

QUALITY OF TIFTON AND ALFALFA HAYS AND EQUINE BODY BIOMETRICS

Kátia de Oliveira

Universidade Estadual Paulista - Unesp, Dracena, SP, Brazil,

<https://orcid.org/0000-0002-0785-9016>

Denise Tsuzukibashi

Universidade Estadual Paulista - Unesp, Botucatu, SP, Brazil.

Daniele Floriano Fachioli

Universidade Estadual Paulista - Unesp, Botucatu, SP, Brazil,

<https://orcid.org/0000-0002-4116-9289>

Marcos Jun Watanabe

Universidade Estadual Paulista - Unesp, Botucatu, SP, Brazil,

<https://orcid.org/0000-0002-7317-4250>

João Henrique Silva Vera

Universidade Estadual Paulista - Unesp, Botucatu, SP, Brazil,

<https://orcid.org/0000-0002-8804-0570>

Juliana da Silva Barros

Universidade Estadual Paulista - Unesp, Botucatu, SP, Brazil,

<https://orcid.org/0000-0003-4612-751X>

Email correspondente: juliana.1234barros@gmail.com

Ciniro Costa

Universidade Estadual Paulista - Unesp, Botucatu, SP, Brazil,

<https://orcid.org/0000-0003-1854-2927>

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Abstract

This paper targets the quality of tifton bermudagrass (*Cynodon* spp.) and alfalfa (*Medicago sativa*) hays in isoproteic and isoenergetic diets on horse body biometrics. Six equines, between 6.5 ± 0.5 years old and with an mean body weight of 354 ± 50 kg, were used in this study. The treatments consisted of six diets and the experimental delineation was the Latin square (6x6). The diet's forage was composed by a combination of hays, in a factorial scheme (2x3), with two forages (tifton bermudagrass and alfalfa) and three types of hay (A, B and C). There was a significant effect in the variables body weight, abdominal girth, fecal water content, and fecal production for the forage type. Greater digestibility coefficient of dietary dry matter values were identified in the diets composed by type A and B alfalfa hay ($64.71 \pm 2.38\%$ and $63.45 \pm 2.41\%$, respectively), that have significantly surpassed ($P = 0.0061$) the results obtained for the type C hay diets. It was possible to conclude that the alfalfa hay provided a reduction in the horse's body weight and thoracic perimeter, what can be explained by the greater verified digestibility, as well as the lesser fecal water retention with the diets containing only the legume.

Keywords Abdominal Girth, Body Weight, Digestibility, Forage, Horse.

QUALIDADE DOS FENOS DE TIFTON E ALFAFA E A BIOMETRIA CORPORAL DE EQUINOS

Resumo

Objetivou-se estudar a qualidade dos fenos de capim-tifton (*Cynodon* spp.) e alfafa (*Medicago sativa*), em dietas isoproteicas e isoenergéticas, sobre a biometria corporal de cavalos. Foram utilizados seis equinos, com idade entre 6.5 ± 0.5 anos e peso corporal médio de 354 ± 50 kg. Os tratamentos consistiram em seis dietas e o delineamento experimental foi o quadrado latino (6x6). O volumoso da dieta foi composto por combinações de fenos, em esquema fatorial (2x3), sendo duas forragens (capim-tifton e alfafa), três tipos de feno (A, B e C) além de, concentrados comerciais para equinos e mistura mineral. As variáveis peso corporal, perímetro do abdômen, teor de umidade nas fezes e produção fecal apresentaram efeito significativo para o tipo forrageiro. O grupo de cavalos alimentados com feno de alfafa apresentou menores valores para peso corporal (349.23 ± 39.69 kg), perímetro do abdômen ($174,27 \pm 8,18$ cm), teor de umidade nas fezes ($72.65 \pm 0.79\%$) e produção fecal (2.42 ± 0.10 kg). Identificaram-se maiores coeficientes de digestibilidade da matéria seca nas dietas compostas por fenos de alfafa dos tipos A e B com valores de $64.71 \pm 2.38\%$ e $63.45 \pm 2.41\%$, respectivamente, do que os resultados obtidos para as dietas com fenos tipo C ($P = 0.0061$). Concluiu-se que o feno de alfafa proporcionou redução no peso corporal e perímetro torácico dos cavalos, e pode ser explicado por causa da melhor digestibilidade verificada, bem como a menor retenção de água fecal das dietas contendo apenas a leguminosa.

Palavras-chave Cavalo, Degradabilidade, Forragem, Perímetro abdominal, Peso corporal.

INTRODUCTION

The feeding strategy which is adopted to feed athletic horses can have an increasing effect on their body weight. Historically, the use of forage in diets of performance horses has been limited (JANSSON and HARRIS, 2013). Horses that consume diets containing a high forage proportion end up having an increment in the mass of ingesta present in the large intestines due to the increase in water retention in the gastrointestinal tract (GIT) as well as the amount of undigested fibrous material (OLIVEIRA, PAGLIOSA and SALVADOR, 2019). In this way, researchers and handlers have been recommending forage consumption restriction three days before intense short-duration exercise, with the objective to decrease the called ballast weight referred to the gastrointestinal tract content and, thus, the possible reduction of the energy spent during the race (BRUNNER et al., 2012).

Harris et al. (2017) suggested that horses should be fed at least 15 g dry matter (DM) of forage per kg of body weight (BW) and day in order to support natural behavior and health. However, for some horses, even this amount of grass or legume forage may exceed energy requirements especially during a weight loss program and therefore replacing part of the forage ration with low energy straw could be a good alternative (Jansson et al., 2021).

Research has indicated that the ingestion level of the offered forage seems not to be the most important factor for the body weight increase in horses. Muhonen, Philippeau and Julliand (2022) considered the forage bromatological composition as a primordial characteristic. In this sense, Muhonen and Julliand (2023) verified a body weight increase in athletic horses fed with oat concentrate and late-harvested mature grass haylage and lucerne and mature grass haylage in comparison to early-harvested grass haylage and mature grass haylage, which might be explained by the upper intake of less-digestible fiber.

From all the literature consulted, there were no reference with the objective to evaluate the effect of the nutritional quality of the Tifton Bermudagrass and alfalfa hays in the feeding of athlete horses. In this sense, this research objective was to study the effect of Tifton 85 Bermudagrass and alfalfa hays in isoproteic and isoenergetic diets on horses body biometrics.

MATERIALS AND METHODS

The experiment was conducted at the RR horse farm, at the town of Sao Pedro, Sao Paulo, Brazil. Six male mixed-breed equine were used, portraying an mean age of 6.5 years and mean body weight (BW) of 354 ± 50 kg. The treatments consisted of six diets, constituted by concentrate and forage, in which the diet's forage portion was made of hay combinations in a factorial scheme 2×3 , that is, two forages (Tifton 85 Bermudagrass and alfalfa) and three hay qualities (A- high, B- medium and C- poor). The experimental delineation was the Latin quarter 6×6 (six horses and six periods), totaling six repetitions per treatment. Each experimental period was composed of seven days, to give the horses time to adapt to the diet, followed by three days of measurements (collection phase). During the three-day adaptation period, 33.33% of the old diet was replaced by the new one, stipulated by the experimental rotation of the Latin square. The variables that were measured on the horses were body weight (BW), abdominal girth (AG), water consumption (WC), fecal water content (FW), total serum protein concentration (TP), fecal production (FP) and digestibility coefficient of dietary dry matter (DCDM).

This research was approved by the Ethical Comity for Animal Use (ECAU) of UNESP, Dracena Campus, protocol number 44/2012.

Tifton Bermudagrass and alfalfa hays from types A, B and C, commercial concentrate for equines (Proequi 13® Rolled Grains - Guabi®), containing 12.04% crude protein (CP) and 3.2 Mcal/kg digestible energy (DE) were purchased to concoct the experimental diets, as well as equine mineral supplement (Guabiphos® Centauro 80 - Guabi), that was offered in the amount of 50 g/animal/ day on the first daily meal, during the whole experimental period.

The nutritional quality of the Tifton Bermudagrass and alfalfa hays, for types A, B and C, used in the experimental diets are shown on [table 1](#). The hays, soybean meal and concentrate samples were analyzed for the dry matter (DM), ether extract (EE) and crude protein (CP), according to the Association of Official Analytical Chemists (AOAC, 1984), the neutral detergent fiber (NDF) acid detergent fiber (ADF), lignin and cellulose were analyzed following the methodology described by Van Soest, Robertson and Lewis (1991).

The study adopted the total dry matter ingestion index (DMI) of 2.10% of BW in a 50-55:45-50 concentrate to forage relation. The diets were formulated to provide the minimum nutritional requirements for horses in intense training (NRC, 2007), and

Table 1. Bromatological composition on a percentage in dry matter (%DM) and digestible energy content (DE) in megacalories per kilogram (Mcal/kg) of the ingredients used on the experimental diets balance.

| Feedstuff | DE (Mcal.kg ⁻¹) | Nutrient (% MS) | | | | |
|--------------------------------------|--------------------------------|-----------------|-----------|--------|------------------|------------------|
| | | CP ⁴ | Cellulose | Lignin | NDF ⁵ | ADF ⁶ |
| Commercial mixture ¹ | 3.20 | 12.04 | 12.62 | 3.28 | 37.57 | 15.90 |
| Soybean meal ¹ | 3.17 | 51.33 | 9.55 | 2.09 | 23.46 | 11.64 |
| Soybean oil ² | 9.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Tifton Bermudagrass hay ³ | | | | | | |
| Type A | 2.56 | 13.28 | 34.93 | 5.73 | 72.62 | 40.66 |
| Type B | 2.49 | 11.80 | 32.67 | 6.30 | 74.57 | 38.98 |
| Type C | 2.40 | 10.79 | 36.29 | 5.74 | 78.74 | 42.03 |
| Alfalfa hay ³ | | | | | | |
| Type A | 2.65 | 20.06 | 25.47 | 10.91 | 49.55 | 36.39 |
| Type B | 2.48 | 18.61 | 31.28 | 12.18 | 58.99 | 43.46 |
| Type C | 2.30 | 17.70 | 34.10 | 12.67 | 62.75 | 46.77 |

¹Energy calculated according to the National Research Council - NRC (2007) using the DE = 4.07 - 0.55 x ADF equation; ²DE obtained by the NRC (2007) nutritional composition table, ³Energy calculated according to the NRC (2007) using the DE = 2.118 + (0.01218 x CP) - (0.0093 x ADF) - (0.00383 x (NDF - ADF)) + (0.04718 x EE) ether extract - (0.0262 x MM) ash; ⁴CP = crude protein; ⁵NDF = neutral detergent fiber; ⁶ADF = acid detergent fiber.

to be isoproteic (15.7% CP) and isoenergetic (2.9 Mcal/kg). The protein and energy content corrections between the experimental diets were achieved by addition of soybean meal and soybean oil, respectively (Table 2).

The feeding was provided in three equal meals composed of concentrate and forage, and they were offered at 7 am, 1 pm and 7 pm. Fifteen minutes before the meals were offered, the leftover feedstuffs, when present, were collected and weighted. The horses were kept in 9 m² individual stalls, with cemented floors covered

Table 2. Centesimal composition, bromatological and energetic content in megacalories per kilogram (Mcal/kg) of the experimental diets.

| Ingredients (%) | Experimental Diets ¹ | | | | | |
|--|---------------------------------|------|------|------|------|------|
| | TA | TB | TC | AA | AB | AC |
| Commercial mixture ² | 46.0 | 45.3 | 44.9 | 49.9 | 47.6 | 45.7 |
| Soybean Meal | 8.0 | 9.4 | 10.2 | 0.0 | 2.4 | 4.0 |
| Soybean Oil | 0.0 | 0.0 | 0.0 | 0.2 | 2.4 | 4.6 |
| Tifton Hay | 46.0 | 45.3 | 44.9 | 0.0 | 0.0 | 0.0 |
| Alfalfa Hay | 0.0 | 0.0 | 0.0 | 49.9 | 47.6 | 45.7 |
| CP ³ (%) | 15.7 | 15.6 | 15.5 | 16.0 | 15.8 | 15.6 |
| NDF ⁴ (%) | 52.6 | 53.0 | 54.6 | 43.6 | 47.7 | 49.0 |
| DE ⁵ (Mcal.kg ⁻¹) | 2.9 | 2.9 | 2.8 | 2.9 | 2.9 | 2.9 |

¹Experimental Diets: TA= type A Tifton Bermudagrass, TB= type B Tifton Bermudagrass, TC= type C Tifton Bermudagrass; AA= type A Alfalfa, AB= type B Alfalfa, AC= type C Alfalfa; ²Equine commercial concentrate (Proequi 13 Rolled Grains - Guabi); ³CP= crude protein; ⁴NDF= neutral detergent fiber; ⁵DE= digestible energy.

with wood shavings, automatic water supply, and concrete feeders for concentrate and mineral supplement consumption. The hays were offered in hay bags.

The physical activity was performed for five days per week, with an hour duration per training session (six minutes at walk, 30 minutes at trot, 18 minutes at gallop and six minutes at walk), classified as intense work according to the NRC (2007). On the weekends, the horses were allowed free access to a sand ring for 2 hours.

Furthermore, in the beginning of the experiment, the horses were treated with a broad spectrum ivermectin-based dewormer, 200 ug/kg by body weight (Equizol® 1.3%) and, in the beginning of each experimental period, the horses BW were monitored to allow for possible adjustments in the consumption of the experimental diets; thus, the horses body fat composition (fat content) was measured for the exercise program evaluation, adapting when necessary. For that, the fat thickness (FT) was assessed in centimeters (cm), adopting the transcutaneous technique with the ultrasound ALOKA model SSD-500, 3.5 MHz 13 cm long transducer. The measurement was performed at the tail insertion, placing the probe five centimeters lateral to the spinal axis and seven centimeters cranial to the tail base. The fat percentage was estimated with the following equation: % Body Fat = $2.47 + 5.47 \times (FT \text{ in cm})$, in which the means in the beginning and in the end of the experiment were $27.0 \pm 6.9\%$ and $26.8 \pm 6.4\%$, respectively. The ultrasound evaluations were performed after the animals' skins were cleaned and the area prepared with soybean oil on the animal's left side by the same habilitated professional. The measurements were obtained while the horses were standing on a plane and paved floor.

The variables that were measured on the horses were body weight (BW), abdominal girth (AG), water consumption (WC), fecal water content (FW), total serum protein concentration (TP), fecal production (FP) and digestibility coefficient of dietary dry matter (DCDM). The animals were weighted daily (portable electronic scale KM3-N with bars COIMMA®) before the first meal was provided, at 7 am, during the whole experimental period. The abdominal girth was assessed daily after the animals were weighted, using a metric tape measure, and this measurement was taken at the level of the 18th thoracic vertebra. The horses' water consumption was verified also every day, in the morning, during the whole experimental period, using graduated water recipients (500 mL-graduated bucket) and offering free-choice water.

During three days after the adaptation period, the horses total feces were collected directly from the ground and weighted every eight hours to quantify the FP (OLIVEIRA et al., 2021). The feces were homogenized and sampled in 100 g., and were placed in plastic bags, identified and stored at a 15°C temperature for posterior dietary DM and DCDM analyses. The FW was calculated from the determination of the horses fecal DM and fecal production, plus the feed amount (forage + concentrate) consumed per animal, using the following equation: % FW = 100 - fecal DM; and the dietary DCDM was calculated with the following equation: % DCDM = 100 x (kg consumed feedstuffs - kg fecal production)/ kg consumed feedstuffs).

On the last day of the collection phase, a jugular venous blood sample was collected in an empty red top tube to obtain the serum, in the morning, before the first daily meal, for the TP determination. These tubes were centrifuged (Centribio® centrifuge) at a 1,087 x g constant speed for 04 minutes. After this procedure, they were placed in 1.5 ml plastic tubes, identified and stored in a -16°C temperature freezer until the time to be processed. The serum total protein concentration was obtained using the Biuret reagent kit (Labtest®) and the reading was performed with a spectrophotometer (Celm model SBA-200) at laboratory of Department of Animal Science at ESALQ/USP, in Piracicaba/SP.

The data were analyzed for normal distribution, and it was considered normal when $W \geq 0.90$. The variables were submitted to the variance analysis of the computer program Statistical Analysis System (SAS, 2018) for data analysis. The comparison between means was performed by the Tukey test, and the main effect probability was used through the F test. All tests used a 5% significance level. The interaction among factors was not significant.

RESULTS

[Table 3](#) shows the mean values for body weight (BW) and abdominal girth (AG) for the studied equines. There was no effect ($P > 0.05$) of the experimental diets on the animals' BW and AG, but there was a significant difference ($P < 0.05$) for the forage type as the main effect. The horse group which was being fed with Tifton Bermudagrass hay presented superior values in the variables BW and AG, which were 367.75 ± 42.88 kg and 177.05 ± 8.14 cm, respectively; while the horses that consumed alfalfa hay obtained a reduction in these variables, with values of 349.23 ± 39.69 kg for

Table 3. Mean and standard deviation of the body weight (BW) and of the abdominal girth (AG) for equines submitted to experimental diets.

| Variable | Diets with Tifton Hay | | | Diets with Alfalfa Hay | | |
|----------|----------------------------|------------------|------------------|----------------------------|------------------|------------------|
| | A | B | C | A | B | C |
| BW (kg) | 366.88 ±48.82 | 372.44 ±37.38 | 363.94 ±44.05 | 350.06 ±36.59 | 348.56 ±45.46 | 349.07 ±49.65 |
| | Mean ¹ | | | Mean ² | | |
| | 367.75 ^A ±42.88 | | | 349.23 ^B ±39.69 | | |
| AG (cm) | 176.23 ±8.21 | 177.09 ±9.04 | 177.81 ±7.59 | 174.50 ±9.20 | 173.71 ±7.54 | 174.57 ±7.82 |
| | Mean ¹ | | | Mean ² | | |
| | 177.05 ^A ±8.14 | | | 174.27 ^B ±8.18 | | |

^{A,B} Means with different letters on the same line differ between themselves by the F test.

BW and 174.27 ± 8.18 cm for AG. Such responses resulted in a difference, between the means of the experimental groups containing Tifton Bermudagrass and alfalfa hay as a dietary source of forage, of 18.52 kg (P= 0.032) and 2.78 cm (P= 0.048) for BW and AG, respectively.

Table 4 shows the means which refer to water consumption (WC), fecal water content (FW) and serum total protein concentration (TP) for the equines. There was no significant effect (P> 0.05) for WC and TP in the comparisons between treatments and, also for the forage type which was present in the diets, as a main effect. However, it was observed an effect of the experimental diets on the fecal water content (P< 0.0001)

Table 4. Mean and standard deviation of the water consumption (WC), fecal water content (FW) and the serum total protein concentration (TP) of the equines eating isoproteic and isoenergetic diets submitted to three Tifton Bermudagrass¹ and alfalfa² hay types (A, B and C).

| Variable | Tifton Bermudagrass Hay | | | Alfalfa Hay | | |
|--------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|
| | A | B | C | A | B | C |
| WC (L) | 37.04 ±13.97 | 43.52 ±16.13 | 33.94 ±9.69 | 36.04 ±12.15 | 41.37 ±15.41 | 38.96 ±16.87 |
| | Mean ¹ | | | Mean ² | | |
| | 37.82±13.83 | | | 38.70±14.58 | | |
| FW (%) | 74.97 ^a ±2.79 | 73.58 ^b ±1.72 | 73.73 ^b ±1.99 | 73.95 ^{ab} ±1.20 | 71.93 ^c ±3.67 | 72.07 ^c ±1.76 |
| | Mean ¹ | | | Mean ² | | |
| | 74.09 ^A ±2.24 | | | 72.65 ^B ±2.56 | | |
| TP (g·dL ⁻¹) | 7.68 ±0.58 | 7.75 ±0.59 | 7.63 ±0.60 | 7.63 ±0.70 | 7.67 ±0.43 | 7.72 ±0.43 |
| | Mean ¹ | | | Mean ² | | |
| | 7.69±0.56 | | | 7.67±0.51 | | |

L= liters; %= percentage; g·dL⁻¹= grams per deciliter; ^{a,b,c} Means with different letters on the same line differ (P< 0.05) between themselves by the Tukey test; ^{A,B} Means with different letters on the same line differ (P< 0.005) between themselves by the F test.

or on the statistic comparison for the forage type ($P= 0.0004$).

Horses which ate type B alfalfa hay as a forage source showed a smaller fecal water retention, with an mean value of $71.93 \pm 0.85\%$ FW. The diets which contained hays classified as type A presented higher values of FW, resulting in a $74.97 \pm 0.85\%$ content for the Tifton Bermudagrass and $73.95 \pm 0.85\%$ for the alfalfa hay, what surpassed the results obtained for the diets with types B and C forages. In the comparison between the forage type present in the diets as a main effect, the Tifton Bermudagrass hay that was offered to the horses resulted in a greater fecal water content (FW tifton= $74.09 \pm 0.79\%$) than the alfalfa hay, with an mean value of $72.65 \pm 0.79\%$ FW ($P= 0.0004$).

Table 5 indicates the mean values of the fecal production for the equines and of the digestibility coefficient of dry matter (DCDM) for the experimental diets. A significant effect between treatment was verified for the variables FP ($P= 0.0013$) and DCDM ($P= 0.0061$). In addition, a statistic difference was detected for the forage type present in the diets, as a main effect, for FP ($P= 0.0181$).

The higher FP occurred in the groups with diets containing type C Tifton Bermudagrass and alfalfa hays, resulting in 2.71 ± 0.11 kg and 2.62 ± 0.12 kg, respectively. When we compared the forage type as a main effect, the animals that ingested alfalfa hay (FP alfalfa= 2.42 ± 0.10 kg) presented a lower FP in relation to the horses that ingested Tifton Bermudagrass hay (FP tifton= 2.58 ± 0.10 kg). In relation to the dietary DCDM, the diets composed by types A and B, with $64.7 \pm 2.38\%$ and $63.45 \pm 2.41\%$ values, significantly surpassed ($P= 0.0061$) the results obtained for diets with

Table 5. Means and standard deviation of fecal production (FP) and digestibility coefficient of dry matter (DCDM) for equines fed with isoproteic and isoenergetic diets, submitted to three Tifton Bermudagrass¹ and alfalfa² hay types (A, B and C).

| Variable | Tifton Bermudagrass Hay ¹ | | | Alfalfa Hay ² | | |
|-------------------------------------|--------------------------------------|---------------------|--------------------|------------------------------|--------------------|--------------------|
| | A | B | C | A | B | C |
| FP (kg in DM.day ⁻¹) | 2.54 ^{ab} | 2.48 ^{bc} | 2.71 ^a | 2.30 ^c | 2.35 ^c | 2.62 ^{ab} |
| | ± 0.35 | ± 0.36 | ± 0.42 | ± 0.24 | ± 0.23 | ± 0.17 |
| | Mean ¹ | | | Mean ² | | |
| | 2.58 ^A ± 0.39 | | | 2.42 ^B ± 0.26 | | |
| DCDM (%) | 61.88 ^{ab} | 62.16 ^{ab} | 59.30 ^b | 64.71 ^a | 63.45 ^a | 58.97 ^b |
| | ± 4.13 | ± 6.93 | ± 8.24 | ± 3.66 | ± 5.80 | ± 5.63 |
| | Mean | | | Mean ² | | |
| | 60.98 ± 6.85 | | | 62.44 ± 5.72 | | |

kg in DM.day⁻¹ = kilograms in dry matter per day; % = percentage; ^{a,b,c} Means with different letters on the same line differ ($P < 0.05$) among themselves by the Tukey test; ^{A,B} Means with different letters on the same line differ ($P < 0.05$) among themselves by the F test.

type C hays.

DISCUSSION

The BW values ([Table 3](#)) resultant from the consumption of Tifton Bermuda grass and alfalfa hays as a forage source by the horses confirmed that, in isoproteic and isoenergetic diets, the forage type interferes in the BW. Muhonen and Julliand (2023) observed a variation on the equine BW which might be explained by the lower intake of less-digestible fiber, resulting in less mass of undigested organic matter and, in total, less water in the large intestine.

In the present paper, the observed 18.52 kg weight loss corresponded to a 5.2% BW decrease. Similar results were obtained in a previous study using sedentary fistulated horses comparing the effect of feeding lucerne haylage, young grass haylage and the more conventional mature grass haylage and concentrate diet on the osmolality and viscosity of the equine hindgut digesta and the BW and fluid balance of the horse (MUHONEN, PHILIPPEAU and JULLIAND, 2022).

It is important to highlight that, in the paper published by Muhonen, Philippeau and Julliand (2022) the experimental diets composed by diet with early harvested and late harvested grass haylage with an 80:20 energy ratio resulted in reduction on the ingested NDF. On the present research, the diets containing alfalfa hay in comparison with the Tifton Bermudagrass hay provided a reduced NDF consumption of 1.1 kg. Thus, besides the reduction in NDF consumption, the diets with alfalfa hay presented a better fiber quality and, consequently, diminished the indigestible material content in the digestive tract. In this way, it can be considered that the best results over the BW resulted from the reduced NDF consumption and this fiber's superior nutritious quality, provided by the diets that contained alfalfa hay.

The abdominal girth result showed that forage type influence on this variable in equines eating isoproteic and isoenergetic diets ([Table 3](#)). In this sense, horses that consumed diets with alfalfa hay, independently of its type, resulted in a 2.78 cm reduction in this measurement. This situation can be explained by the lower NDF content in alfalfa hay when compared with the Tifton Bermudagrass hay ([Table 1](#)).

The absence of a statistic effect for the horses' water consumption can be justified by the fact that the animals received isoproteic diets, since the water ingestion is related to the protein content of the diets offered to the animals, according to

Oliveira et al. (2017). At the same time, by Muhonen, Philippeau and Julliand (2022) did not verify any difference on the water consumption by equines in diets constituted by different forage types. It is worth highlighting that the 15.7% CP mean content proposed to the experimental diets, promoting a 3.3 g CP/kg BW/ day estimated protein ingestion, is above 1.7 g CP/kg BW/day, but within the 3.4 g CP/kg BW/day maximal consumption, recommended by the NRC (2007). According to Oliveira (2013), the CP ingestion within these limits by athlete horses is important to avoid an increase in the daily water requirement due to the excessive metabolic nitrogen, which needs to be eliminated via urine.

In relation to the fecal water retention, Richardson and Murray (2016) reported that dietary fiber content has a direct effect over the fecal water content, due to fluid shift by the fibrous fraction present in the feces. Oliveira et al. (2015) affirmed that the fecal water retention increases with consumption of grass hay, what can justify higher FW mean for the feces resulting from the Tifton Bermudagrass hay ingestion (Table 4).

In addition, Ribeiro et al. (2011) verified an increase in the fecal water retention as the alfalfa started to be added to the diet, and justified that the fecal water content was influenced by the legume's higher crude protein content. Such explanation fails to justify what happened in the present research, because both groups of experimental diets, containing alfalfa or Tifton Bermudagrass hays, were corrected to be isoproteic (Table 2). In this sense, it can be affirmed that lower water retention in the feces of the horses which consumed alfalfa hay (Table 4) occurred due to the distinct composition of the forages fibrous fraction.

Water ingestion and fecal water content were quantified to verify if there were alterations resulting from the experimental diets and, consequently, if there was interference on the horses' body biometrics (BW and AG). Thus, analyzing the results for tables 3 and 4, it is possible to infer that the BW reduction and AG smaller measurement verified for the horses which consumed diets with alfalfa hay did not occur in function of water ingestion, but resulted from the reduction in fecal water retention, Richardson and Murray (2016) affirmed that the water retained in the animal's digestive tract provides a higher BW and a greater abdominal distention.

The information obtained from the serum total protein concentration (Table 4) in horses fed with Tifton Bermudagrass and alfalfa hays as a forage source made possible the statement that the equine blood volume remained unaltered, not being

influenced by the evaluated feeding. Therefore, the diet has not interfered in the animal's ability to maintain the fluid balance (DANIELSEN et al., 1995). A diet effect on TP concentration during exercise was shown with lower TP when horses were fed an ad libitum hay diet, indicating a larger movement of water from the gastrointestinal tract to the plasma volume compared to a limited hay diet (CAVALLINI et al., 2022).

The animals digest and utilize the components, such as protein and energy, of the diet low in fiber better than the high fiber content diets, then, the physical properties and chemical composition of forages have higher impact on BW than forage DM intake per se (MUHONEN, PHILIPPEAU and JULLIAND, 2022). The hay quality is inversely related to the forage NDF content, what agrees with the results presented in the current research. Moreover, the NDF values presented on table 1, associated to the table 5 results, reinforce the affirmation by Lewis (1985) that, usually, legume hays present a higher nutritional value and a greater digestibility than grass hays. Similarly, Grev et al. (2019) obtained a higher DCDM value for adult horses fed with alfalfa hay for reduced lignin (64.4%) compared to non-reduced lignin alfalfa hay (61.7%).

Through the association of the results from tables 3, 4 and 5 can infer that the lower values for BW and AG ([Table 3](#)) for equines fed with alfalfa hay in isoproteic and isoenergetic diets occurred due to the lower fecal water retention, as well as the reduced amount of indigestible mass in the digestive tract. Thus, it should be taken into consideration that the alfalfa hay ingestion as a dietary forage source resulted in a lower fecal production and promoted a greater dietary digestibility. In this sense, in the case of athletic horses, the performance can benefit from this nutritional management, which can become an important ergogenic strategy, since it decreases the ballast weight and abdominal volume in horses.

CONCLUSIONS

The ingested forage type influenced the horses' body biometrics. Independently from the quality, the alfalfa hay as a forage source in isoproteic and isoenergetic diets presented a higher digestibility in relation to the Tifton hay due to the reduction in the equine biometric parameters, such as body weight and abdominal girth.

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