Rev. Bras. Saúde Prod. Anim., Salvador, v.23, 01 - 13, e21472022, 2022
http://dx.doi.org/10.1590/S1519-994021472022

Animal Production and Environment
Received on: 19/10/2021
Accepted on: 12/01/2022

Biochemical profile of castrated and uncastrated male goats supplemented with vitamin E or not

Perfil bioquímico de caprinos machos castrados e não castrados suplementados ou não com vitamina E

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ABSTRACT

The objective of this study was to evaluate the biochemical parameters of goats submitted to castration or not and receiving supplementation with vitamin E or not. A total of 24 goats, uncastrated (12 experimental units) and castrated males (12 experimental units), with average body weight weighing 17.6 kg ± 2.67 kg, were distributed in a completely randomized design in a 2 x 2 factorial arrangement, with two animal conditions (castrated and uncastrated male) and vitamin E supplementation (with and without supplementation), with six replications. There was an effect of treatments (P<0.05) over time for all studied variables. There was an effect of vitamin supplementation (P<0.05) for phosphorus, iron, protein, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALP) and gamma-glutamyltransferase (GGT). Vitamin supplementation increased glucose, creatinine and GTT levels over time, and reduced levels of phosphorus, iron, protein, albumin, AST and ALP. We conclude that vitamin E influenced the biochemical parameters studied, but castration did not change the

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biochemical profile of goats, regardless of whether they were supplemented with vitamin E or not.
Key words: clinical parameters, small ruminants, supplementation

RESUMO

Objetivou-se avaliar os parâmetros bioquímicos de caprinos submetidos ou não a castração e recebendo ou não suplementação com vitamina E. Um total de 24 caprinos, machos não castrados (12 unidades experimentais) e machos castrados (12 unidades experimentais), com peso corporal médio de 17,6 kg ± 2,67 kg, foram distribuídos em delineamento inteiramente casualizado em arranjo fatorial 2 x 2, sendo duas condições animais (macho castrado e não castrado) e suplementação com vitamina E (com e sem suplementação), com seis repetições. Houve efeito dos tratamentos (P<0,05) ao longo do tempo para todas as variáveis estudadas. Houve efeito da suplementação vitamínica (P<0,05) para fósforo, ferro, proteína, glicose, aspartato aminotransferase (AST), alanina aminotransferase (ALP) e gama-glutamiltransferase (GGT). A suplementação vitamínica elevou os níveis de glicose, creatinina e GTT ao longo do tempo, e reduziu os níveis de fósforo, ferro, proteína, albumina, AST e ALP. Concluímos que a vitamina E influenciou nos parâmetros bioquímicos estudados, porém a castração não alterou o perfil bioquímico de caprinos, independentemente de estarem suplementados ou não com a vitamina E.
Palavras-chave: parâmetros clínicos, pequenos ruminantes, suplementação

INTRODUCTION

The State of Maranhão in Brazil has potential for goat livestock since the soil and climate characteristics are beneficial for the development of animals. However, the production systems within the State are still old and with low technological adherence, in addition to irregular nutrition due to the low quality of food, constituting factors which compromise the productive performance and the health of goat herds, and in turn hindering the economic development of the activity in which efficient measures are needed to reduce production costs (Teixeira et al., 2016).

The metabolic state of the animals can be evaluated through clinical examinations correlating with different paraclinical tests, mainly evaluating the metabolic and hormonal profile, antioxidant indices and mineral levels. Deficiency of metabolites and vitamins can delay reproductive development, fetal growth and milk production (Samimi et al., 2021).

Testosterone exerts an androgenic effect on the development and maintenance of the male reproductive system, and an anabolic effect on the development of muscles, kidneys and liver (Olaifa, 2018), regulation in the oxidative phase of carbohydrates and improves lipid metabolism (Gupta et al., 2008), which may cause changes in the hematological parameters of the animals (Olaifa, 2018).

Castration is a procedure in which the testicles are removed or the vessels that irrigate the testicles are cut, and these procedures vary according to the castration method used (Mohammed & James, 2013).

Rev. Bras. Saúde Prod. Anim., Salvador, v.23, 01 - 13, e21472022, 2022
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Vitamin E can be integrated into animal feed in different ways, with the most common and active form in foods being α-tocopherol (Mendonça Júnior et al., 2011), and is related to several functions in the body. Camargo et al. (2010) state that this vitamin acts in protecting the lipid membrane, receptors and cellular components involved in modulating the immune response of animals.

Knowing that the effects of vitamin E, administered intramuscularly in goats can cause metabolic changes and the clinical status of the animals, it is necessary to analyze the impacts that this supplementation will cause in neutered and non-castrated animals which are supplemented or not with vitamin E through serum biochemistry. Thus, the objective of this study was to evaluate the biochemical parameters of goats submitted to castration or not and receiving supplementation with vitamin E or not.

MATERIALS AND METHODS

The experiment was carried out in the Sheep and Goat Sector of the Federal Institute of Education, Science and Technology of Maranhão, Campus São Luís – Maracanã, located in the city of São Luís, MA, Brazil (2º37'01" south and 44º16'19" west). A total of 24 goats, 12 non-castrated and 12 castrated males, with an initial age of eight months and without a defined racial pattern (SPRD), with a mean body weight (BW) of 17.6 kg ± 2.67 kg, were finished after 90 days in a confinement system, with the first 15 days of the experimental period destined for adaptation of the animals to the diets and facilities, and 75 days for data collection. The castration procedure was performed using burdizzo-type castrating pliers. All procedures with animals were carried out in accordance with the regulations of the Animal Use Committee of the State University of Londrina (217/2014).

The animals were identified with earrings and dewormed using 10 $g.kg^{-1}$ of moxidectin (Cydectin, Fort Dodge Animal Health, Campinas, SP, Brazil) at a dose of 1ml.50 kg^{-1} of body weight (BW) and were then distributed in pens measuring 1.50 m x 1.70 m (one animal per pen) with a concrete floor, covered with sawdust, according to a completely randomized design in a 2 x 2 factorial arrangement, with six replications. The investigated treatments consisted of two animal conditions (castrated and non-castrated males) and vitamin E supplementation (with and without supplementation).

The experimental diets were based on Tifton 85 grass hay (Cynodon *spp*.) and concentrated ration of ground corn and soybean meal, being an isoprotein and isoenergetic diet (Table 1). The diets were formulated for animals with 20 kg BW for an average daily gain of 150 $g.day^{-1}$, according to the NRC (National Research Council, 2007) with daily adjustment, allowing for leftovers of up to 15%. Water and mineral mixture were fed ad libitum to the animals. The feed was supplied twice a day at 8:00 and 17:00 h.

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Rev. Bras. Saúde Prod. Anim., Salvador, v.23, 01 - 13, e21472022, 2022
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Table 1. Composition of experimental diets (g.Kg^{-1} DM*) of castrated and uncastrated goats with no defined racial pattern (SPRD), supplemented with vitamin E

Ingredients	Animal Condition	
	Castrated	Uncastrated
Tyfton-85 Hay	382.8	425.8
Corn grounded	367.0	369.9
Soybean meal	147.3	113.6
Wheat meal	91.3	80.1
Limestone	11.3	10.3
Mineral supplement[2]	0.3	0.3
Chemical Composition		
Dry Matter	871.2	870.6
Crude Protein	150.0	136.7
Neutral Detergent Fiber	382.6	380.1
Acid Detergent Fiber	201.4	200.1
Ethereal Extract	34.6	35.4
Total Digestible Nutrients	666.7	670.9
Phosphorus	7.2	3.3

*DM: Dry Matter. [2]Composition: Ca: 13.4%; P: 7.5%; Mg: 1%; S: 7%; Cl: 21.8%; Na: 14.5%; Mn: 1100 mg.Kg^{-1}; Fe: 500 mg.Kg^{-1}; Zn: 4600 mg.Kg^{-1}; Cu: 300 mg.Kg^{-1}; Co: 40 mg.Kg^{-1}; I: 55 mg.Kg^{-1}; Se: 30 mg.Kg^{-1}

Vitamin E supplementation occurred once a week throughout the experimental period. A dose of 3 mL of vitamin E (acetate-alpha-tocopherol), equivalent to 300 mg, was administered subcutaneously. The animals of the treatments without supplementation received placebo (saline solution) to be submitted to the same stress.

Next, 4 mL of blood were collected from each lamb in the morning (before feeding and water) under aseptic conditions by jugular vein puncture, in vacutainer-type flasks without anticoagulante on days 0, 15, 30, 45, 60 and 75. The vials were kept in an inclined position without disturbance immediately after blood collection. The samples were centrifuged at 3000 rpm for 10 minutes to separate the clear serum, which was collected in 2 mL microtubes and stored at -20 °C for further analysis.

Laboratory analyzes were performed at the CERNITAS Laboratory (a laboratory accredited by MAPA, belonging to the National Network of Agricultural Laboratories, ordinance no. 60, of 22/04/2008). Serum concentrations of total calcium (Labtest method), phosphorus (Basques-Lustosa method), magnesium (Labtest method), iron (Goodwil method), total protein (Biuret method), albumin (Bromocresol Blue method) urea (Urease method) and creatinine (Kinetic method) were evaluated, as well as the plasma glucose content (Orthotoluidine method) and the serum activities of the enzymes aspartate-amino-transferase - AST (Reitman-Frankel method), gamma-glutamyl -transferase - GGT (modified Szasz method) and alkaline phosphatase - ALP (Labtest method). All samples were analyzed in a semiautomatic spectrophotometer at specific wavelengths for each constituent and in a selective ion doser.

Data were submitted to analysis of variance using the F test and the comparison of means test using Tukey's

ISSN 1519 9940

Rev. Bras. Saúde Prod. Anim., Salvador, v.23, 01 - 13, e21472022, 2022
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test at the 5% level by the SPSS version 22 statistical software program (IBM Corporation, New York, USA).

RESULTS

Mineral profile

There was no effect of the interaction between castration and vitamin E supplementation (P>0.05) in relation to blood calcium and magnesium concentrations (Table 2). An effect (P>0.05) was verified over time for all variables of the mineral profile in animals that received supplementation.

Table 2. Mean values for mineral profile of castrated and uncastrated goats, supplemented or not with vitamin E

Variable	Treatment	Days						EPM	P-value	
		0	15	30	45	60	75		T[1]	D[2]
Calcium, mg.dL^{-1}	CYE	10.47	11.30	10.42	10.13	10.90	10.88	0.20	0.242	0.127
	CNE	10.55ab	11.28a	10.28ab	10.10b	10.10b	10.23ab	0.20		
	UYE	9.95	11.03	10.75	10.22	10.67	11.02	0.20	0.172	0.088
	UNE	9.47b	10.78a	10.15ab	10.47ab	10.70a	10.55ab	0.19		
Phosphorus, mg.dL^{-1}	CYE	6.10a	6.13a	2.00c	2.70bc	3.45b	3.28b	0.15	0.668	<0.001
	CNE	6.78b	7.95a	2.28c	2.60c	2.17c	2.37c	0.15		
	UYE	6.69a	6.03a	2.30b	2.58b	3.45b	3.45b	0.18	0.999	<0.001
	UNE	6.17a	6.55a	2.37c	2.40bc	3.43bc	3.60b	0.18		
Magnesium, mg.dL^{-1}	CYE	1.98	1.80	1.72	2.12	1.82	1.96	0.11	0.421	0.399
	CNE	2.05ab	1.70b	2.37a	1.86b	2.08ab	2.07ab	0.11		
	UYE	1.98ab	1.73b	1.90ab	2.35a	1.71b	1.98ab	0.08	0.430	0.655
	UNE	1.95	1.98	2.20	1.89	2.12	2.03	0.08		
Iron, mg.dL^{-1}	CYE	145.85a	114.15b	33.71d	29.96d	80.50c	80.33c	3.75	0.982	<0.001
	CNE	151.60a	116.11b	33.19d	31.83d	77.17c	75.33c	3.94		
	UYE	144.08a	137.17a	36.33c	29.75c	95.20b	83.33b	3.48	0.356	<0.001
	UNE	159.65a	145.50a	35.50c	33.56c	56.90bc	71.16b	3.48		

CYE: Castrated with vitamin E. CNE: Castrated without vitamin E. UYE: Uncastrated with vitamin E. UNE: Uncastrated without vitamin E. [1]: Treatment. [2]: Days.
Different lowercase letters indicate significant difference in serum analyte concentrations over time.

Protein and energy profile

There was an effect of time on serum protein concentrations for the treatment without castration (P<0.05) (Table 3). There was an effect of time between treatments for albumin, urea and creatinine concentrations (P<0.05), with the last two showing peaks at 45 days. There was an effect of time and vitamin supplementation (P<0.05) for glucose, with a peak at 45 and 60 days.

Table 3. Mean values for protein and energy profile of castrated and uncastrated goats, supplemented with vitamin E or not

Variable	Treatment	Days						EPM	P-value	
		0	15	30	45	60	75		T[1]	D[2]
Protein, mg.dL^{-1}	CYE	6.57a	5.96ab	5.68ab	5.16b	5.60ab	5.83ab	0.24	0.839	0.313
	CNE	5.82	6.43	5.92	5.36	5.32	5.40	0.25		

ISSN 1519 9940

RBSPA Revista Brasileira de Saúde e Produção Animal

Rev. Bras. Saúde Prod. Anim., Salvador, v.23, 01 - 13, e21472022, 2022
http://dx.doi.org/10.1590/S1519-994021472022

	UYE	6.58a	5.72ab	5.12b	4.50b	6.68a	6.23a	0.11	0.064	0.028
	UNE	5.17b	5.62ab	5.49ab	6.68ab	6.80ab	6.82a	0.12		
Albumin, g.dL^{-1}	CYE	1.88b	2.48a	0.93b	1.33b	2.17a	2.15a	0.09	0.370	<0.001
	CNE	1.84a	2.10a	1.02b	1.55b	1.95a	2.12a	0.09		
	UYE	1.95b	2.48a	1.11c	1.30c	1.75bc	1.71bc	0.06	0.503	<0.001
	UNE	1.75ab	2.22a	1.15b	1.32b	2.02a	2.20a	0.06		
Urea, g.dL^{-1}	CYE	42.83	37.33	37.67	45.81	46.17	46.20	1.98	0.269	0.025
	CNE	39.83b	36.67c	41.17abc	53.80a	51.70ab	51.67ab	2.01		
	UYE	43.50	31.33	43.67	42.40	42.67	42.72	2.70	0.291	0.016
	UNE	41.67ab	33.67b	44.50ab	52.00a	49.55a	49.50a	2.75		
Creatinine, g.dL^{-1}	CYE	0.72ab	0.67b	0.83ab	0.92ab	0.93a	0.93a	0.04	0.252	0.010
	CNE	0.63	0.65	0.99	1.00	0.68	0.69	0.04		
	UYE	0.62b	0.68b	0.82ab	0.99a	0.98a	0.97a	0.04	0.811	<0.001
	UNE	0.70b	0.73ab	0.97a	0.97a	0.88ab	0.89ab	0.04		
Glucose, g.dL^{-1}	CYE	41.67c	50.96bc	76.17b	148.72a	168.13a	60.00bc	3.70	0.648	<0.001
	CNE	63.23bc	54.38c	81.93b	118.62a	139.41a	73.67bc	3.70		
	UYE	65.41bc	67.41bc	90.11b	125.44a	137.74a	63.00c	3.84	0.049	<0.001
	UNE	70.10c	42.15c	56.73c	122.64b	150.49a	59.17c	3.84		

CYE: Castrated with vitamin E. CNE: Castrated without vitamin E. UYE: Uncastrated with vitamin E. UNE: Uncastrated without vitamin E. [1]: Treatment. [2]: Days.
Different lowercase letters indicate significant difference in serum analyte concentrations over time.

Enzyme profile
There was an effect of vitamin supplementation (P<0.05) for aspartate aminotransferase (AST) and alanine aminotransferase (ALP), peaking at 75 days and 45 and 60 days, respectively (Table 4). For the gamma-glutamyltransferase (GGT) variable, there was an effect (P<0.05) of the animal condition at 45 days (fourth collection).

Table 4. Mean values for enzymatic profile of castrated and uncastrated goats, supplemented with vitamin E or not

Variable	Treatment	Days						EPM	P-value	
		0	15	30	45	60	75		T[1]	D[2]
AST, UI.L^{-1}	CYE	70.33b	72.33b	49.41c	39.91c	68.83b	159.86a	2.44	0.001	<0.001
	CNE	81.17b	82.00b	65.00bc	58.80c	73.83b	139.41a	2.28		
	UYE	72.00b	70.66b	50.16c	46.00c	67.16bc	132.97a	2.35	0.022	<0.001
	UNE	80.16b	77.80b	68.66bc	56.00c	64.83bc	139.22a	2.41		
ALP, UI.L^{-1}	CYE	48.44a	55.32a	25.20b	54.51a	59.22a	41.01b	1.88	0.002	<0.001
	CNE	63.31b	72.04ab	26.81c	64.23ab	75.07a	35.81c	1.88		
	UYE	43.20b	49.50b	29.80c	69.83a	62.50a	37.16bc	2.77	0.362	<0.001
	UNE	59.80a	61.00a	30.20c	59.84a	57.50ab	41.66bc	2.77		
GGT, UI.L^{-1}	CYE	37.33b	37.67b	60.50a	39.33b	41.00b	61.19a	1.90	0.927	<0.001
	CNE	41.17b	41.00b	54.83a	36.50b	35.83b	66.22a	1.90		
	UYE	40.50b	40.50b	52.17b	32.17c	37.17c	67.17a	1.90	0.652	<0.001
	UNE	38.16b	40.50b	50.50ab	45.66b	41.66b	60.53a	1.93		

ISSN 1519 9940

Rev. Bras. Saúde Prod. Anim., Salvador, v.23, 01 - 13, e21472022, 2022
http://dx.doi.org/10.1590/S1519-994021472022

AST: aspartate aminotransferase. ALP: alanine aminotransferase. GGT: gama-glutamyltransferase. CYE: Castrated with vitamin E. CNE: Castrated without vitamin E. UYE: Uncastrated with vitamin E. UNE: Uncastrated without vitamin E. [1]: Treatment. [2]: Days.
Different lowercase letters indicate significant difference in serum analyte concentrations over time.

DISCUSSION

Minerals are of fundamental importance for ruminants, being present in fluids and tissues, and having basic functions such as tissue structure, assisting in acid-base balance, osmotic pressure and cell membrane permeability (Soetan et al., 2010). Calcium (CA) levels (Table 2) were within the normal range for goats, ranging from 9.5 to 11.4 $mg.dL^{-1}$ throughout the entire experimental period, which is consistent with findings in other studies (Duarte et al., 2011; Kaneko et al., 2008; Lima et al., 2021).

Castrated animals showed differences ($P<0.05$) over time for CA levels, but no information was found in the consulted literature to justify the observed fact, since castration alters hormonal metabolism and animal development.

The main functions of calcium are to form and maintain teeth and bones, muscle contraction, regulate hear rate, transmit nerve impulses and secrete hormones, and it has a direct interaction with phosphorus (P), in which the excess of both minerals can lead to a decrease in their absorption (Fayer & Bengoumi, 2018).

Serum phosphorus (P) concentrations for all treatments (Table 2) until the second collection remained within the reference values (4.2 to 9.1 $mg.dL^{-1}$) (Kaneko et al., 2008), however, there was a decrease in the concentration from the third collection onwards, which ranged from 2 to 3.6 $mg.dL^{-1}$ between the treatments studied, and this fact may have occurred due to the physiological variation of the animal due to its advancing age (Lima et al., 2021).

Magnesium (Mg) values were within the range reported for small ruminants by Chester-Jones et al. (1990) and Boyd (1984) (1.92 mg/dL^{-1} and 2.19 to 2.92 mg/dL^{-1}, respectively). Soetan et al. (2010) reports that this mineral is absorbed in the intestine and subsequently transported to the blood, cells and tissues, where problems in the digestive tract and kidneys can influence absorption, and consequently, changes in serum magnesium levels. Weakening of the hair and roughness is observed when Mg is deficient in the diet in young animals, leading to the animals having a stunted appearance, and in extreme cases convulsions leading to death (Merck, 1986).

The serum iron (Fe) concentration in this study ranged from 29.8 to 159.7 $mg.dL^{-1}$ (Table 2), being close to the reference values ranging from 39 to 207 $mg.dL^{-1}$ (Kaneko et al., 2008; Smith, 1997). Concentrations above 218 $mg.dL^{-1}$ can be considered an overload, which can cause liver damage, with advancing age of the animal being a greater susceptibility to this overload (Kaneko et al., 2008).

Vitamin E supplementation can reduce iron toxicity (Kaneko et al., 2008) in addition, reduced testosterone levels can decrease iron absorption, and its incorporation mainly occurs in red blood cells and hemoglobin synthesis (Olaifa, 2018).

In a study carried out by Lima et al. (2021) with goats fed on native pasture with or without mineral supplementation, the authors found higher values than those found in this study for iron, which ranged from 189.3

ISSN 1519 9940

Rev. Bras. Saúde Prod. Anim., Salvador, v.23, 01 - 13, e21472022, 2022
http://dx.doi.org/10.1590/S1519-994021472022

to 293.7 mg.dL^{-1}, and from 167.5 to 245.7 mg.dL^{-1}, respectively. The authors reported that the highest concentrations for non-supplemented animals came from involuntary soil ingestion, since there is a shortage of forage at some time of the year.

All groups showed a decrease in total protein concentration on some of the days studied (Table 3), differing from the reference value range, which is 6.4 to 7.0 mg.dL^{-1} for goat species (Kaneko et al. al., 2008). The concentration of total proteins is reduced in liver failure, intestinal and kidney disorders, hemorrhage or dietary deficiency (Gonzalez & Silva, 2008). It was observed that the total protein content increased throughout the experiment, remaining within the reference values in the last two collections, mainly for non-castrated animals. In evaluating the biochemical profile of lactating goats fed diets containing 0, 7, 14 and 21% of crude glycerin from the production of biodiesel from frying oil, Santos et al. (2018) found that the different crude glycerin percentages vin the diet did not affect the concentrations of total proteins.

Serum albumin concentration was below the acceptable range for all treatments according to Raju et al. (2015) (14.74 gL^{-1}), and this can predict an indication of liver dysfunction, although there are other parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALP), alkaline and acid phosphatase that assertively investigate this dysfunction (Tripathi et al., 2008).

The increase in urea levels (Table 3) throughout the collections, especially for castrated animals, corroborates Al-Zghoul et al. (2008) and Olaifa & Opara (2011), since urea acts as a source of nitrogen for protein biosynthesis, where the intense breakdown of protein leads to ammonia formation, which can cause intoxication in the animal (Harmeyer & Martens, 1980; Olaifa & Opara, 2011). Castration causes inflammatory trauma in the animal, leading to changes in protein absorption, and consequently, an increase in urea production.

When studying different times after castration, Kayode & Obot (2017) observed that the urea concentration in the blood of goats increased due to the increase in cortisol levels, as this stress caused by castration increases the breakdown of proteins and nucleic acids in the muscles due to the increase in this hormone.

Soul et al. (2019) state that creatinine is formed in skeletal muscle through the degradation of serum phosphocretin for energy production, in which creatinine levels are directly proportional to the amount of muscle mass (Carlos et al., 2015). Therefore, an increase in creatinine levels was observed over time, which may be related to a reduction in thyroxine activity (Soul et al., 2019) in turn, castrated animals without supplementation had a drop in creatinine levels from 60 days of collection (Table 3), leading to the belief that they had maximized efficiency energy use.

The results found herein were superior to those found by Olaifa (2018) for creatinine levels (Table 3). This same author reports that creatinine is removed from the blood through glomerular filtration and by proximal tubular secretion through the kidneys, and is commonly used as an indicator to presuppose renal function.

It is observed that vitamin E supplementation tended to increase glucose levels in goats (Table 3), corroborating Ziaei (2014) that when

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supplementing goats up to the level of 50 mg.kg^{-1} of vitamin E, glucose considerably increased. Some studies indicate that weaning, rumen development, dietary and physiological changes cause changes in ruminant blood glucose (Baldwin et al., 2004; Mccarthy & Kesler, 1956; Redlberger et al., 2017). The literature reports that the blood glucose of adult goats ranges from 60 to 190 mg.dL^{-1} (Kaneko, 1989; Pérez et al., 2003; Yanaka et al., 2012).

The aspartate aminotransferase (AST) concentrations found ranged from 39.9 to 159.9 IU.L^{-1} (Table 4), differing from the values adopted as reference for goats, which is from 167 to 513 IU.L^{-1} (Kaneko, 1989). However, there is still a lot of divergence in the literature regarding AST variations as a function of the age of the animal (Yanaka et al., 2012). Gwaze et al. (2012) report that food availability can affect AST levels, because it is correlated with muscle growth, and with correct liver functioning.

The concentration of alanine aminotransferase (ALP) levels when the animals received the supplementation ranged from 25.2 to 75.0 IU.L^{-1} (Table 4), being below the reference range for the goat species, which is 93 at 387 IU.L^{-1} (Kaneko, 1989). However, serum ALP values in goats can increase up to tem-fold without liver damage (Yanaka et al., 2012), and the elevation can occur during periods that involve increased bone activity, such as the growth phase (Gwaze et al., 2012).

Gwaze et al. (2012) reported negative interaction of ALP with increased age of the animals, since this enzyme is highly related to the calcification process, so bone development is accelerated when the animals are young, and Kaneko et al. (2008) claim that this enzyme also helps in the development of the gastrointestinal tract.

The gamma glutamyltransferase (GGT) concentrations found ranged from 32.2 to 67.2 IU.L^{-1} (Table 4) throughout the experimental period, in agreement with the reference values of Pérez et al. (2003) from 26 to 77 IU.L^{-1}.

CONCLUSIONS

Castration did not influence the biochemical profile of goats, regardless of whether they were supplemented with vitamin E or not. Vitamin supplementation increased glucose, creatinine and gamma-glutamyltransferase (GTT) levels over time, and reduced the amount of phosphorus, iron, protein, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALP).

ACKNOWLEDGEMENT

The authors thank the Instituto Federal do Maranhão – Campus Maracanã, the Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão – FAPEMA and the Coordination for the Improvement of Higher Education Personnel – CAPES, for their financial and logistical support.

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ISSN 1519 9940