT occurs the last stage of pregnancy.

The clinical signs found here are compatible with the findings of previous studies (Amirul et al, 2016; Barakat et al, 2007; Herfnawy et al, 2011; Souto et al, 2013; Vasava et al, 2016). The higher intensity of the clinical signs may be related to the higher concentration of βHB in this period along with other factors, as reported by Marutsova et al. (2015), who observed a higher concentration of βHB at the end of gestation than during lactation in dairy cows. However, the highest occurrence of subclinical forms has been observed during lactation, particularly in the first 10 or 15 days because of an increased energy demand in high-yield goats. In this period most goats develop mild or subclinical ketosis, similar to what happens in dairy cattle (Marutsova et al, 2015; Mattheus, 2009; Pichler et al, 2014).

Therefore, βHB assessment is important to detect the goats which are at a high risk of developing PT and the detection should be carried out at least three weeks before parturition. The occurrence of subclinical form was higher than the clinical form during the lactation period, which is similar to what has been reported in dairy cows (González; Silva, 2006) and based on these findings, professionals should be equipped to provide solutions to prevent economic losses.

The significant βHB elevation from the 20th dap onwards observed in the G3 group is associated with an inability to meet energy demands at the end of pregnancy due to rapid fetal growth (Macedo et al., 2015; Souto et al., 2013). This

elevation was observed to be three times the initial value *prepartum* until parturition. Previous studies confirm that βHB is an indicator of hyperketonemia. However, it is not only a sign of PT but has the capacity to act in a multifunctional way in the disease development process as well, as can be seen in the reduction of the female's ability to use ketone bodies as energy source, justifying the fact that this disease occurs more frequently during late pregnancy than early lactation, when the energy demand is higher (Dore et al., 2015; Harmeyer & Schlumbohm, 2006).

The βHB concentration of subclinical G2 group was also provided by Binev et al. (2014) and Feijó et al. (2016) in a study on subclinical PT. This finding is characterized by an elevation of ketone bodies in the bloodstream without manifestation of any clinical symptoms. Increase in βHB concentration occurred moderately in the G2 group, particularly during parturition and lactation, which was corroborated by the findings of Sadjadian et al. (2013). They related that PT, during lactation in dairy goats, also called ketosis by some authors, occurs due to the requirement for high MY that is more than the diet could offer, considering that in this period, there is the inability of the females to consume enough feed to meet their needs.

Despite significant statistical differences between the groups, the similar behavior of NEFA might be attributable to the variation in the intensity of the metabolic alterations that occur in each set of individuals. Cajueiro (2014) observed a similar behavior in healthy dairy goats, attributing this increase until parturition to an increased energy demand for colostrum production, labor stress, and reduction of dry matter intake. Whereas, Barbosa et al. (2009) observed an increase in NEFA concentration *prepartum* due to the involvement of the endocrine system, such as the action of lipolytic hormones in this period.

The higher NEFA concentrations of the G3 group, when compared to G2 and G1, occur due to the greater lipid mobilization to meet the greater demand of the female for nutrients *prepartum*. This observation was also reported by Barakat et al. (2007), Souto et al. (2013), and Sakha (2016), who found a marked serum NEFA increase in goats and sheep with PT.

The high NEFA values observed in G2 group as compared to the G1 control also occurred due to energy imbalance during the days near the parturition. These findings were reported by Feijó et al. (2016) in ewes with subclinical ketosis, who verified a NEFA elevation *prepartum*, with this increase being maintained after parturition. This change in the NEFA concentration, *postpartum*, according to Rios et al. (2006), may be associated with energy feed deficiency as well as the high energy requirements in the goat during early lactation and during the period of higher MY; this change may, in turn, lead to the mobilization of body reserves with a subsequent increase in free fatty acids.

The negative energy balance (NEB) found during the transitional period in this study was corroborated by the reports of Marutsova and Marutsova (2016). According to these authors, the NEFA increase in newly-born cattle with subclinical ketosis may be a result of lipolysis, assisted by insulin resistance during early lactation, which may have occurred because of the fatty acid formation exceeding the capacity of metabolization by the liver.

The absence of significant difference between the groups and their average values within the range of normality diverge from most of the published results on the glycemic profile of small ruminants with PT and also from the little information present on goats undergoing the transitional period with this disease. However, Souto et al. (2013) reported that a large majority of goats with clinical PT has normal glycemic concentrations, although they may have different glycemic profiles.

Several other studies have also reported different glycemic profiles in small ruminants, however, hypo- or hyperglycemia are prominent. This non-glucose-specificity as a parameter to diagnose PT may be due to this variable undergoing strong homeostatic control and remaining within the normal range even in diseased animals. These results suggest that the hormonal mechanism is responsible for maintaining normoglycemia (Henze et al., 1998; Sakha, 2016; Santos et al., 2011; Sargison et al., 1994).

Therefore, glycemia results in this study are not a good indicator of the metabolic disorder under consideration. However, Bani Ismail et al. (2008) have identified significantly lower glucose concentration in goats with subclinical PT as compared to those in the control group. Nevertheless, a similar study was conducted on goats with subclinical ketosis that showed significantly reduced serum glucose during the pregnancy and lactation period (Gupta et al., 2008).

The significant increase in glucose in the three groups at parturition is due to the stress elevation of cortisol in the bloodstream, as was reported by Santos et al. (2012), Araújo et al. (2014), and Lima et al. (2016). According to Barbosa et al. (2009), the glucose increase in dairy goats on the day of parturition may be a result of increased glucagon, glucocorticoid, and catecholamine concentrations, which favor gluconeogenesis and glycogenolysis, leading to depletion of hepatic glycogen stores.

In spite of significant elevation in the concentration of glucose in G2 and G3 groups at parturition, these values were within the range of normality for goats (Kaneko et al., 2008). However, hyperglycemia was observed in the control group that might be a result of a pronounced drop in the insulin concentration in the healthy animal group because members of this group are capable of increased production on glucose by the liver.

The absence of a significant statistical difference in fructosamine concentrations in the G3 and G1 groups might be related to the glucose behavior, which presented little variation during all assessment times, except at parturition (Souto et al., 2013). Therefore, this glycemia is within the reference limits for the goats, which is confirmed by the same author abovementioned, who reported normoglycemic goats with PT.

As fructosamine is formed, when glucose reacts non-enzymatically with amine groups of proteins, mainly albumin, its blood concentration is controlled by the balance between the synthesis and elimination of these protein compounds and glucose. However, if the albumin concentration is within the normal range, the values of fructosamine are related to the glycemic levels that have changed three and four weeks before. Thus, the levels of fructosamine increase in prolonged hyperglycemia or hyperproteinemia, but may reduce in an inverse condition (Bernstein, 1987; Jensen et al., 1993; Kaneko et al., 2008; Santos et al., 2011).

On the other hand, The lower concentrations of fructosamine in subclinical G2 group in relation to control group is different from that reported by Yatoo et al. (2015) in goats with subclinical PT; the authors found high concentrations of fructosamine, followed by an increase in the albumin concentration. However, in case of metabolic conditions, this variable has not been often described in the literature, which suggests the need for further investigations to confirm the diagnostic value of fructosamine in subclinical PT cases.

The significant increase in fructosamine at parturition, which is maintained during lactation, might probably because of the fructosamine that is formed, when glucose binds irreversibly with amine groups of albumin and other blood proteins (Ambruster, 1987). In other words, its concentration in this study is probably directly related to the serum protein concentration, in view of the normoglycemia.

The observations made during lactation, which indicated no significant difference between the assessment times, are in agreement with observations of Campos et al. (2007) who concluded that this variable is not a good indicator of the narrow interval between the values. On the other hand, Brito et al. (2006) reported that fructosamine is a useful indicator of metabolic status, when they verified that dairy sheep had a similar glucose metabolism behavior during the energy deficit of the transitional period, reflecting a risk situation for PT.

Regarding cholesterol, the absence of significant difference between the groups demonstrates that the disease and its subclinical form were not able to influence the concentrations of this variable during the transitional period. In the same way, Bani Ismail et al. (2008) and Gupta et al. (2008) found no significant increase in cholesterol concentrations in goats with subclinical PT during pregnancy and/or lactation. Although Santos et al. (2011) also found cholesterol values within the

reference limits for sheep, the values were in the lower limit of normality. The authors considered that there is a tendency to reduce the concentration of this variable in animals affected by clinical PT. This change suggests that the liver's ability to secrete this compound into the bloodstream as lipoprotein is compromised, thereby inducing accumulation of liver fat. On the other hand, Feijó et al. (2016) reported that the total serum cholesterol concentrations are elevated during the period of subclinical induction ketosis in sheep. They attributed this elevation to oxidation of beta-lipid reserves that led to an increase of acetyl-CoA.

In spite of gradual decrease in cholesterol concentrations observed until parturition, with a significant decrease at this time eventually reached a general average concentration below the goat reference value (Kaneko et al., 2008). According to González and Silva (2006), the hepatic cholesterol synthesis is related to the level of cholesterol ingested in the diet.

Another factor that calls attention is the similar behavior of cholesterol and insulin; insulin was also found in lower concentrations at parturition. This may justify the reduction in cholesterol concentration observed as the enzyme, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, is known to control cholesterol synthesis. The active form of the enzyme is dephosphorylated and the inactive form in phosphorylated. Insulin promotes dephosphorylation, and thus, activates cholesterol synthesis. With the decrease of insulin at parturition, there is a decrease in the dephosphorylation of the enzyme with a consequent decrease in cholesterol synthesis (González & Silva, 2006).

The absence of differences of triglyceride concentrations between the groups, G3 and G1 is corroborated by Santos et al. (2011), who similarly found this variable within the reference values for sheep with clinical PT for animals that either recovered or died. However, these results differ from those reported by Barakat et al. (2007), who observed a decrease in phospholipids and triglycerides in animals clinically affected by PT. This correlates with the low amount of triglycerides, which occur as a result of the reduction of appetite in sick animals. Albay et al. (2014) also found that a significant increase of triglyceride values occurs in goats with clinical PT as compared to in healthy animals.

The significant drop in triglyceride concentrations from the stage of parturition is in agreement with results reported by Lima et al. (2016), according to whom lowest concentrations were found *postpartum* in dairy goats because of increased MY, lower fatty acid availability, lipolysis to obtain energy, and a greater contribution of circulating triglycerides to the mammary gland for fat synthesis in milk during early lactation.

The significant reduction of insulin concentration in G3 and G2 as compared to G1 is mainly related to the NEB *prepartum*. This finding corroborates results of Henze et al. (1998) in sheep with PT; who reported sheep to have lower insulin and high βHB concentrations as compared to the values in pregnant and healthy sheep. Henze et al. (1994) and Yatoo et al. (2015) also observed a decrease in insulin concentrations in sheep with subclinical and clinical PT during late gestation and at the onset of lactation as a result of the inability of pancreatic β-cells to secrete adequate amount of this hormone.

A reduction of insulin concentration observed in the G1 pregnant and healthy goats near parturition was similar reported by Moallem et al. (2012), whereby the insulin concentration in serum of pregnant ewes carrying three fetuses began to fall five weeks before parturition. This may have occurred because of the homeorrhetic regulation to save glucose for the brains and fetal-placental units of pregnant females. Therefore, Brockman (1979) reported that the low insulin concentrations in lactating goats and cows and in pregnant sheep may decrease the use of glucose by extra mammary and extrauterine tissues to save it for MY and fetal growth.

As far as the effect of time is concerned, significant insulin reduction occurs during the parturition and lactation period, but is more pronounced in the subclinical and clinical groups. Similar observations were made by Lomax et al. (1979) in lactating dairy cows that presented lower serum concentrations of insulin as compared to non-lactating animals. This characteristic in high MY animals could be a predisposing factor in the onset of ketosis, since a decline in the availability of

glucose and acetate to peripheral tissues, except for the mammary glands that are responsible milk synthesis, could be accompanied by increased NEFA mobilization and hepatic ketogenesis.

The higher cortisol concentrations in the G2 and G3 groups, particularly in the subclinical group, were observed to be related to the metabolic changes during the *peripartum* stage. According to the studies of Kulcsár et al. (2006) and Anoushepour et al. (2014), there is an increase in cortisol concentrations during late gestation in sheep carrying twins while having subclinical PT. In addition, Yatoo et al. (2015) also found increased cortisol concentrations in goats with subclinical ketosis during their lactation periods, which may be due to an increase in the hypothalamic-pituitary-adrenal activity related to this metabolic disorder. On the other hand, Bani-Ismail et al. (2008) and Gurdogan et al. (2014) did not observe any significant changes in cortisol concentrations in goats and sheep with subclinical PT, respectively. However, Henze et al. (1994) and Gupta et al. (2008) observed a reduction in the concentration of cortisol during lactation as compared to during pregnancy in sheep and goats with subclinical ketosis (a condition not observed in this study).

The higher cortisol concentrations in G2 during lactation could be justified by the greater occurrence of the subclinical form in the time after parturition, which is a factor that triggers stress. The mean cortisol concentration in the G3 group was lower than in G2, although it was higher, when compared to the concentration in the control group. This trend occurred probably due to the therapeutic intervention provided to goats suffering from clinical PT.

In addition, the elevation of this variable in G3 is corroborated by the findings of Hefnawy et al. (2011) and Souto et al. (2013), who found similar behavior in goats with clinical PT as a result of the increased release of this hormone by the adrenal gland. This may also be to the inability of the fatty liver cells – as in the case of PT – to metabolize circulating cortisol. Several studies have also found elevation in cortisol concentration in small ruminants with clinical PT (Andrews, 1997; Ford et al., 1990; Gurdogan et al., 2014; Henze et al., 1998; Sakha, 2016)

According to Cajueiro (2014) and Lima et al. (2016), who worked with small ruminants undergoing the transitional period, the cortisol elevation at parturition is most expressed in the clinical and subclinical animals. Silva Filho (2016) also reported that this increase is related to the stress of *partum*, which is rendered important by the stimulation of energy production from the adipose tissue and the release of amino acids from the breakdown of muscle protein.

Regarding free T3 and T4 concentrations, no significant difference between the groups indicates that there was no influence of these hormones on the etiopathogenesis of clinical and subclinical PT. Bani Ismail et al. (2008) studied goats with subclinical PT to report a similar concentration of free T3 and T4 as compared to healthy goats.

However, other authors report divergent results, such as Gupta et al. (2008) reported a significant decrease in T3 and T4 concentrations in goats with the subclinical PT; yet in goats with ketosis during lactation, there was an increase of their concentration in relation to healthy goats, suggesting a thyroid hypofunction, especially during the last trimester of gestation, while in lactation, increased concentrations suggest an overactive thyroid. According to Kulcsár et al. (2006), who found low concentrations of thyroid hormones in sheep with subclinical PT, this is due to an increase in the degree of inactivation in the peripheral tissues. Alternatively, following a decrease in the activation capacity of T4, a decrease of transformation of T4 and T3 occurs. These changes represent an important mechanism in adapting to NEB.

In addition, results of Macedo et al. (2015), who concluded that clinical PT in sheep causes marked changes in the metabolism of these hormones. According to Hefnawy et al. (2011), who also found a significant decrease in T4 in goats with clinical PT, this reduction is possibly related to an excessive secretion of cortisol, characterizing a negative correlation between these variables.

As for the effect of assessment times, higher T3 concentrations during lactation and higher T4 concentrations during the late pregnancy stage are not corroborated by the findings of another study on healthy goats that showed that T3 concentrations increased during gestation and lactation, while those of T4 decreased during these periods as compared to

animals in the dry period. This variation of results is due to thyroid hyperactivity in relation to the parathyroid at late gestation, which becomes quiescent during *prepartum* (Gupta et al., 2008).

Contreras et al. (1999) found low concentrations of T4 in cows *prepartum* stage but did not find differences between these concentrations. As far as T3 concentrations are concerned, they were within the parameters of normality, but these values were higher during the gestation period. This study also points out that it is difficult to attribute the thyroid hormone plasma variations to a specific factor, since there are many factors involved, such as genetics, number of lactations, milk production, diet, and climatic conditions among other factors.

There are few reports on the free T3 and T4 hormone concentrations during the transitional period in ruminant females with clinical or subclinical PT. The results of our study will thus serve as a basis for further studies that provide an understanding of the behavior of these hormones in the etiopathogenesis of this disease.

The occurrence of the subclinical form of the pregnancy toxemia was expressive as compared to the clinical form during the transitional period of dairy goats. This represents its effect on the production capacity of these animals. Therefore, the occurrence of clinical pregnancy toxemia was more frequent in the period before parturition, whereas the subclinical form assumed greater epidemiological importance during lactation of dairy goats. It is thus essential to intervene as soon as possible to avoid further economic losses.

5. Conclusion

The clinical and subclinical forms of pregnancy toxemia triggered marked changes in serum concentrations of βHB, NEFA, insulin, and cortisol. The βHB assessment validated the possibility of detecting goats that are at a high risk of developing pregnancy toxemia approximately three weeks before parturition.

It is necessary to continue this line of research and expand the knowledge regarding the biochemical peculiarities of pregnancy toxemia, particularly in its subclinical form. The pioneering nature of this research is as follows, the risk of pregnancy toxemia during the transitional period in different groups of dairy goats in order to evaluate its effect on the dairy economy of goat breeding.

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