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Clinical biochemistry profile of American Quarter Horse broodmares fed Tifton-85 (*Cynodon* spp.) hay and haylage[1]

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ABSTRACT.- Guimarães T.C., Rezende A.S.C., Costa M.L.L., Ferreira M.G., Miranda A.L.S., Botelho A.F.M., Soto-Blanco B. & Melo M.M. 2019. **Clinical biochemistry profile of American Quarter Horse broodmares fed Tifton-85 (*Cynodon* spp.) hay and haylage.** *Pesquisa Veterinária Brasileira 39(5):317-323*. Escola de Veterinária, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, Cx. Postal 567, Campus Pampulha, Belo Horizonte, MG 31270-901, Brazil. E-mail: mariliamartinsmelovet@hotmail.com

The conservation of haylage (a pre-dried feed) can be challenging, since there is an increased risk of mould growth, which can contaminate this foodstuff with mycotoxins. However, when the hygienic quality is secured, haylage enhances grass palatability and provide enough supply of dry matter throughout the year. Due to the lack of information regarding its effect on blood parameters in horses fed exclusively with this foodstuff, the aim of this study is to provide information regarding its use in comparison to hay and ensure that it does not affect horses' biochemical profile. Twelve Quarter Horse broodmares were distributed into two groups, each fed with Tifton-85 (*Cynodon* spp.) hay or haylage for a period of 28 days, and the biochemical profile was done in five different times (T0 before the experiment started and, chronologically, seven days apart - T1, T2, T3 and T4), It was analyzed total protein (TP) and its fractioning; enzymes alanine aminotransferase, aspartate aminotransferase and γ-glutamyl-transferase; endogenous catabolism products urea and creatinine; and ions calcium and phosphorus. Mycotoxins in haylage were also investigated and remained below the legislation thresholds. Only TP was higher in the last sampling (T4) of the haylage group, which may be related to the foodstuff's higher protein digestibility. No differences were observed between serum enzymes, urea, creatinine and Ca/P from both experimental groups. Haylage has proven to be safe, when well prepared for horses, without causing impairing side effects, as shown by the normal serum biochemistry parameters presented in this study.

INDEX TERMS: Biochemistry, clinics, American Quarter Horse, Tifton-85, *Cynodon* spp., hay, haylage, equine, forage, clinical chemistry, horses, nutrition.

RESUMO.- [**Perfil bioquímico clínico de matrizes Quarto de Milha alimentadas com feno e *haylage* Tifton 85 (*Cynodon* spp.)**] A conservação do *haylage* (alimento pré-seco) pode ser desafiadora, considerando o aumento do risco de crescimento de fungos, com consequente produção de micotoxinas. Entretanto, quando a qualidade da higiene e armazenamento é assegurada, o *haylage* aumenta a palatabilidade da forragem e fornece suplemento de matéria seca suficiente ao longo do ano. Devido à falta de informação relativa aos efeitos dessa alimentação nos parâmetros sanguíneos de equinos alimentados exclusivamente com essa dieta, o objetivo do presente estudo é avaliar o perfil bioquímico sanguíneo dos equinos após administração da haylage em comparação com feno. Doze matrizes Quarto de Milha foram distribuídas em dois grupos, cada um recebendo feno ou *haylage* de Tifton 85 (*Cynodon* spp.) por um período de 28 dias. O perfil bioquímico foi realizado em cinco tempos (T) diferentes (T0, antes do início do experimento e cronologicamente, a cada

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sete dias após o fornecimento das dietas - T1, T2, T3 e T4) para análise de proteína total (PT) e seu perfil fracionado, das enzimas alanina aminotransferase, aspartato aminotransferase, γ-glutamil-transferase, dos produtos de catabolismo creatinina e ureia e, dos íons cálcio e fósforo. Micotoxinas no *haylage* foram investigadas e mantiveram-se abaixo dos limites determinados pela legislação brasileira. O perfil bioquímico revelou, somente, elevação da PT em T4 no grupo que recebeu *haylage*, o que pode estar relacionado à sua maior digestibilidade proteica. Nenhuma diferença foi observada nos outros parâmetros estudados em ambos os grupos experimentais. Conclui-se que *Haylage* é comprovadamente seguro, quando bem preparado para equinos, sem causar efeitos na saúde geral, conforme demonstrado pelos exames bioquímicos no presente estudo.

TERMOS DE INDEXAÇÃO: Bioquímica, clínica, matrizes, Quarto de Milha, feno, *haylage*, Tifton 85, *Cynodon* spp., cavalos, equinos, forragem, hematologia, nutrição.

INTRODUCTION

Tifton-85 (*Cynodon* spp.) is a sterile hybrid resulting from crossings between Tifton-68 and Tifton-292. It was developed by the United States Department of Agriculture as a collaborative project with the University of Georgia, and registered in October 1992 (Burton et al. 1993). Tifton-85 is largely employed in hay-making due to its favorable structural characteristics, such as fast dehydration, primary presence of thin stems, and a high leaf/thatch ratio (Domingues 2009).

In recent years, haylage has been studied around the world (Müller & Udén 2007, Müller 2011, Guimarães et al. 2016, Harris et al. 2017). Haylage is a pre-dried feed, and an intermediate between hay and silage. Its processing is based on partial drying and fermentation of forages in aerobic conditions. Such a processing of forages can provide various benefits, especially in the intensive farming systems, such as the enhancement of its palatability and certification, that enough dry matter (DM) will be offered to the animal despite seasonal variations. In addition, replacement of high starch containing concentrate with energy dense fibrous feeds (silage and haylage) is a potential solution for several clinical problems in horses and ruminants, such as colic, laminitis and acidosis. Energy dense fibrous feeds have been shown to enhance volatile fatty acid production in the hindgut, and present high palatability (Richardson & Murray 2016).

Considering that haylage contains higher water content than hay, its conservation is challenging by the considerable risk of mould growth, which can contaminate the foodstuff with mycotoxins, accounted for respiratory diseases, mycotoxicosis, skin disorders and reproductive failure in horses (Müller et al. 2011).

The main toxigenic fungi proliferations include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus* and *Fusarium moniliforme* (Kamphues 2013, Wambacq et al. 2016).

Employing haylage in the equine farming system is only welcomed by horse owners if its quality of conservation and nutritional values are established. Nutritional values of fermented forages are often better than those of dry hay, because of the growth stage of the harvested grass and the smaller losses of leaves during haylage processing (Bergero et al. 2002, Schenck & Müller 2014).

There is a lack of information regarding the effect of haylage on blood parameters in horses fed exclusively with this foodstuff. Blood biochemical paneling might provide information regarding animal's health status (Rezapour et al. 2016). Therefore, the aim of this study is to provide comparative biochemical blood values of broodmares fed with hay and haylage of Tifton-85 (*Cynodon* spp.) to ensure that haylage is capable of maintaining horses' normal blood parameters.

MATERIALS AND METHODS

Ethics statement. This study was approved by the Committee of Ethics and Use of Animals (Protocol 552010), at the Federal University of Minas Gerais (UFMG), in compliance with the Ethics Principles in Animal Experimentation.

Feed production. The experiment was conducted in farms of the Itaúna district, in the State of Minas Gerais, Brazil. The two evaluated treatments comprised haylage or hay of Tifton-85 (*Cynodon* spp.) diets, both supplemented with mineral salt, water *ad libitum*, and concentrate. Concentrate was manufactured on the farm, with a 94.82 DM content, 70% corn, 15% whit bran, 5% soy bean, 3% calcitic limestone, 2% mineral salt, 1% dicalcium phosphate and 4% soy oil. All animals were weighed weekly and received the daily diet considering 2% of their body weight, in the proportion of concentrate: hay or haylage of 50% each. Both hay and haylage were administrated 3 times a day, at 7am, 13pm and 17pm. Concentrate was provided twice a day, at 9am and 15pm.

For both feedstuffs, grass was harvested at 30 days of growth, wilted and tedded on the field until a 70% DM content was achieved. For haylage production, a biological additive (Silobac®) was added, according to the manufacturer's recommendations, consisting of diluting 2g of the product in 2L of water to inoculate each ton of forage intended for haylage-making. This product supplied *Lactobacillus plantarum* and *Pediococcus pentosaceus* in a concentration of 2.5×10^5 CFU per gram of forage, for each bacterium species. The purpose of this addition was to balance the microbial population in the material for haylage production, improving anaerobic fermentation. Approximately 1.3kg of green mass was wrapped in polyethylene bags (40 x 60cm) and vacuum sealed according to the technique described earlier (Kung Junior et al. 2010). Diet composition is described in Table 1 and haylage composition is established in Table 2. To verify possible fungi development and consequent mycotoxin production, visual inspection of hay and haylage was performed, along with mycotoxin investigation, since visual inspection alone is considered insufficient to make such a determination (Raymond et al. 2000).

Table 1. Chemical composition of the diet supplied for the horses

Diet (concentrate + forage)/Compounds		
	Hay	Haylage
Dry matter - DM (%)	94.54	95.71
Organic matter (% DM)	91.20	90.85
Ash (% DM)	8.80	9.15
Crude protein (% DM)	11.44	12.71
Neutral detergent fiber corrected for ash (% DM)	55.16	54.77
Acid detergent fiber corrected for ash (% DM)	20.41	22.58
Hemicellulose (% DM)	34.74	32.29
Gross energy (Mcal/kg DM)	4.72	4.85

Data based on dry matter.

Table 2. Chemical composition Tifton-85 haylage (*Cynodon* spp.) in different opening times (days)

Parameters	0d	1d	3d	7d	14d	28d	56d
Dry matter	26.78	71.70	71.98	73.20	70.62	70.45	72.28
IVDDM (%)	38.01	38,42	37.51	36.98	35.00	37.18	36.05
Crude protein (%)	17.63	19.06	18.91	19.65	17.18	17.09	17.71
% N-NH3/3 total	0.39	0.41	0.67	0.73	1.04	1.24	1.10
pH	5.48	6.08	5.19	5.53	5.37	5.42	5.21
NDF (%)	74.16	74.31	72.23	74.25	73.21	73.24	73.33
ADF (%)	32.33	31,03	31.58	31.87	32.15	31.31	31.96
HEM (%)	41.83	43.27	40.64	42.38	41.56	40.93	41.27
LIG (%)	3.28	3.56	3.36	3.59	3.79	3.56	3.39
Acetic acid (g/kg MS)	0.91	1.17	0.60	2.02	2.14	1.85	2.79
Propionic acid (g/kg/MS)	0.27	0.10	0.03	0.11	0.08	0.10	0.08
Butyric acid (g/kg/MS)	0.77	-0.70	0.18	0.36	0.42	0.23	0.20
Lactic acid (g/kg/MS)	2.26	0.35	3.01	10.24	11.44	16.15	26.06

d = days, IVDDM = *In vitro* digestibility of dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, HEM = hemicelluloses, LIG = lignin.

Haylage underwent mycotoxin evaluation to discard the possibility of any effect on the blood results. Veratox kits (Neogen Food Safety, Lansing/MI, USA) were used to quantify aflatoxins, fumonisins and zearalenone. A spectrophotometric reading was performed (ELISA plate reader in 650nm) on the grass after harvesting and on haylage 56 days after its production in order to assess the fermentation potential (Costa 2012). Analysis took place in the Laboratory of Veterinary Toxicology housed in the Veterinary College of UFMG.

Animals and groups. Twelve healthy Quarter Horse broodmares, with mean weight of 452±46.80kg and ranging in age from eight to 12 years, were used. Horses were randomly distributed into two groups (n=6): Group 1 underwent the hay-based diet, and Group 2, the haylage-based diet. Animals were kept in $9m^2$ stalls and were allowed a one-hour stroll within a known enclosure. Animals were weighed weekly for the calculation of the feedstuffs for maintenance requirements (NRC 2007). Haylage and hay were supplied for the first time to the animals during the experimental period, since their prior diet consisted of Tifton-85 (*Cynodon* spp.), mineral salt and water *ad libitum*. Hay and haylage were provided three times a day, and concentrate, twice a day. Before feeding the horses, haylage was visually inspected for mould.

Blood samples and biochemistry profile. Blood sampling was performed once a week within the 28- day experimental period through venipuncture of the jugular vein, using vacuum tubes without anticoagulant. The first sample was collected on time zero (T0) before the animals were introduced to their new experimental diets, and then after 7 (T1), 14 (T2), 21 (T3) and 28 (T4) days. All samples were collected in the morning, before the concentrate was fed.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl-transferase (GGT), alkaline phosphatase (AP), urea and creatinine, calcium (Ca) and phosphorus (P) were determined in sera samples using commercial kits (Bioclin, Belo Horizonte/MG, Brazil) and a semi-automated analyzer (TP Analyzer Basic, Thermoplate, São Paulo/SP, Brazil). Total protein (TP) was estimated through refractometry, and protein profile (serum protein electrophoresis) was accomplished using horizontal electrophoresis in agarose gel at 12% and TRIS buffer. Gels were stained with Amido black (Sigma-Aldrich, St. Louis/MO, USA) and discolored in a series of ethanol and acetic acid, with a 35-minute gel run. Reading was made through a scanner with a software (Celm CE-250, São Paulo/SP, Brazil). The concentration of protein in each fraction (g/dL) was determined through percentage multiplication of each fraction obtained in the total protein concentration.

Statistical analysis. Experimental design was completely randomized, with an arrangement of subdivided parcels in which a parcel was the randomized groups (1 or 2) and the subdivided parcels were moments of blood sampling, with six repetitions (animals in each group). Variables were tested for normality of distribution. AST, ALT, GGT and creatinine were transformed by $\log^{10}$ (x+1). Urea, Ca and AP presented non-parametric distribution and were analyzed by Mann-Whitney test, (for group comparison), and by Friedman test (for moments' comparison). All other variables were analyzed by ANOVA with Tukey test. A $P<0.05$ value was accepted as a statistically significant difference for all the tests.

RESULTS AND DISCUSSION

This study provides the effects on blood parameters of horses fed with haylage compared to hay diet. Haylage is a pre-dried feed processed with partial drying and aerobic fermentation. Since it has a greater water content than hay, its conservation has a higher risk of mould growth and mycotoxins production that may impair animal health (Müller et al. 2011).

The levels of mycotoxins in the present study were equivalent between grass and haylage (Table 3), which allows inferring that haylage production conditions were appropriate. These concentrations are lower than the tolerance levels estimated for horses, which are 5mg/kg for fumonisin (Diener 1996), 10µg/kg for aflatoxins (Basalan et al. 2004) and 1000µg/kg for zearelenone (Juhász et al. 2001). Mufatto et al. (2016) observed that pre-drying Tifton-85, while adopting at least a 29-day fermentation period is directly related to improvement of microbiological quality in the foodstuff.

Blood biochemical panel was used to define the health status of animals, providing information regarding tissue injury, organ malfunction, adaptation to nutritional and physiological challenges, and during specific metabolic dysfunctions. Therefore, blood biochemistry can provide grounds for the interpretation of liver, kidney, bone and muscular adjustments. Total protein, AST, GGT, ALT provide information of liver function, whereas urea and creatinine are associated with kidney lesions, Ca and P are related to basic cellular function (Gonzáles & Silva 2006).

Serum enzymes are presented in Table 4. Discrete differences were observed amongst evaluated times, but GGT, ALT and AST, remained close to the established parameters for the species as

Table 3. Mycotoxins concentrations found in the Tifton-85 and haylage at day 56 compared with established tolerance levels for horses

Micotoxins	Tifton-85	Haylage (56d)	Tolerance levels for horses
Aflatoxin	0.95 µg/kg	0.95 µg/kg	10µg/kg*
Fumonisin	0.60 mg/kg	0.60mg/kg	5mg/kg**
Zearalenone	0.71 µg/kg	0.71 µg/kg	1000 µg/kg***

* Basalan et al. 2004, ** Diener 1996, *** Juhasz et al. 2001.

Table 4. Mean values and standard deviation of GGT (UI/L), AST (UI/L), ALT (UI/L) of horses after hay administration (Group1-G1) and haylage of Tyfton-85 (Group 2-G2)

Time	GGT		AST		ALT	
	G1	G2	G1	G2	G1	G2
T0	12.65 ± 3.19 ^{B}	12.90 ± 4.71	262.27 ± 123.72	177.41 ± 11.48 ^{AB}	7.06 ± 3.31	8.14 ± 9.11
T1	13.50 ±6.82 ^{B}	14.27 ±9.28	227.18 ± 101.30	138.39 ± 39.06 ^{B}	8.95 ± 6.61	9.11 ± 12.01
T2	17.36 ± 4.52 ^{AB}	16.65 ± 4.17	200.43 ± 97.81	145.17 ± 37.74 ^{B}	9.74 ± 6.63	15.24 ± 11.43
T3	20.73 ± 6.35 ^{AB}	17.81 ± 6.67	231.89 ± 121.64	158.50 ±69.04 ^{B}	5.72 ±3.09	6.77 ±2.79
T4	22.66 ±3.14 ^{A}	19.24 ± 9.10	259.97 ± 96.02	230.23± 83.20 ^{A}	9.23 ± 3.10	10.83 ± 4.10

$^{A, B, AB}$ Means followed by the same uppercase letters do not differ between sample times (columns); means followed by the lowercase letters do not differ between groups (lines) (Friedman, P<0.05).

slight variations were observed. Only the hay group presented significant rise (P<0.05) of GGT (12.6±3.19 to 22.7±3.14U/L) (Fig.1). Haylage group showed similar values, 12.9±4.71U/L (T0) e 19.2±9.10U/L (T4), but without statistic increase. Although discrete alterations occurred, all values remained within the normal limit for equine (9 to 26U/L) according to Padilha et al. (2017), demonstrating that no cholangitis or cholestasis occurred.

In regards to AST, no alterations were observed in the group that was fed with hay. The groups treated with haylage presented a discrete decrease that persisted for three weeks (T1, T2 and T3). Mean values observed in both G1 and G2 ranged respectively from 200.43U/L to 259.97U/L and 138.39U/L to 230.23U/L, similarly to those reported by Souza et al. (2016) of 140U/L and Padilha et al. (2017) with means of 246.34U/L. According to Kaneko & Harvey (1997) all values remained within the normal range (226-366 U/L).

ALT remained unchanged in both groups and times. G1 mean levels were from 5.72 to 9.23U/L, while G2 from 6.77 to 15.24U/L. Previous reports indicate mean values between 5.13 U/L (Souza et al. 2016) and 12.6U/L (Ural et al. 2009). According to Kaneko & Harvey (1997) all values remained within the normal range (3-23U/L). Both GGT and ALT are mostly present in liver cells, and the fact that both serum levels were considered normal is an indicator of the animals' health. Hay and haylage did not alter the physiology of the liver.

ALT activity in horses' liver is considered to be low, and as so it can't be used separately from other serum biochemistry evaluations, only in association is enzymes, such as AST and GGT. It is important to highlight that AST, although not organ-specific, is frequently used in routine exams for the detection of liver injury. After careful analysis of hepatic enzymes, it is clear that haylage supply for 28 days is not able to cause any type of injury or lesion to the hepatic tissue, or even stimulation or production of enzymes.

Ca mean values observed in both G1 and G2 ranged respectively from 2.76 to 3.84mmol/L and 2.77 to 3.84mmol/L,

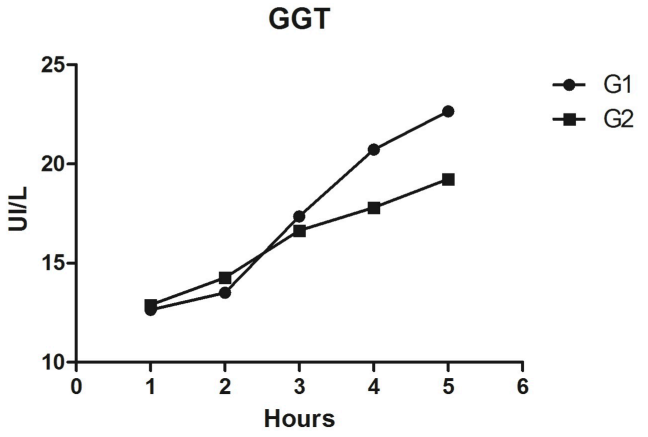

Fig.1. GGT (UI/L) serum relationship in horses that received hay (G1) and haylage of Tifton-85 (G2).

very similar to 2.43 to 3.10mmol/L described by Kaneko & Harvey (1997) and did not change between time and/or experimental groups (Table 5).

P mean values observed in both G1 and G2 ranged respectively from 3.38 to 4.28mg/dL and 3.51 to 3.80mg/dL inside the normal limits (3.1-5.6mmol/L) described by Kaneko & Harvey (1997).

These findings represent that both hay and haylage provide sufficient diet content to maintain ionic balance and energetic metabolism. Such a result is yet another confirmation of the haylage proper nutritional balance for horses.

Urea and creatinine levels had discrete variations between analyzed times (Table 6). Urea had a significant decrease in T3 and, highest levels of creatinine were seen in T3 for both groups (1.60±0.22, hay group) and (1.54±0.38, haylage group). All the creatinine values remained in the normal range for the species (creatinine 1.0 to 2.0mg/dL); however, the urea values presented variations and are above the values

described by Kaneko & Harvey (1997) (10 to 24mg/dL). These variations were expected, due to protein intake and test methodology, with no clinical significance (Souza et al. 2009, Padilha et al. 2017). These results indicate that the feeding and protein intake caused minor variations in urea and creatinine levels but hay and haylage consumption did not result in kidney lesions.

Table 5. Mean values of calcium (Ca) (mmol/L) and phosphorus (P) (mmol/L) of horses after hay administration (Group1-G1) and haylage of Tyfton-85 (Group 2-G2)

Time	Ca		P	
	G1	G2	G1	G2
T0	2.76 ± 0.002	2.77 ± 0.002	3.38 ± 0.86	3.52 ± 0.47
T1	3.83 ± 0,024	3.82 ± 0.002	4.28 ± 0.48	3.51 ± 0.57
T2	3.85 ± 0.004	3.58 ± 0.02	3.77 ± 0.36	3.63 ± 0.7
T3	3.84 ± 0.015	3.84 ± 0.02	3.88 ± 0.50	3.71 ± 0.7
T4	3.82 ± 0.055	3.84 ± 0.019	4.21 ± 0.45	3.80 ± 0.54

Friedman, P<0.05.

Table 6. Mean values and standard deviation of urea (mg/dL) and creatinine (mg/dL) of horses after hay administration (Group 1-G1) and haylage of Tyfton-85 (Group 2-G2)

Time	Urea		Creatinine	
	G1	G2	G1	G2
T0	42.82 ± 4.96 ^{A}	41.54 ± 7.36 ^{AB}	1.37 ± 0.21 ^{ABC}	1.26 ± 0.22 ^{AB}
T1	62.80 ± 3.47^{A}	60.28 ± 6.96 $_{A}$	1.54 ± 0.33 ^{AB}	1.45 ± 0.27 ^{AB}
T2	30.91 ±2.85 ^{B}	35.73 ±7.31 ^{BC}	1.18 ± 0.09 ^{BC}	1.13 ± 0.05 ^{B}
T3	32.61 ± 4.97 ^{B}	34.31 ± 1.11 ^{BC}	1.60 ± 0.22 ^{A}	1.54 ± 0.38 ^{A}
T4	31.80 ± 3.97 ^{B}	31.80 ± 1.34^{C}	1.10 ± 0.08 ^{C}	1.21 ±0.14 $_{AB}$

$^{A, B, C, AB, BC, ABC}$ Means followed by the same uppercase letters do not differ between sample times (columns); means followed by the lowercase letters do not differ between groups (lines) (Friedman, P<0.05; Urea Tukey, P<0.05).

Protein fractions were similar between groups as described in Tables 7 and 8; however, protein distribution was different from previously reported. Protein fractioning revealed nine distinct bands, denominated albumin, α1b-globulin, α1b-globulin, α2a-globulin, α2b-globulin, β1-globulin, β2a-globulin, β2b-globulin and γ-globulin. In the present work, albumin represented the highest percentage of serum proteins, followed by γ-globulin, β2a-globulin, β1-globulin, β2b-globuin and α1 and α2 fractions. In a study using 126 horses, the highest levels were also found in albumin and γ-globulin, followed however by α2, β1 and β2 globulin, and α1 (Riond et al. 2009). These differences may be associated with different breeds, sexes, ages and diet between the studies, revealing the importance of future work to determine normal range values for Quarter horses.

Total protein concentration (Table 8) presented no statistical difference between experimental groups and time of analysis and ranged from 5.57 to 6.67g/dL in G1 and from 6.10 to 7.70g/dL in G2. All levels remained within normal parameters for the equine species. Nonetheless, Moore-Colyer & Longland (2000) highlighted a higher digestibility of haylage in comparison to hay, which may elicit the rise of TP in the haylage group, not seen in the current study. Since there was no significant difference between the groups regarding protein concentrations, it may be inferred that the experimental diets were not able to alter their pattern.

The relation between albumin and globulin is presented in Figure 2. This is an important measure that grants data regarding dysproteinmeias and the systematic classification of electrophoretic profile (Riond et al. 2009). In the present work, no differences were found between groups, showing that protein profile is unchanged by haylage. This methodology is

Table 7. Mean values and standard deviation for albumin (Alb) (g/dL), α 1a (g/dL), α 1b (g/dL), α 2a (g/dL) and α 2b (g/dL) from horses serum, after administration of hay (Group 1-G1) and haylage (Tifton-85) (Group 2-G2)

Time	Albumin		α 1a		α 1b		α 2a		α 2b	
	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2
T0	2.23 ± 0.38 ^{A}	2.25 ± 0.71	0.27 ± 0.09 ^{AB}	0.27 ± 0.09	0.12 ± 0.05	0.15 ± 0.11	0.13 ± 0.08 ^{B}	0.11 ± 0.07	0.27 ± 0.20	0.25 ± 0.25
T1	1.28 ± 0.36 ^{B}	1.74 ± 0.42	0.17 ± 0.12 ^{B}	0.20 ± 0.10	0.12 ± 0.08	0.12 ± 0.10	0.21 ± 0.07 ^{AB}	0.21 ± 0.05	0.24 ± 0.15	0.25 ± 0.13
T2	1.78 ± 0.62 ^{ABb}	2.40 ± 0.53 ^{a}	0.27 ± 0.05 ^{AB}	0.23 ± 0.06	0.14 ± 0.07	0.16 ± 0.04	0.17 ± 0.11 ^{AB}	0.22 ± 0.12	0.28 ± 0.11 ^{a}	0.22 ± 0.14
T3	1.87 ± 0.48 ^{AB}	2.06 ± 0.51	0.28 ± 0.06 ^{A}	0.28 ± 0.09	0.19 ± 0.10	0.17 ± 0.02	0.25 ± 0.15 ^{AB}	0.23 ± 0.11	0.31 ± 0.18 a	0.34 ± 0.21
T4	2.11 ±0.27 ^{A}	2.34 ± 0.28	0.21 ± 0.11 ^{AB}	0.26 ± 0.06	0.13 ± 0.04 ^{b}	0.21 ± 0.08 ^{a}	0.38 ± 0.23 ^{A}	0.27 ±0.11	0.16 ± 0.13 ^{a}	0.31 ± 0.14

Means followed by the same uppercase letters do not differ between sample times (columns); means followed by the lowercase letters do not differ between groups (lines) (Tukey, P<0.05).

Table 8. Mean values and standard deviation for β 1 (g/dL), β 2a (g/dL), β 2b (g/dL), γ globulins (g/dL) and total protein (TP) (g/dL) from horses serum, after administration of hay (Group 1-G1) and haylage (Tifton-85) (Group 2-G2)

Time	β 1		β 2a		β 2b		γ globulinas		Total protein	
	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2
T0	0.63 ± 0.26	0.74 ± 0.23 ^{B}	1.40 ± 0.49 ^{Aa}	0.93 ± 0.42 ^{b}	0.86 ± 0.42 ^{Ab}	1.22 ±0.26 ^{Aa}	0.86 ± 0.21	1.11 ± 0.39	6.67 ± 0.82	7.70 ± 0.80
T1	0.73 ± 0.30 ^{b}	1.29 ± 0.51 ^{Aa}	1.00 ± 0.30 ^{AB}	1.14 ± 0.47	0.51 ± 0.24 ^{AB}	0.59 ± 0.11 ^{B}	1.15 ± 0.49	1.22 ± 0.23	5.57 ± 1.40	6.10 ± 0.84
T2	0.61 ± 0.19	0.84 ± 0.30 ^{B}	0.89 ± 0.24 ^{B}	0.90 ± 0.34	0.56 ± 0.35 ^{AB}	0.53 ±0.28 ^{B}	1.11 ± 0.25	1.07 ± 0.31	5.80 ± 0.9	6.60 ± 1.47
T3	0.84 ± 0.24	0.92 ± 0.26 ^{AB}	1.28 ± 0.26 ^{AB}	1.18 ± 0.45	0.45 ± 0.09 ^{B}	0.64 ± 0.25 ^{B}	1.27 ± 0.5	1.25 ± 0.27	6.57 ± 1.8	6.90 ± 1.29
T4	0.77 ± 0.11	0.92 ± 0.10 ^{AB}	1.01 ± 0.19 ^{AB}	1.06 ± 0.20	0.37 ± 0.07 ^{Bb}	0.76 ± 0.25 ^{Ba}	1.07± 0.15	1.29 ± 0.23	6.20 ± 0.5	7.53 ± 0.62

$^{A, B, AB}$ Means followed by the same uppercase letters do not differ between sample times (columns); $^{a, b}$ means followed by the lowercase letters do not differ between groups (lines) (Tukey, P<0.05).

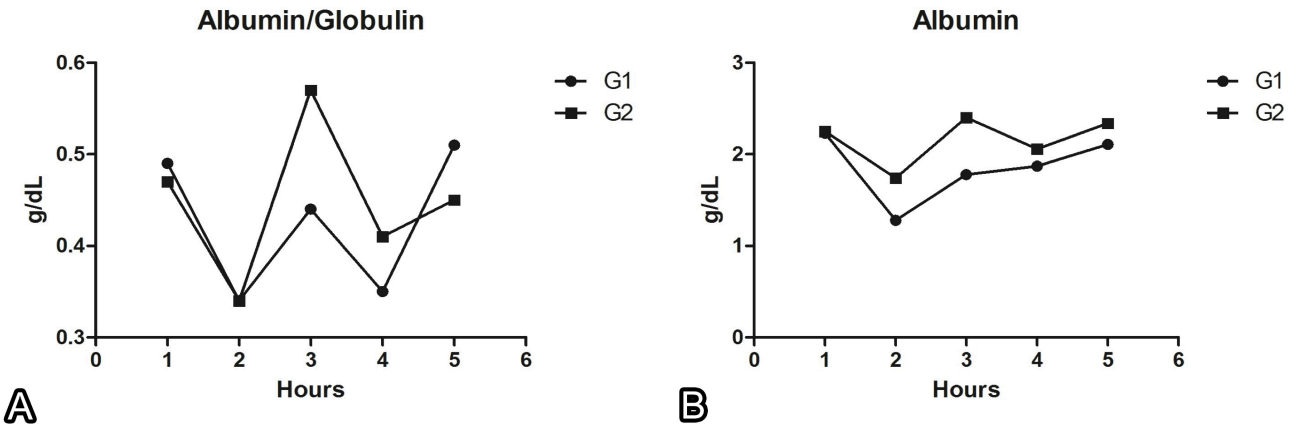

Fig.2. (**A**) Albumin/Globulin ratio from horses that received hay (G1) and haylage of Tifton-85 (G2). (**B**) Serum albumin relationship in g/dL from horses that received hay (G1) and haylage (G2).

simple and useful as a screening test for the species, informing the clinician of valuable information in changes of such fractions that may help characterize dysproteinemias in the equine patient (Riond et al. 2009).

CONCLUSION

The use of haylage of Tifton-85 (*Cynodon* spp.) with 70% of DM for 28 days is a safe and adequate alternative feeding for Quarter Horse broodmares as it did not cause alterations in horses' biochemical profiles when compared to hay.

Conflict of interest statement.- The authors have no competing interests.

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