Revista Brasileira de Zootecnia
Brazilian Journal of Animal Science
© 2017 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., 46(2):118-122, 2017

Retinol and mineral status in grazing foals during the dry season

Maria Lindomárcia Leonardo da Costa[1*], Adalgiza Souza Carneiro de Rezende[2], Ivan Barbosa Machado Sampaio[2], Vinícius Pimentel Silva[3], Juliano Martins Santiago[4], Vany Perpétua Ferraz[5], Eloísa de Oliveira Simões Saliba[2], Ângela Maria Quintão Lana[2]

[1] *Universidade Federal da Paraíba, Departamento de Zootecnia, Areia, PB, Brazil.*
[2] *Universidade Federal de Minas Gerais, Escola de Veterinária, Departamento de Zootecnia, Belo Horizonte, MG, Brazil.*
[3] *Universidade Federal Rural do Rio de Janeiro, Departamento de Nutrição Animal e Pastagens, Seropédica, RJ, Brazil.*
[4] *Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, Serra Talhada, PE, Brazil.*
[5] *Universidade Federal de Minas Gerais, Departamento de Química, Belo Horizonte, MG, Brazil.*

ABSTRACT - The objective of this study was to examine serum retinol and some mineral (zinc, iron, calcium, phosphorus, magnesium, and copper) levels in seven Mangalarga Marchador colts aged 329.48 days, reared on pasture in the city of Montes Claros, MG, Brazil, during the dry season. Equines were evaluated for four periods of 45 days during the total study period of five months. The foals had access to *Panicum maximum* cv. Tanzania pasture, while water and mineral salt lick were provided *ad libitum*. Blood samples were taken to determine serum retinol and levels of minerals, along with pasture samples to quantify beta-carotene in the grass. The results indicated that retinol levels varied (2.87 to 1.97 µg/dL) and remained below the standard levels. The levels of zinc, iron, calcium, and phosphorus did not vary significantly with average values of 36.79 µg/dL, 77.32 µg/dL, 10.33 mg/dL, and 9.99 mg/dL, respectively. However, zinc and calcium remained below standard concentrations of 60-120 µg/dL and 10.8-13.5 mg/dL, respectively, since the beginning of the study. On the other hand, copper and magnesium levels decreased over time (97.76 to 77.56 mg/dL and 2.86 to 2.21 µg/dL, respectively), but showed normal levels for horses during the research. Beta-carotene in grass showed a downward trend over time from 38.53 to 0.09 mg/kg of dry matter. The results also indicated a significant relationship between serum retinol and serum copper levels. Vitamin A supplementation is necessary whenever foals are fed pastures during the dry season.

Key Words: equine, vitamin A, pasture

Introduction

Grazing horses derive vitamin A from provitamin A carotenoids present in forages, but the efficiency of this conversion is relatively poor in these animals (Greiwe-Crandell et al., 1997). The most common provitamin A carotenoids in plant-based feedstuffs are β-carotene, α-carotene, and β-cryptoxanthin. The provitamin A carotenoids in major feed ingredients used in livestock diets have not been assessed for almost 50 years (Pickworth et al., 2012).

Beta-carotene is converted into retinol by enzymes in the intestine (Bondi and Sklan, 1984). Iron and zinc are important in the activation of these enzymes and affect the bioconversion of retinol and carotenoids (During et al., 1999; Blomhoff and Blomhoff, 2006).

Serum retinol levels reflect the status of vitamin A in livestock since they are homeostatically controlled from the hepatic reserves (Ross and Harrison, 2007). In addition, the synergistic effect of the minerals in the metabolism of this vitamin is quite intricate (Geor et al., 2013).

Inadequacy of vitamin A, particularly in pastures during the dry season, is expected to compromise the foal growth (Gay et al., 2004) because retinol is involved in several functions such as vision, bone development, reproduction, and integrity of mucosal and epithelial surfaces (Ortega et al., 2010).

The objective of this study was to examine serum retinol and some mineral (zinc, iron, calcium, phosphorus, magnesium, and copper) levels in Mangalarga Marchador grazing foals during the dry season.

Material and Methods

All procedures used in this experiment were approved by the Ethics Committee in Animal Experimentation of Universidade Federal de Minas Gerais (case no. 194/08).

Received: January 21, 2016
Accepted: November 5, 2016
***Corresponding author:** lindomarciacosta@gmail.com
http://dx.doi.org/10.1590/S1806-92902017000200006

How to cite: Costa, M. L. L.; Rezende, A. S. C.; Sampaio, I. B. M.; Silva, V. P.; Santiago, J. M.; Ferraz, V. P.; Saliba, E. O. S. and Lana, A. M. Q. 2017. Retinol and mineral status in grazing foals during the dry season. Revista Brasileira de Zootecnia 46(2):118-122.

Copyright © 2017 Sociedade Brasileira de Zootecnia. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The present study was conducted from April to October, in the city of Montes Claros (16°44'06" S and 43°51'42" W) in the state of Minas Gerais, Brazil. The average monthly rainfall level registered during the dry season was 8.75 mm. Seven male Mangalarga Marchador foals aged 329.48±21.15 days and weighting 224.42±46.29 kg were included in the experiment. Prior to the trial and every other month until the end of experiments, the foals were treated against ectoparasites with deltamethrin (10 mL diluted into 5 L of water for aspersion of each animal) and against endoparasites with ivermectin (1.6 g for 100 kg of weight) at a strictly maintained dosage according to the manufacturers' recommendation. Foals were kept on five hectares of *Panicum maximum* cv. Tanzania pasture, while mineral salt (Table 1) and water were provided *ad libitum*.

Every 45 days for approximately five months, blood and grass samples were collected from the animals and pasture, respectively, to evaluate serum retinol and mineral levels in the animals and beta-carotene in the forage.

Blood sampling was done through jugular puncture using 10-mL vacutainer tubes with no anticoagulant. The tubes were immediately placed into an ice chest protected from light with aluminum foil to prevent the oxidation of retinol. The collected blood was allowed to clot and then was centrifuged at 3000 rpm for 20 min at 4 °C to separate the serum fraction. Serum samples obtained from each foal were then pipetted and placed into amber cryogenic tubes, packed in aluminum foil, and frozen at –20 °C until they were thawed for retinol analysis. Retinol was extracted according to the procedure described by Pesce and Kaplan (1987) and retinol concentration was determined using high-performance liquid chromatography.

The extraction of minerals from blood consisted in diluting 500 µL of serum from the animals in 10-mL flasks containing 0.1% Triton X-100 (Merck Millipore, Darmstadt, Germany). Serum mineral evaluation followed the inductively coupled plasma optical emission spectrometry (ICP-OES) using the technique by Rocha et al. (2009).

Table 1 - Chemical composition of *Panicum maximum* cv. Tanzania during the experimental period

Item	Experimental period (days)			
	1	45	90	135
Dry matter (g kg^{-1} of fresh matter)	408.3	428.0	384.5	878.3
Crude protein (g kg^{-1} DM)	79.4	55.9	60.0	59.8
Neutral detergent fiber (g kg^{-1} DM)	689.2	656.1	722.0	805.7
Acid detergent fiber (g kg^{-1} DM)	393.4	369.1	419.0	502.0
ADIN (g kg^{-1} DM)	32.8	25.7	22.3	20.8
Ash (g kg^{-1} DM)	143.4	129.3	105.7	110.3
Calcium (g kg^{-1} DM)	7.5	6.1	5.3	5.4
Phosphorus (g kg^{-1} DM)	1.3	1.5	1.6	1.2
Gross energy (Mcal kg^{-1})	4.78	4.07	4.10	3.98

DM - dry matter; ADIN - acid detergent insoluble nitrogen.
Mineral salt composition (guaranteed analysis kg^{-1}): calcium, 140 g; phosphorus, 75 g; sodium, 137 g; magnesium, 14 g; sulfur, 14 g; iron, 1200 mg; manganese, 1200 mg; zinc, 3000 mg; copper, 1250 mg; cobalt, 60 mg; iodine, 130 mg; selenium, 30 mg; and fluorine (max.), 750 mg.

Forage samples were randomly and manually collected from different areas of the pasture simulating equine feeding behavior according to Gardner (1986). The grass samples were wrapped in black plastic bags and then cut with scissors in an environment protected from light. The material was packed into several layers of black plastic, wrapped in aluminum foil and frozen at –20 °C for analysis of beta-carotene according to the AOAC (1995).

Retinol concentrations did not show normality or homoscedasticity; therefore, they were analyzed through non-parametric Friedman test. Serum minerals followed a completely randomized block design comparing four treatments (1, 45, 90, and 135 days) in seven blocks (foals), followed by Student-Newman-Keuls test. Beta-carotene in *Panicum maximum cv.* Tanzania grass followed a completely randomized design with four treatments (1, 45, 90, and 135 days) and four replications each, assessed by Student-Newman-Keuls test. Pearson's correlation coefficient was determined between beta-carotene and crude protein contents in grass and serum retinol. All data were analyzed using the software SAEG (Sistema para Análises Estatísticas e Genéticas, version 8.0). To study the association among the variables (minerals, retinol, and beta-carotene), principal-components multivariate analysis of the software INFOSTAT, version 2008, was used.

Results and Discussion

During the dry season, serum retinol concentration of the foals dropped ($P<0.05$) over the study period (Table 2).

In this research, concentrations of retinol were lower than the standard value of 10 µg/dL reported by Lewis (2000), implying that foals in the present experiment had hypovitaminosis A with minimum liver stock levels and, probably, their hepatic reserves were mobilized to maintain serum retinol levels (Ball, 1998). Foals in this study were probably using their vitamin A reserves before the trial started, as the preceding 30 days had no rain at all, thus reducing the amount of beta-carotene in the pasture (Table 2).

Another relevant factor in the results was the category used in the present experiment, because young animals have little hepatic reserve, which makes them more susceptible to vitamin A deficiency when compared with adult animals. The liver reaches its peak storage of vitamin A only in adulthood (Taipina, 2001).

Álvarez et al. (2015) reported similar results when evaluating mares reared in a continuous grazing system in an area of silvopastoral system during the spring in Central Spain. Those authors found blood retinol concentration of 6.58 μg/mL, which indicates hypovitaminosis A. In contrast, in Southeastern Brazil, Garcia et al. (2006) found no difference (P>0.05) in serum retinol in pregnant mares kept on pasture during the dry season.

The results also indicated that the concentration of zinc did not vary (P>0.05) during the observed period. However, the values, in general, were suboptimum when compared with the standard values of 60-120 μg/dL according to Wichert et al. (2002). Although mineral salt was offered *ad libitum*, zinc deficiency was detected in the animals. This may be attributed to levels lower than those mentioned by the manufacturer or, in spite of having free access to the mineral lick, the foals consumed less of it. Rahman et al. (2002) studied different proportions of vitamin A and zinc in humans and came to the conclusion that there was synergism involving these nutrients. Zinc levels affected the synthesis of retinol-binding protein and beta-carotene to retinol conversion. Thus, it can be inferred that, in the present trial, low serum retinol could have been influenced by limited amounts of serum retinol transported to tissues as a result of zinc deficiency or the capacity of the animal to convert pasture beta-carotene into retinol.

Iron levels did not vary (P>0.05) during the study period. From 45 days onward, serum iron levels decreased numerically to values below the standard concentrations for horses (73 to 140 μg/dL) according to the NRC (2007), which indicates deficiency of this mineral.

Calcium and phosphorus concentrations did not vary (P>0.05) either during the observed period. Serum calcium values were always lower than the values of 10.8-13.5 mg/dL suggested by Garcia-Lopez et al. (2001). Low concentrations of calcium may result in poor skeletal development and further lead to problems such as osteopenia in growing animals (Geor et al., 2013). The phosphorus levels were higher than the values reported by Mundim et al. (2004) for equines (4.21±1.2 mg/dL). According to the NRC (2007), horses show higher phosphorus levels at birth, which decrease over time until reaching standard concentration as adults.

The results also indicate that serum magnesium concentrations varied (P<0.05) during the research with a decline observed after a third of the study period and values remaining stable until the final collection. In spite of the decline, the levels were well above the values of 1.6-2.0 mg/dL mentioned by the NRC (2007). These values showed that the foals had no magnesium deficiency. Serum copper levels decreased (P<0.05), but copper concentrations were well within 50-150 μg/dL for horses according to Wichert et al. (2002).

With no consistent rain during the experimental period, the nutritional value of the *Panicum maximum* cv. Tanzania grass declined as the grasses matured, which may also have influenced beta-carotene concentration. The study also indicated that during the short spell of rainfall volume (21 mm) recorded on the 90th day of the study, the grasses responded rapidly, leading to an enhancement in beta-carotene levels, although there was no significant response in serum retinol levels in the foals. This occurrence can be explained by low rainfall, which did not allow homogeneous and efficient budding in the grazing area and, consequently, did not provide sufficient amounts of this nutrient to the animals. In addition, horses have low capacity of converting beta-carotene into retinol with efficiency of only 33% of the amount ingested (Bondi and Sklan, 1984). Insufficient beta-carotene intake associated with low nutritional quality of the forage during the experimental period resulted in loss of serum retinol in foals. A correlation of 0.83 was found between crude protein and serum retinol, which suggests that the animals need to feed on quality forage with adequate crude protein concentrations to maintain retinol levels.

There was positive correlation of 0.81 between crude protein and beta-carotene in grass. This high correlation is because carotenoids protect photosynthetic organisms against potentially harmful photo-oxidative processes and are essential structural components of the photosynthetic antenna and reaction-center complexes. The beta-carotene

Table 2 - Average serum concentration of retinol and minerals in foals and beta-carotene levels in grass

Period (days)	Retinol[1] (μg/dL)	Zn (μg/dL)	Fe (μg/dL)	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)	Cu (μg/dL)	Beta-carotene (mg kg^{-1} DM)
1	2.87a	39.56a	94.02a	10.64a	10.34a	2.86a	97.76a	38.53a
45	3.08a	38.21a	85.18a	10.41a	9.53a	2.77a	79.54b	12.75c
90	1.99b	34.10a	63.24a	10.37a	9.51a	2.18b	79.56b	22.17b
135	1.97b	35.30a	66.83a	9.90a	10.53a	2.21b	77.56b	0.09d
CV (%)	-	20.73	26.63	6.36	9.58	12.32	10.18	30.88

CV - coefficient of variation.
[1] Friedman test (P<0.05).
Means within columns followed by different letters are different by SNK test (P<0.05).

color is masked by chlorophyll in photosynthetic tissues (Bartley and Scolnik, 1995).

In this research, we observed a relationship of retinol, minerals, and beta-carotene levels using a non-inferential principal-component multivariate analysis (Figure 1). Calcium levels were not considered in the multivariate analysis since the coefficient of variation was quite low at 7.2%.

Serum iron levels inversely varied in relation to beta-carotene in grass, i.e., the higher the beta-carotene levels in grass, the lower iron levels were found in serum.

Serum phosphorus levels seemed to be independent of grass quality, but zinc levels were highly correlated with phosphorus levels. Recent studies have indicated that copper levels are correlated with retinol levels. These results are in accordance with the findings of Van De Weyer et al. (2010), who found similar deficient results in beef cows on pastures for this micronutrient.

We found copper as the most associated serum element with retinol, contrary to current literature findings which consider zinc the mineral most associated with retinol.

The reduction of beta-carotene in grass, inflicted by the weather along the dry season, causes a decrease in serum copper, and, consequently, further reduction in serum retinol.

Castenmiller and West (1998) stated that levels of zinc in the body affect the synthesis of retinol-binding protein and beta-carotene conversion into retinol. The correlation between these nutrients were shown by Ahn and Koo (1995) in adult female rats, in which induced zinc deficiency decreased blood levels of retinol.

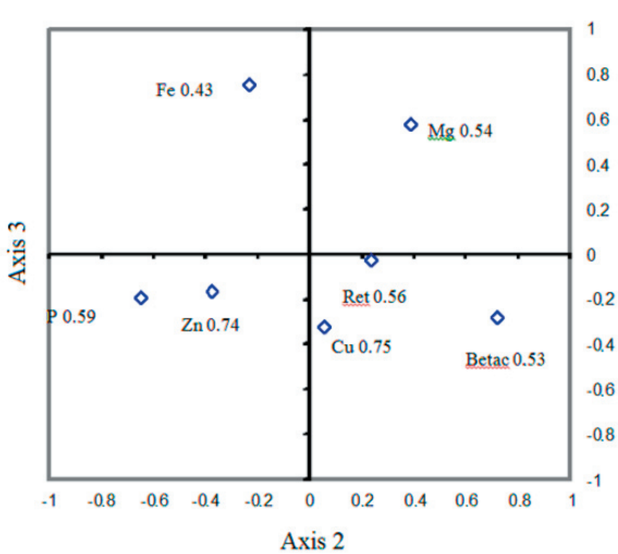

Figure 1 - Relationships between levels of minerals, beta-carotene (Betac), and retinol (Ret).

However, in the present study, the mineral identified as the main component positively correlated with retinol was copper. Similar results were obtained by Root et al. (1999) when studying women in China whose main source of vitamin A was beta-carotene from fresh vegetable diets.

Simpson et al. (2004) listed copper-dependent liver proteins in ewes treated with different dietary copper levels. They found an increase in aldehyde dehydrogenase enzyme expression in the oxidation of retinal into retinoic acid. The retinol-binding protein had its activity increased by 1.5-2.0 times with higher dietary copper levels. Those authors claimed that aldehyde dehydrogenase binds to free retinal and retinol-binding protein bound to retinal.

Foals in the present study were probably using liver retinol to keep homeostasis. To complete its metabolic pathway, stored retinol must be oxidized into retinal and then into retinoic acid, the latter step being copper-dependent, which corroborates the present findings.

Zinc plays an important role in the metabolic pathway of vitamin A, mainly in its transportation to blood vessels. However to have vitamin A acting in epithelial tissues and in cell transcription, the biochemical steps involved require the presence of copper.

Conclusions

The practical recommendation is to supplement foals with vitamin A whenever they are kept on pastures during the dry season.

References

Ahn, J. and Koo, S. I. 1995. Effects of zinc and essential fatty acid deficiencies on the lymphatic absorption of vitamin A and secretion of phospholipids. Journal of Nutrition Biochemistry 6:595-603.

Álvarez, R.; Meléndez-Martínez, A. J.; Vicario, I. M. and Alcalde, M. J. 2015. Carotenoids and fat-soluble vitamins in horse tissues: a comparison with cattle. Animal 9:1230-1238.

AOAC - Association of Official Analytical Chemistry. 1995. Official methods of analysis. 16th ed. AOAC International, Arlington, VA.

Ball, G. 1998. Bioavailability and analysis of vitamins in foods. Chapman & Hall, London.

Bartley, G. E. and Scolnik, P. A. 1995. Plant Carotenoids: Pigments for photoprotection, visual attraction and human health. The Plant Cell 7:1027-1038.

Blomhoff, R. and Blomhoff, H. K. 2006. Overview of retinoid metabolism and function. Journal of Neurobiology 66:606-630.

Bondi, A. and Sklan, D. 1984. Vitamin A and carotene in animal nutrition. Progress in Food & Nutrition Science 8:165-191.

Castenmiller, J. J. M. and West, C. E. 1998. Bioavailability and bioconversion of carotenoids. Annual Review of Nutrition 18:19-38.

During, A.; Fields, M.; Lewis, C. G. and Smith, J. C. 1999. β-carotene 15, 15'dioxygenase activity is responsive to copper and iron concentrations in rat small intestine. Journal of the American College of Nutrition 18:309-315.

Garcia, T. R.; Rezende, A. S. C.; Sampaio, I. B. M.; Lana, A. M. Q. and Moura R. S. 2006. Concentrações séricas de retinol em éguas gestantes em haras do Vale do Rio Doce e Sul de Minas Gerais. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 58:1174-1180.

Garcia-Lopez, J. M.; Provost, P. J.; Rush, J. E.; Zicker, S. C.; Burmaster, H. and Freeman, L. M. 2001. Prevalence and prognostic importance of hypomagnesemia and hypocalcemia in horses that have colic surgery. American Journal of Veterinary Research 62:7-12.

Gardner, A. L. 1986. Técnicas de pesquisa em pastagens e aplicabilidade de resultados em sistemas de produção. Embrapa, Brasília.

Gay, L. S.; Kronfeld, D. S.; Grimsley-Cook, A.; Dascanio, J. J.; Ordakowski-Burk, A. O.; Splan, R. K.; Dunnington, E. A. and Sklan, D. J. 2004. Retinol, β-carotene and β-tocopherol concentrations in mare and foal plasma and in colostrum. Journal of Equine Veterinary Science 24:115-120.

Geor, R. J.; Harris, P. A. and Coenen, M. 2013. Equine applied and clinical nutrition. Saunders Elsevier, Philadelphia.

Greiwe-Crandell, K. M.; Kronfeld, D. S.; Gay, L. S.; Sklan, D.; Tiegs, W. and Harris, P. A. 1997. Vitamin A repletion in thoroughbred mares with retinyl palmitate or beta-carotene. Journal of Animal Science 75:2684-2690.

Lewis, L. D. 2000. Nutrição clínica equina: alimentação e cuidados. Roca, São Paulo.

Mundim, A. V.; Teixeira, A. A.; Galo, J. A. and Carvalho, F. S. R. 2004. Blood biochemical profile and osmolality in horse used for work in urban centers. Bioscience Journal 20:135-142.

NRC - National Research Council. 2007. Nutrient requirements of horses. 6th ed. National Academy Press, Washington.

Ortega, R. M.; Mena, M. C. and Andrés, P. 2010. Vitamina A. p.547-569. In: Tratado de nutrición: Bases fisiológicas y bioquímicas de la nutrición. 2nd.ed. Hernández, A. G. Médica Paramericana, Madrid.

Pesce, A. J. and Kaplan, L. A., 1987. Methods in clinical chemistry. Mosby-Year Book Publisher, Washington.

Pickworth, C. L.; Loerch, S. C.; Kopec, R. E.; Schwartz, S. J.; Fluharty, F. L. 2012. Concentration of pro-vitamin A carotenoids in common beef cattle feedstuffs. Journal of Animal Science 90:1553-1561.

Rahman, M.; Wahed, M. A.; Fuchs, G. J.; Baqui, A. H. and Alvarez, J. O. 2002. Synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutrition in children. American Journal of Clinical Nutrition 75:92-98.

Rocha, O. G. F.; Moura, R. S.; Costa, M. L. L.; Rezende, A. S. C. and Melo, M. M. 2009. Metodologia analítica para determinação de cobre, zinco e ferro em soro equino por espectrometria de emissão por plasma indutivamente acoplado. p.22. In: Revista Brasileira de Toxicologia (suplemento). Sociedade Brasileira de Toxicologia, Belo Horizonte.

Root, M. M.; Hu, J.; Stephernson, L. S.; Parker, R. S. and Campbell, C. 1999. Determinants of plasma retinol concentrations of middle-aged women in rural China. Nutrition 15:101-107.

Ross, A. C. and Harrison, E. H. 2007. Vitamin A: Nutritional aspects of retinoids and carotenoids. p.1-39. In: Handbook of vitamins. Zempleni, J.; Bucker, R. B.; Mccormick, D. B. and Suttie, J. W., eds. CRC Press, New York.

Simpson, D. M.; Beynon, R. J.; Robertson, D. H. L.; Loughran, M. J. and Haywood, S. 2004. Copper-associated liver disease: A proteomics study of copper challenge in a sheep model. Proteomics 4:524-536.

Taipina, M. S. 2001. Efeito da radiação sobre a vitamina A e o beta-caroteno de fígado bovino e suíno. Dissertação (M. Sc.). Universidade de São Paulo, São Paulo.

Van De Weyer, L. M.; Hendrick, S. and Waldner, C. L. 2010. Serum micronutrient concentrations in beef cows before and after the summer grazing season. Canadian Journal Animal Science 90:563-574.

Wichert, B.; Frank, T. and Kienzle, E. 2002. Zinc, copper and selenium intake and status of horses in Bavaria. Journal of Nutrition 132:1776 -1777.