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Effects of L-Arginine Supplementation on Lactating Mares and the Development of Foals

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ABSTRACT

***Background*:** Most animal species are able to produce Arginine (Arg) under normal conditions. However, in some situations, its degradation can be higher than its production. For example, during a period of lactation or disease, there is an increase in the consumption of Arg. In this case, endogenous production is not enough for the animal's demands. Indeed, Arg supplementation in animals has several benefits for the animal's body, such as the increase of angiogenesis, improvements in immunity and the reproductive system, as well as the stimulation of lactogenesis. During the early phase of growth, a deficiency of Arg could cause a reduction in the growth rate and metabolic activity of animals. Therefore, this amino acid is considered essential in some phases of the life of animals. However, very few studies of the supplementation of this amino acid in horses have been carried out. The aim of the present study was to characterize the effects of supplementing lactating mares and their foals with Arg.

***Materials, Methods & Results*:** Lactating mares (n = 10) were divided into two groups (control group: n = 3 / supplemented group: n = 7) and maintained exclusively under grazing. The supplemented group received 50 g of Arg during the lactation period. Samples of milk and blood from mares and blood from foals were collected at different phases of the lactation period. The following parameters were measured in milk: Glutamine (Gln); Glutamate (Glu); protein; fat; casein; lactose; urea and total solids. The following parameters were measured in blood: Gln; Glu; total plasmatic protein (TPP); albumin; urea; creatinine; uric acid; triglycerides; total cholesterol; calcium (C); phosphorous (P); magnesium (Mg) and ferrous (Fe). In addition, the biometric parameters of Wither Height (WH), Chest Perimeter (CP), Cannon Bone Circumference (CBC) and Fat Percentage (FP) of foals were obtained. A significant increase of Gln was observed in the milk in both groups ($P < 0.05$). The highest concentration of Gln was detected in the third month of the lactation period in the supplemented group (~ 2.26 mmol/mL), and the control group (~ 1.91 mmol/mL) during the same period. Gln did not alter in the blood ($P > 0.05$), although Glu was higher in the control group in the first month of the lactation period (~ 0.21 mmol/mL) ($P < 0.05$). An increase in uric acid (~ 0.19 mmol/L) in both groups on the day of birth ($P < 0.05$). In the supplemented group, increases in triglycerides (~ 0.60 mmol/L), Ca (~ 2.90 mmol/L) and Mg (~ 0.52 mmol/L) were observed in the first month of the lactation ($P < 0.05$). At birth, foals exhibited high levels of urea (~ 4.67 mmol/L) and uric acid (~ 0.21 mmol/L), and low levels of P (~ 2.02 mmol/L) ($P < 0.05$). The levels of Gln in the blood of foals remained between 0.50 and 0.70 mmol/mL throughout the lactation period ($P > 0.05$). Even when they were added (Gln + Glu), no differences were observed ($P > 0.05$). However, when the biometric parameters were analyzed, significant variations were detected in almost all characteristics (weight, WH, CP and CBC). In particular, the control group exhibited higher body mass and CP in the fifth month, when compared with the group of foals born from supplemented mares ($P < 0.05$). The FP did not alter in either of the groups analyzed ($P > 0.05$).

***Discussion*:** The results indicate that the supplementation of lactating mares with Arg produced few alterations in the parameters analyzed for both mares and foals. In addition, the supplementation did not produce side effects among the supplemented animals.

Keywords: horse, functional amino acid, biomarkers, biometric measures, body composition.

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INTRODUCTION

Arginine (Arg) is a versatile amino acid that is used by different tissues in several metabolic functions, including the production of urea, creatinine and other blood biomarkers [11,19,21]. Although the subject is significant in relation to the metabolism of animals, very few studies of the effects of Arg supplementation in horses have been carried out.

Supplementation with Arg has been widely used in domestic animals to increase its presence in the blood, since it is a precursor of antioxidative compounds and polyamines for different functions [2,12]. It has been shown that supplementation with Arg can stimulate angiogenesis in the mammary gland of different animal species. In addition, it contributes to the formation of polyamines, which are important regulators of lactogenesis and protein synthesis for the production of milk [4,11,16,18,19]. In this context, it has been demonstrated that the absorption of Arg by mammary tissue is important for angiogenesis and for the production of other amino acids during the lactation period [19,25]. In most animal species, the presence of Arg in milk is low. Supplementation of pregnant females (near to or immediately after the birth) could stimulate greater milk production, and consequently increase the concentration of this amino acid in their milk.

However, there are very few studies that address supplementation with Arg, blood biomarkers and the development of foals. Therefore, the aim of the present study was to investigate the effects of supplementation with Arg during the lactation period by identifying alterations in the composition of milk and biomarkers of the metabolism of mares and their offspring.

MATERIALS AND METHODS

Quarter horse adult multiparous mares (n = 10), aged between 4 and 12 years, were divided into two groups: the control group (n = 3) and the supplemented group (n = 7). These mares were kept in pastures of Massai grass (*Panicum maximum*) throughout the pregnancy and lactation periods, with free access to both water and mineralized salt.

Animals in the supplemented group received the following on a daily basis: 50 g of L-Arginine[1] (L-Arginine®, minimum of 98 % Arginine) and 200 g of commercial concentrate[2]. The mares in the control group only received 200 g of concentrate daily. After 3-4 months of life, the foals had free access to creep feeder.

For milk sampling, the foals were separated from their mothers for 30 minutes and placed in a paddock. After this period, the mares were milked in the presence of the foals, using clean and sanitized bottles. No oxytocin was used for this procedure. The samples were divided into two aliquots: the first was acidified with 10 % perchloric acid and neutralized with potassium hydroxide to determine the Gln and Glu. The second aliquot was used to determine the constituents (protein, fat, casein, lactose, urea and the total solids) of the milk using automatic equipment Bentley Combi B2300[3].

Blood samples were collected before the beginning of the supplementation, on the day of parturition (minimum of 8 h before), and at the first, third and fifth months of the lactation period. These samples were collected by jugular venipuncture using heparinized vacuum tubes. Subsequently, the samples were divided into two aliquots. One of these aliquots was used to determine the Gln and Glu after acidification and neutralization, whereas the other was centrifuged to extract plasma, which in turn was used to analyze the blood biomarkers. The Gln and Glu present in the milk and blood were determined by the enzymatic method [13]. Blood biomarkers (total plasma protein (TPP), albumin, urea, creatinine, uric acid, triglycerides, total cholesterol, Ca, P, Mg and F) were assessed using a semi-automatic device (Doles D-250, Doles, Brazil) and a commercial kit Doles® [4].

The foals were assessed based on the following parameters: Wither Height (WH); Chest Perimeter (CP) and Cannon Bone Circumference (CBC) (obtained with a hipometer and a tape measure). The weight of the foals was obtained using a weighing scale, and the Fat Percentage (FP) was determined based on the method described by Westervelt *et al*. (1976).

The data were analyzed by ANOVA and the Student-Newman-Keuls test of multiple comparisons, using SigmaStat 3.0 software. In both tests, the significance level was set at 5 %. The results are presented as mean and standard deviation values.

RESULTS

A significant increase of Gln was observed in the milk in both groups ($P < 0.05$). The highest concentration of this amino acid was detected in the third month of the lactation period in the supplemented group (~ 2.26 mmol/mL). This value was approxi-

mately 15 % higher than that found in the control group (~ 1.91 mmol/mL) during the same period (Table 1). When the values of Gln and Glu were added, an increase of 18 % in the supplemented group was observed in the third month of the lactation period. The FP, the levels of protein and total solids were higher in samples collected on the day of birth ($P < 0.05$), while the percentage of lactose was higher between the first and third months of the lactation ($P < 0.05$). The urea and casein present in the milk of mares did not alter throughout the study ($P > 0.05$).

Gln did not alter in the blood ($P > 0.05$), although Glu was higher in the control group in the first month of the lactation period (~ 0.21 mmol/mL) ($P < 0.05$). An increase in uric acid (~ 3.20 mg/dL) was observed in both groups on the day of birth ($P < 0.05$), whereas this same acid was present in lower levels in both groups during the first month of lactation. In the supplemented group, increases in triglycerides (~ 0.60 mmol/L), Ca (~ 2.90 mmol/L) and Mg (~ 0.52 mmol/L) were observed in the first month of the lactation ($P < 0.05$). On the other hand, biomarkers such as TPP, albumin, total cholesterol and Fe did not alter in either group during the five months of lactation ($P > 0.05$) [Table 2].

In the analysis of the blood biomarkers of suckling foals, significant differences were only detected for a few biochemical parameters. At birth, foals exhibited high levels of urea (~ 4.67 mmol/L) and uric acid (~ 0.21 mmol/L), and low levels of P (~ 2.02 mmol/L) ($P < 0.05$). The levels of Gln remained between 0.50 and 0.70 mmol/mL throughout the lactation period ($P > 0.05$). Even when they were added (Gln + Glu), no differences were observed ($P > 0.05$). However, when the biometric parameters were analyzed, significant variations were detected in almost all characteristics (weight, WH, CP and CBC). In particular, the control group exhibited higher body mass and CP in the fifth month, when compared with the group of foals born from supplemented mares ($P < 0.05$). The FP did not alter in either of the groups analyzed ($P > 0.05$) [Table 3].

Table 1. Concentrations of different biomarkers in milk of mares supplemented or not with L-Arginine.

Biomarker	Parturition Day (n = 10)	Supplementation					
		50 g of Arginine/day (n = 7)			No supplemented Group (n = 3)		
		1st month	3rd month	5th month	1st month	3rd month	5th month
Glutamine (mmol/mL)	0.60 ± 0.18^{d}	$2.10 \pm 0.34^{a,b}$	2.26 ± 0.25^{a}	$1.07 \pm 0.19^{b,c,d}$	$1.41 \pm 0.28^{a,b,c,d}$	$1.92 \pm 0.35^{a,b,c}$	$1.10 \pm 0.28^{a,b,c,d}$
Glutamate (mmol/mL)	0.42 ± 0.108^{b}	$1.06 \pm 0.17^{a,b}$	1.28 ± 0.17^{a}	$0.75 \pm 0.20^{a,b}$	$1.10 \pm 0.28^{a,b}$	$0.97 \pm 0.09^{a,b}$	$0.82 \pm 0.08^{a,b}$
Gln+Glu (mmol/mL)	1.02 ± 0.27^{d}	$3.16 \pm 0.47^{a,b}$	3.53 ± 0.30^{a}	$1.82 \pm 0.37^{b,c,d}$	$2.51 \pm 0.53^{a,b,c}$	$2.89 \pm 0.32^{a,b,c}$	$1.92 \pm 0.36^{a,b,c,d}$
Fat (%)	2.00 ± 0.28^{a}	0.82 ± 0.16^{b}	0.47 ± 0.11^{b}	1.02 ± 0.21^{b}	1.01 ± 0.12^{b}	0.37 ± 0.05^{b}	0.70 ± 0.04^{b}
Protein (%)	3.98 ± 0.49^{a}	2.03 ± 0.11^{b}	1.64 ± 0.07^{b}	1.99 ± 0.13^{b}	2.37 ± 0.18^{b}	1.81 ± 0.08^{b}	1.66 ± 0.08^{b}
Lactose (%)	4.55 ± 0.38^{a}	6.44 ± 0.02^{a}	6.62 ± 0.03^{a}	3.25 ± 1.37^{b}	6.19 ± 0.08^{a}	6.40 ± 0.19^{a}	6.45 ± 0.12^{a}
Total solids (%)	14.79 ± 1.37^{a}	10.13 ± 0.27^{b}	9.56 ± 0.13^{b}	7.36 ± 0.99^{b}	10.42 ± 0.21^{b}	9.43 ± 0.29^{b}	9.64 ± 0.03^{b}
Urea (mmol/L)	1.71 ± 0.41	2.47 ± 0.38	3.31 ± 0.19	2.54 ± 0.34	2.70 ± 1.04	3.29 ± 0.17	2.73 ± 0.09
Casein (%)	4.86 ± 1.54	1.28 ± 0.09	0.99 ± 0.06	1.86 ± 0.11	1.57 ± 0.14	1.12 ± 0.07	1.03 ± 0.05

[abcd]Different letters in the same row indicate that $P < 0.05$ by Tukey test.

Table 2. Concentrations of different biomarkers in blood of mares supplemented or not with L-Arginine.

Biomarker	Parturition Day (n = 10)	Supplementation					
		50 g of Arginine/day (n = 7)			No supplemented Group (n = 3)		
		1st month	3rd month	5th month	1st month	3rd month	5th month
Glutamine (mmol/mL)	0.54 ± 0.04	0.45 ± 0.03	0.41 ± 0.05	0.45 ± 0.08	0.46 ± 0.10	0.42 ± 0.03	0.46 ± 0.02
Glutamate (mmol/mL)	0.16 ± 0.01^{a,b}	0.16 ± 0.02^{a,b}	0.17 ± 0.01^{a,b}	0.14 ± 0.03^{a,b}	0.21 ± 0.01^{a}	0.15 ± 0.01^{a,b}	0.12 ± 0.01^{b}
Blood [Gln+Glu] (mmol/mL)	0.66 ± 0.05	0.61 ± 0.03	0.58 ± 0.04	0.59 ± 0.08	0.66 ± 0.09	0.57 ± 0.01	0.57 ± 0.03
Urea (mmol/L)	6.01 ± 0.60	5.58 ± 0.55	3.56 ± 0.29	3.81 ± 0.37	5.82 ± 1.66	3.95 ± 0.87	4.07 ± 0.81
Creatinine (µmol/L)	98.12 ± 5.30	99 ± 6.19	100.78 ± 7.07	97.24 ± 7.96	117.57 ± 9.72	85.75 ± 11.49	107.85 ± 10.61
Acid Uric (mmol/L)	0.19 ± 0.01^{a}	0.11 ± 0.01^{e}	0.13 ± 0.01^{b,c,d}	0.15 ± 0.01^{b}	0.11 ± 0.01^{e}	0.13 ± 0.01^{b,c,d,e}	0.15 ± 0.01^{b,c}
TPP (g/L)	81.10 ± 7.50	104.10 ± 17.10	77.10 ± 1.80	81.00 ± 8.70	111.80 ± 22.30	75.00 ± 4.20	88.80 ± 5.50
Albumin (g/L)	36.50 ± 2.90	39.70 ± 1.60	37.10 ± 1.30	34.50 ± 4.70	34.60 ± 7.30	38.40 ± 2.10	36.10 ± 8.30
Total Cholesterol (mmol/L)	4.28 ± 1.24	1.66 ± 0.21	2.36 ± 0.09	1.15 ± 0.18	1.37 ± 0.37	1.98 ± 0.28	1.01 ± 0.22
Triacylglicerol (mmol/L)	0.56 ± 0.07^{a,b}	0.60 ± 0.10^{a}	0.27 ± 0.05^{a,b,c}	0.21 ± 0.05^{c}	0.46 ± 0.13^{a,b,c}	0.30 ± 0.02^{a,b,c}	0.12 ± 0.11^{c}
Calcium (mmol/L)	2.66 ± 0.05^{a,b}	2.90 ± 0.12^{a}	2.35 ± 0.13^{b}	2.56 ± 0.14^{a,b}	2.48 ± 0.09^{a,b}	2.38 ± 0.12^{a,b}	2.88 ± 0.24^{a,b}
Phosphorus (mmol/L)	1.08 ± 0.12^{c,d,e,f}	2.10 ± 0.28^{a,b}	1.79 ± 0.02^{a,b,c,d}	1.58 ± 0.05^{a,b,c,d,e,f}	2.13 ± 0.46^{a}	1.88 ± 0.19^{a,b,c}	1.67 ± 0.06^{a,b,c,d,e}
Magnesium (mmol/L)	0.44 ± 0.01^{d,e,f}	0.52 ± 0.01^{a}	0.44 ± 0.01^{c,d,e}	0.46 ± 0.01^{b,c}	0.48 ± 0.01^{b}	0.41 ± 0.01^{f}	0.46 ± 0.01^{b,c,d}
Ferrous (µmol/L)	1.92x10^{4} ± 0.11x 10^{4}	1.42x10^{4} ± 0.20x10^{4}	1.53x10^{4} ± 0.42x10^{4}	2.09x10^{4} ± 0.22 x10^{4}	1.4x10^{4} ± 0.2x10^{4}	1.8x10^{4} ± 0.13x10^{4}	1.44x10^{4} ± 0.06x10^{4}

^{abcdef}Different letters in the same row indicate that $P < 0.05$ by Tukey test.

Table 3. Concentrations of different biomarkers (from the birth to the fifth month of life) in suckling foals from mares in lactation supplemented or not with L-Arginine.

Biomarker	Parturition day (n = 10)	Supplementation					
		50 g/day (n = 7)			Control (n = 3)		
		1st month	3rd month	5th month	1st month	3rd month	5th month
Glutamine (mmol/mL)	0.59 ± 0.07	0.59 ± 0.04	0.48 ± 0.03	0.41 ± 0.04	0.49 ± 0.13	0.55 ± 0.09	0.53 ± 0.02
Glutamate (mmol/mL)	0.18 ± 0.02	0.17 ± 0.01	0.19 ± 0.02	0.16 ± 0.02	0.20 ± 0.06	0.17 ± 0.03	0.17 ± 0.02
Gln+Glu (mmol/mL)	0.77 ± 0.09	0.76 ± 0.04	0.68 ± 0.04	0.57 ± 0.03	0.69 ± 0.19	0.72 ± 0.06	0.71 ± 0.04
Urea (mmol/L)	4.67 ± 0.58a	1.79 ± 0.36b	2.54 ± 0.32[a],b	4.36 ± 0.77[a],b	1.57 ± 0.32b	2.36 ± 0.57[a],b	2.92 ± 0.26[a],b
Creatinine (µmol/L)	114.04 ± 14.14	108.73 ± 8.84	121.99 ± 1.77	150.28 ± 8.84	117.57 ± 12.38	129.06 ± 12.38	161.77 ± 5.30
Acid Uric(mmol/L)	0.21 ± 0.02a	0.14 ± 0.01b	0.16 ± 0.01[a],b	0.16 ± 0.01[a],b	0.16 ± 0.01[a],b	0.16 ± 0.01[a],b	0.17 ± 0.01[a],b
TPP (g/L)	69.80 ± 7.90	94.80 ± 19.80	79.50 ± 2.60	82.30 ± 6.30	74.10 ± 1.80	78.90 ± 2.60	77.90 ± 4.10
Albumin (g/L)	36.20 ± 1.40	33.40 ± 1.70	36.90 ± 1.90	37.80 ± 3.80	32.50 ± 1.90	38.60 ± 2.90	40.80 ± 1.90
Total Cholesterol (mmol/L)	5.84 ± 1.00	6.09 ± 1.04	3.52 ± 0.26	2.38 ± 0.20	5.65 ± 2.28	4.51 ± 0.14	3.07 ± 0.38
Triacylglicerol (mmol/L)	0.86 ± 0.34	0.30 ± 0.14	0.17 ± 0.04	0.33 ± 0.04	0.18 ± 0.08	0.17 ± 0.07	0.57 ± 0.67
Calcium (mmol/L)	2.53 ± 0.06	3.10 ± 0.31	2.72 ± 0.11	2.71 ± 0.09	2.90 ± 0.34	2.63 ± 0.10	2.68 ± 0.13
Phosphorus (mmol/L)	2.02 ± 0.26c	3.28 ± 0.26[a],b	2.79 ± 0.25[a],b,c	2.07 ± 0.10[a],B,C	3.53 ± 0.49a	2.73 ± 0.40[a],b,c	2.90 ± 0.57[a],b,c
Magnesium (mmol/L)	0.46 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.45 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.47 ± 0.01
Ferrous (µmol/L)	3.04x104 ± 0.50x104	1.80x104 ± 0.45x104	1.94x104 ± 0.50x104	1.95x104 ± 0.20x104	1.39x104 ± 0.45x104	1.89x104 ± 0.17x104	2.05x104 ± 0.07x104
Whiter height (cm)	87.70 ± 0.96d	96.20 ± 1.49c	108.00 ± 0.83b	115.80 ± 1.90a	96.33 ± 0.88c	105.33 ± 1.20b	115.66 ± 0.88a
Chest perimeter (cm)	79.60 ± 1.15e	96.00 ± 1.54d	111.20 ± 1.35c	121.60 ± 3.66b	95.33 ± 0.33d	114.00 ± 1.15c	129.33 ± 0.88a
Cannon bone circumference (cm)	11.70 ± 0.15d	12.60 ± 0.18c	13.80 ± 0.20b	14.90 ± 0.18a	12.16 ± 0.16c,d	13.83 ± 0.16b	15.00 ± 0.28a
Body mass (Kg)	40.50 ± 2.14e	72.80 ± 3.87d	115.00 ± 4.03c	150.20 ± 13.45b	71.66 ± 0.33d	122.66 ± 4.33c	179.00 ± 3.78a
Body fat (%)	9.45 ± 0.02	9.45 ± 0.03	9.51 ± 0.01	9.39 ± 0.03	9.44 ± 0.08	9.51 ± 0.01	9.43 ± 0.03

[abcd]Different letters in the same row indicate that $P < 0.05$ by Tukey test.

DISCUSSION

The results for both groups of mares and their offspring demonstrated that the supplementation of lactating mares with 50 g of Arg for a period of five months produced few alterations in the concentration of biomarkers in the blood and milk of mares and in the blood of foals. However, it is important highlight that a large amount of the Arg supplemented in adult animals is metabolized in the intestine before it reaches the portal circulation [26] and consequently, may only lead to a few modifications in the metabolism. In previous studies, Arg (100 g/day) was supplemented at the end of pregnancy (three last weeks) and in the early lactation period (first week) and combined with that already present in the basal diet, but did not produce alterations during gestation [17,23]. In this present study, the mares received more Arg (50 g/day) than that present in the food, which was of excellent quality but probably exhibited a low percentage of dry matter. It is important to note that the supplementation period was five months and some effects of amino acid supplementation are expected in relation to the metabolism of lactation. Finally, the supplementation did not produce side effects among supplemented animals, which is significant, given that supplementation with large amounts of Arg can cause digestive disorders in animals, such as diarrhea [21].

Studies with Quarter horse mares have shown that they produce approximately 10 kg of milk/day, and the amount, percentage of protein (PP) and FP of this milk reduces during the lactation period [7]. Others authors reported that the values of the PP and casein were 4.13 % and 2.02 %, respectively, 24 h after birth [6]. Similar results were found in the present study for the samples analyzed on the day of birth. Conversely, detected PP, FP and lactose values of 12.83 %, 1.25 % and 4.83 %, respectively [8]. Upon comparison of these results with those of the present study, it is clear that the PP was higher, while the results for the other two parameters (FP and lactose) were similar.

The concentration of minerals present in milk undergoes similar alterations to those of the PP and FP. In a study with Thoroughbred mares, it was shown that the concentration of Mg and Fe was elevated in the colostrum, while C and P were only higher in the first week of lactation, with all values decreasing after this period [8]. With regards to Gln, its concentration is elevated in the colostrum and in the days following the birth, but a reduction is observed from then until the end of the lactation period [13]. These findings are similar to those reported for other amino acids such as Arg and Lysine [5,6]. Variations in the concentrations of Gln and Glu have previously been reported [5,6,13]. However, these differences are related to the methods used to determine the amino acids. Gln is measured more accurately by the enzymatic method than by other methods such as High Performance Liquid Chromatography (HPLC).

In the present study, an increase of the Gln present in milk was observed in both groups after parturition (~ 0.60 mmol/mL), until the third month of lactation, before decreasing until the end of the lactation period. It is important to highlight that the supplemented group exhibited the highest level of Gln and Gln + Glu in the first and third months of lactation. As previously reported, Gln and Pro are amino acids that are widely used in the production of Arg. Therefore, it is important that animals have significant levels of these two amino acids during the lactation period, since the milk is poor in Arg [2,25].

Unfortunately, the concentration of Arg in the milk was not measured. However, determining the Gln and Glu is a good indicator of the quality of milk. Indeed, to identify the effects of Arg supplementation on the mares and their foals, it was necessary to assess blood biomarkers associated with the metabolism of Arg, urea and creatinine, which can contribute to a better understanding of the effects of this supplementation [11].

A previous study of Standardbred mares in an intensive system of rearing showed that both GLN and glutamine synthetase (GS) were reduced at the end of the lactation period [13]. Conversely, in the present study, Arg supplementation did not alter the levels of Gln in the blood of mares. This result differed from that obtained using laboratory animals, in which supplementation with Arg reduced the levels of Gln in the blood [3]. The same authors reported that supplementation with Arg could be associated with the inhibition of resynthesis of Gln by different tissues, maintaining the Gln at low levels in the blood of animals.

Curiously, when analyzing the biomarkers of the metabolism of protein, no alternations were observed when comparing both groups of mares. Conversely, other studies [1] reported that urea and creatinine levels are high at birth, but rapidly decrease

to values similar to those reported for adult animals. These variations were not observed in the present study. Probably, these variations are related to the different methodologies used, since it is already known that the time of blood sampling (at birth or after the ingestion of colostrums) can affect the values of blood biomarkers. The values of urea, creatine, TPP and albumin remained within the normal limits for the species, with or without Arg supplementation. This finding was not expected, since it has been demonstrated that Arg supplementation can elevate the level of urea in the blood due to the increased activity of this substance in the liver [3].

In relationship to the biomarkers of the metabolism of fat, the concentration of triglycerides remained high until the first month of lactation in the supplemented group (~ 0.60 mmol/L) and was low in both groups of mares at the end of the fifth month ($P < 0.05$). The level of cholesterol did not change during the lactation period ($P > 0.05$) and its value was similar to that reported for adult animals in maintenance. Other study reported that both triglycerides and cholesterol increase in the first days after birth and mild hypertriglyceridemia can occur, due to the incomplete development of the liver in newborns [1]. This profile of biomarkers of the metabolism of fat indicates that there is a greater availability of energy days after the birth. This energy comes from the large increase in the concentration of cortisol at the moment of parturition and throughout the lactation period [13,22].

Finally, the concentration of minerals in the blood of mares was assessed. It is known that the source of certain minerals in the animal's body is elevated. In order to avoid significant alterations in the concentrations of these minerals, the animal's body needs to overcome several metabolic challenges. The highest concentrations of Ca and Mg ($P < 0.05$) were recorded in the supplemented mares, whereas the concentration of P was highest for the animals in the control group ($P < 0.05$). In all cases, the highest values were observed in the first month of lactation.

There is no clear explanation for these adaptations in the metabolism of minerals. However, the low requirement of this mineral for the formation of the fetus at the end of gestation, as well as the increased availability of milk, could be associated with their high concentration in the early stages of lactation, when foals exhibit a high gain in body weight. These adaptations need to be studied further, since an increase in the consumption of mineral salt is not expected at the beginning of the lactation period.

It is important to note that mares that had free access to salt supplementation with organic minerals, could quickly correct the variations caused by food or milk production. There was a reduction in the concentration of Fe in the first month of lactation, although the differences between treatment types were not significant, remaining within the normal limits for adult animals. It is known that milk is not rich in Fe. The alterations observed herein in the blood of mares could be associated with the presence of this mineral in the milk, which was not analyzed in the present study.

The concentration of Arg altered in the blood and in the skeletal muscles of foals during the lactation period [14]. However, due to the difficulties encountered in measuring this amino acid, biomarkers (TPP, albumin, urea, creatinine and uric acid) that are correlated with this amino acid were determined in the present study. The values of these biomarkers did not alter, with the exceptions of urea and uric acid, which exhibited a high concentration on the day of parturition. However, soon after, the concentration of these minerals reached normal levels and became compatible with the first month of life. This finding was expected since there is an important adaptation to extra-uterine life in these animals after the ingestion of colostrums and the development of different tissues in the first days of life [1]. The increase in the concentration of TPP was also expected, given that the ingestion of colostrums and the stimulation of the immune system lead to an increase in the concentration of globulins.

Biomarkers associated with the metabolism of Arg, such as Gln and Gln + Glu, did not alter during the lactation period in either group. This was an unusual finding, since previous studies have demonstrated that the levels of Glu and Gln alter during this period [14]. The same authors reported that there was an increase in Gln in the blood, which was followed by a reduction in its concentration in the skeletal muscle. Most likely, the variation in the results obtained herein is related to the different systems of rearing.

The concentration of biochemical biomarkers in newborn animals modifies rapidly in the first month of life and must be analyzed with caution [1]. Interestingly, only the P altered, with higher concentrations during the lactation period than at birth.

This finding is significant, since equine milk exhibits few alterations in the levels of P during the lactation period, unlike Ca and Mg [6]. Indeed, equine milk has adequate amounts of Mg but seems to have inadequate amounts of Ca and P [8]. It is known that the maintenance of these minerals in the blood of foals depends on alterations in the metabolism of the bones and liver. In the present study, significant alterations were observed in the concentrations of Ca, P and Mg among mares (Table 2).

Blood iron did not alter ($P > 0.05$) during the period analyzed, although a higher reduction in its concentration was observed in both groups one month after the birth. Similar results were obtained for the mares. This pattern of variation of Fe has previously been observed in foals of different breeds, indicating a significant reduction in the concentration of this mineral in the blood of newborns after the birth [1,9]. Its reduction in the blood of mares is associated with the reduction in the milk, and consequently is associated with the low levels of Fe in foals in the first month of lactation.

The animals studied herein exhibited the expected body growth for the breed in question: a mean growth of about 25 % in height and over 75 % in body mass in the first five months of life. Authors [10] assessed the development of Quarter horse foals. And others [15] studied the development of four different breeds under tropical conditions and demonstrated that the Quarter horses had a WH and CP of ~ 91 cm and ~ 85 cm, respectively, at birth, and ~ 117 cm and ~ 122 cm, respectively, at five months of age. Upon comparison of our data with those previously reported [15], it was notable that the biometric parameters were slightly lower at birth, but similar at five months of age. These data indicate that the supplementation did not affect the development of the animals based on comparisons with the control group and with animals reared in similar conditions.

Considering the animals' weight and the FP, it was clear that the body mass altered in the first five months of the lactation period, while the FP did not. At the end of the experiment, the animals in the control group exhibited a higher CP and body mass than the supplemented animals. This was an unusual finding, since no difference was observed for the other features. This rapid increase in body mass, together with the formation of muscle tissue rich in myoglobin, could also be associated with the reduction of Fe in the blood of foals during the early phases of adaptation to extra-uterine life. However, due to the number of animals used in the present study (n = 10) and the difficulty of maintaining a control group with large numbers of animals (from an ethical point of view), the differences found at five months could be due to the variations. Females of the same breed exhibited similar biometry.

There is little information available regarding the FP in foals, but one previous study with Standardbred foals reported that the FP only alters at sixth months of age [14]. In the present study, the assessments were only performed until the fifth month of life, which may have affected the results. Furthermore, it is important to highlight that young animals exhibit a low FP due to the high metabolic rate associated with this stage of life.

CONCLUSION

The supplementation of mares with Arg did not cause alterations in the biomarkers of blood and milk. In addition, this supplementation did not cause positive or negative effects on the development of the foals of these lactating mares.

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***Ethical approval*.** All procedures performed in studies involving animals were in accordance with the ethical standards of the Ethics Committee for Animal Experimentation of the Universidade Federal Rural de Pernambuco (protocol number: 23082.017404).

***Declaration of interest*.** The authors declare that they have no conflict of interest, and this document is original, and no part of it is submitted anywhere else for conference or publication. The authors Kutschenko M. & Nogueira E.T. state that work at Ajinomoto Brazil and did not participate in experimental activities in this study.

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