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Palatability of teff grass by horses

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Palatability of Teff Grass by Horses

An Undergraduate Honors Thesis

in the

Animal Science Department

Submitted in partial fulfillment of the requirements for the

University of Arkansas

Dale Bumpers College of Agricultural, Food and Life Sciences

Honors Program

by

Rachel Cummins

April, 2014

Dr. Kenneth Coffey, Chair

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Dr. Nancy Jack

Dr. Dirk Philipp
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Abstract

Most forages commonly used to feed horses have potential detriments including blister beetles or excessive fiber concentrations. Teff grass (T), a warm-season annual forage, has the potential to be a good alternative for horses because of its lack of observed disorders. Our objective was to compare preference by horses for T harvested under different conditions with that of bermudagrass (B) harvested at two maturities. Six different forages were evaluated: T harvested at the late vegetative stage (TLV), at late bloom but that incurred 33 mm of rainfall between mowing and baling (TLBR), with caryopsis visible (TES), or at soft dough (TSD), and B harvested at late vegetative (BLV) and mid-bloom (BMB) growth stages. Five mature horses were used in a balanced incomplete block design where each horse received a different combination of 4 forages each day for 6 d. The 4 different forages were suspended in hay nets in each corner of each stall, and each hay was offered at 50% of the average daily hay consumption measured during a 10-d adaptation period. Forage preference as measured by individual forage DM consumption (kg and % of total DM consumed across the 4 forages) was greatest (P< 0.05) from TLV followed by BLV. Preference (kg and % of total DM consumed) of BMB was greater (P< 0.05) than that of TMBR, TES, and TSD, which did not differ from each other (P ≥ 0.63). Therefore, within a specific growth stage, horses apparently preferred teff grass, but effects of maturity and rainfall had a more dramatic effect on preference by horses than forage species.
Literature Review

Forage quality is very important to horse owners, and combines a variety of factors. When looking at forage quality, things animal owners need to consider include palatability, nutritive value and digestibility (Stanier et al., 2010). Palatability is very important when choosing a forage, and is the main focus of this study. Palatability may be affected by a variety of factors including, but not limited to, texture, aroma, succulence, leaf percentage, fertilization, sugar content, tannins, alkaloids, maturity and lignin content (Hoveland, 1996). While palatability may improve intake by the animal, it does not necessarily improve animal performance, and should not be used as a sole indicator of forage quality (Hoveland, 1996). Many horses show a preference for sugar and phosphorous (Stewart, 2005).

Rain damage can be harmful to palatability. Rain damage increased all fiber components excluding hemicelluloses in bermudagrass and orchardgrass (Scarborough et al., 2005) and increased the NDF content of legume hay (Collins, 1983). Intake by cattle was reduced by 10% in response to rain damage on forages (Coblentz, 2006).

Another factor that can affect palatability is maturity. Voluntary intake of teff grass has been found to be less in the late-heading maturities than early-heading and boot stage maturities (Stanier et al., 2010). In that study, voluntary intake appeared to be related to maturity as late-heading teff grass had the greatest NDF and ADF concentrations and boot stage teff grass had the lowest NDF and ADF concentrations. The early-heading teff grass was not different from the late-heading teff grass or the boot stage teff grass in NDF concentrations (Stanier et al., 2010). This suggests that an increase in fiber content is related to a decrease in palatability. Advancing maturity also had a negative effect on the voluntary intake of alfalfa hay (Darlington & Hershberger, 1968).
Alfalfa hay is a common horse forage. Voluntary DM intake of alfalfa by yearling horses was greater than that of coastal bermudagrass and matua bromegrass (LaCasha et al., 1999). Earlier maturities of alfalfa hay have been found to have a greater nutritive value than timothy hay or orchardgrass harvested at comparable maturities. However, the quality of alfalfa hay deteriorates rapidly as it matures, allowing timothy hay to have a greater nutritive value in the more mature forages (Darlington & Hershberger, 1968).

Alfalfa hay is commonly contaminated with blister beetles which emit a chemical called cantharidin, when the beetles are either crushed or handled roughly. Cantharidin is a blistering agent that causes necrosis and ulceration of tissue lining the digestive and renal systems of horses and can also cause colic (Echevarria & Hooser, 2006). A single blister beetle can produce enough cantharidin to kill a horse, and veterinary care for a horse that has ingested cantharidin can be very costly and often ineffective. Cantharidin is very stable in hay and does not decompose over time (Ward, 1997) necessitating disposal of contaminated hay. Alfalfa hay is often used with caution because of the possibility of blister beetles.

Bermudagrass is also a popular forage for horses in some parts of the country. It has greater resistance to rain damage compared with orchardgrass (Scarborough et al., 2005), but bermudagrass is often associated with lower digestibility compared with other forages (Coleman et al., 2003). High temperatures and humidity increase the maturity rate of bermudagrass, causing the thickening of cell walls, which reduces digestibility (Ditsch & Lacefield, 2009). In many instances, the percentage of digestible dry matter of bermudagrass may not exceed 45% (Aiken et al., 1989), and bermudagrass generally contains less CP when compared with alfalfa hay (Sturgeon et al., 2000).
Teff grass is warm-season annual forage recently introduced in the United States from Ethiopia and Eritrea. Teff grass has already gained popularity in the western United States as a horse forage, especially as a forage for horses with metabolic disorders and obesity (Anonymous, 2012). It does not have any observed insect problems, and does not mature as rapidly as bermudagrass (Miller, 2010). Teff grass has lower levels of non-structural carbohydrates compared to cool season forages (Stanier et al., 2010). Teff grass has also been found to be a low input crop, meaning that it can be grown in most locations without insecticides or fungicides (Miller, 2010).

The good qualities of teff grass combined with the detriments of other common horse forages give it the potential to be a viable alternative to bermudagrass and alfalfa hay. Few studies have been conducted examining the palatability of teff grass compared with other common horse forages. The objective of this study is to determine palatability by horses of teff grass when compared with bermudagrass, both at different maturities.

Introduction

Teff grass is warm-season annual forage recently introduced in the United States from Ethiopia and Eritrea. Teff grass has already gained popularity in the western United States as a horse forage, especially as a forage for horses with metabolic disorders and obesity (Anonymous, 2012). Teff grass has the potential to be a viable alternative to other popular horse forages because of its lack of potential disorders. Alfalfa hay is a popular horse forage, but it is commonly contaminated with blister beetles which emit a chemical that can be fatal if consumed by horses (Echevarria & Hooser, 2006). Bermudagrass is another widely-utilized horse forage, however it often has problems with low digestibility due to rapid maturity (Coleman et al.,
Teff grass does not mature as rapidly as bermudagrass (Miller, 2010), and does not have any observed insect problems. Teff grass is lower in non-structural carbohydrates compared with cool-season forages (Stanier et al., 2010), thereby giving it potential as an alternative forage for horses. To be a contender as a replacement of bermudagrass and alfalfa hay, teff grass must first be established as a forage that horses will willingly consume. The purpose of this study is to determine the palatability of teff grass relative to that of bermudagrass at different maturities.

Materials and Methods

All procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol no.13055). Teff grass (T) was planted at the University of Arkansas Watershed Research and Education Center (WREC) according to recommended practices on May 29, 2013. A comparable field of bermudagrass (B), a perennial warm-season grass, was also chosen to provide B hay for comparison with T. The field of B was harvested June 15 and baled for hay to initiate the regrowth process in an attempt to have both forages reaching comparable maturities under similar growing conditions. Both B and T were harvested beginning in late June. The forages included in the study were: T harvested at the late vegetative stage (TLV), T harvested at late bloom but that incurred 33 mm of rainfall between mowing and baling (TLBR), T harvested when the caryopsis was visible (early seed stage; TES), T harvested at soft dough (TSD), B harvested at the late vegetative stage (BLV) and B harvested at the mid-bloom (BMB) growth stage. All forages were allowed to dry in the field to a maximum of 20% moisture and packaged in small-rectangular bales. All bales were stored inside a metal enclosed shed until subsequent feeding.
Five mature horses (511 ± 17.4 kg BW), 2 to 10 yr of age, were housed individually in stalls (3.7 × 3.7 m) at the DEK Equine facility for a 12-d adaptation period followed by a 6-d forage preference evaluation. During the adaption period, the horses were offered BMB and TES. Initially horses were offered 1% of their body weight of each forage divided equally into 2 hay bags. This resulted in a total of 2% of body weight from each forage offered in 4 different hay bags. The bags were placed at random in each corner of their stall, and the amount offered increased daily based on consumption. Triangular tarps were suspended beneath each hay bag to catch forage that was pulled from the bags but not consumed. The average daily DM consumption (ADC) for each horse was determined during the last 5 d of the adaptation period.

The preference portion of the experiment immediately followed the adaptation period and utilized a balanced incomplete block design (Plan 11.6 from Cochran and Cox, 1957) that was repeated twice. The original design was for 3 d, with each horse offered a total of 4 of the 6 forages each day. By repeating the design twice, we were able to offer each forage in combination with each other forage at least twice, and each forage was offered to each horse a total of 4 times during the 6-d period. Each horse had a different combination of 4 forages from each other horse, and the combinations were changed daily based on the experimental design (Figure 1). In order to account for any idiosyncrasies, a number of factors were considered and randomized. First, horses were allocated to a different stall each day based on plans for 5 × 5 Latin Squares with one extra period. This resulted in each horse being housed in each stall at least one day during the study and in only 1 stall a second time. Secondly, the specific corner in which a particular forage was offered was randomized such that the particular forage was offered in all 4 corners of a stall for each individual horse. Each forage was offered at a rate of one-half of the total average daily consumption during the last 5 d of the adaptation period. This is done
to ensure that the horses selected from and established a preference ranking for at least two of the forages each day. For example, if the total consumption of both B and T by horse “X” was 10 kg during the last 5 d of the adaptation period, then horse “X” was offered 5 kg of each of the 4 experimental forages.

Horses were given 2-h exercise periods twice daily in the morning at 0630 and in the evening at 1930. During the morning exercise period, orts were removed and weighed and new forages were placed in the stalls. Each stall door also had a fan to ensure horses were not overheated. Stalls were bedded in sand and cleaned twice daily. No grain was offered during the adaptation period or trial period. Horses had unlimited access to water, even during the exercise periods.

Samples of each hay were taken daily at the time the hay bags were filled and were dried to a constant weight at 50°C. Unconsumed hay was collected daily, weighed, and a representative sample was dried to a constant weight at 50°C. Hay samples from each forage were maintained separately for each day and were ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and analyzed for neutral-detergent fiber, acid-detergent fiber, and acid-detergent lignin (Vogel et al, 1999).

Consumption data were analyzed using PROC GLM of SAS (SAS Institute, Cary, NC). The model included the effects of horse, forage, day, stall, and corner. The effect of stall was included to ensure that location in the barn was not having an effect. The effect of corner was included to determine if horses preferred to consume forages out of a favorite corner. Stall affected ($P < 0.05$) each of the consumption measurements, but corner and day of study did not ($P \geq 0.56$) affect any of the consumption measurements. Therefore the final consumption model included effects of forage, stall, and horse. Means are reported as least-squares means. Pearson
correlation coefficients were also determined among consumption measurements and forage quality analyses using PROC CORR of SAS. The forage quality measurements from each forage on each individual day were matched with consumption of that particular forage on a given day for correlation analyses.

**Results and Discussion**

Weather data affecting the forages in the present study are presented in Table 1. When compared with the 30-yr averages, May of 2013 was relatively wet. This delayed the planting of the teff grass. June of 2013 was unusually dry, which allowed the late vegetative forages to be baled under ideal conditions. However, the dry June also led to issues with growing the later maturities of the forages. Our original intention was to have 3 different maturities each of B and T. However, due to the slow growth rate, only 2 maturities of B were available because of field size limitations. August of 2013 had a greater rainfall compared with the 30 yr average, which delayed the baling of TLBR, TES and TSD. The TLBR also incurred 33 mm of rain damage between mowing and baling.

Forage quality measurements are presented in Table 2. The NDF concentration of TES, TSD and TLBR were not different \((P \geq 0.40)\) from each other, but were greater \((P < 0.05)\) than the NDF concentrations of the other forages. The NDF concentrations of BMB and BLV were greater \((P < 0.05)\) than those of TLV. The greater NDF concentration of TLBR suggests that the rain damage removed soluble components, resulting in NDF concentrations similar to that of a more mature forage. The TES and TSD forages also had the greatest \((P < 0.05)\) ADF concentrations. These are followed by TLBR \((P < 0.05)\). The two maturities of B and TLV were not different from each other \((P \geq 0.14)\), and had the lowest \((P < 0.05)\) ADF concentrations.
Lignin concentrations of TES, TSD and TLBR were greater ($P < 0.05$) than those from BLV and TLV. Lignin concentrations of TSD and TLBR are also not different ($P \geq 0.18$) from the lignin concentrations of TES or BMB. A previous study reported that rain damage increased all fiber components excluding hemicellulose in B and orchardgrass (Scarborough et al., 2005).

Preference of the different hays by horses was expressed in three ways: kg of dry matter consumed per day (kg/d; Figure 2), the amount of each forage consumed as a percentage of the amount of that particular forage offered (% offered daily; Figure 3), and the amount of each forage consumed as a percentage of the total DM intake by each horse (% of DM intake; Figure 4). Preference (kg/d) was greatest ($P < 0.05$) for TLV followed by BLV ($P < 0.05$). The least preferred ($P < 0.05$) forages were TLBR, TES and TSD. The low preference for TLBR, and the fact that the preference for TLBR was not different ($P \geq 0.63$) from that of TES and TSD suggests that the rainfall was just as damaging to preference as the increased maturity of TES and TSD. A study in cattle reported a 10% reduction of intake in response to rain damage on forages (Coblentz, 2006). Preference expressed as a percentage of the total amount offered daily was greatest ($P < 0.05$) for BLV and TLV. The later maturities of T including TLBR were the least preferred forages ($P < 0.05$). This again suggests that the rainfall on TLBR was just as damaging to preference as increasing maturity. Preference expressed as a percentage of the total DM intake was greatest ($P < 0.05$) for TLV. Consumption of TLV was slightly above 50% of the DM intake for horses, which suggests that horses consumed all of the TLV offered, since each forage was offered at half of the estimated ADC. Preference was least ($P < 0.05$) for TLBR, TES and TSD, once again suggesting that rain damage and advanced maturity are equally detrimental to preference by horses.
Forage concentrations of NDF and ADF were both highly and negatively correlated with preference ($P < 0.05$; Table 3). Lignin content was also highly and negatively correlated with preference ($P < 0.05$), but not as highly correlated as NDF and ADF. Hemicellulose content was not correlated with preference ($P \geq 0.11$). In a previous study (Staniar et al., 2010), voluntary intake of T was less from late-heading maturity than from early-heading and boot stage maturities. Concentrations of NDF and ADF were greatest from the late-heading T, lowest from the boot stage T, and intermediate from the early-heading T, which was not different from the late-heading T or the boot stage T in NDF concentrations (Staniar et al., 2010). These results are consistent with the results of our study, which demonstrate that an increase in maturity is detrimental to palatability, and that preference appears to follow closely with NDF and ADF concentrations.

Conclusion

When given a choice of different forages, horses preferred late-vegetative teff grass. However, forage maturity had a larger effect on preference than forage species when forages were compared across different maturities. This conclusion is drawn based on the relatively small difference in preference between bermudagrass and teff grass harvested at a comparable maturity, but a very large negative effect of maturity on preference of both forages. It is also apparent that rain damage can be just as detrimental to palatability as increasing maturity as preference for teff grass harvested at the late bloom stage was never different from preference for the later maturities of teff grass. Strong negative correlations among preference and NDF and ADF support the use of these measures to estimate preference by horses. Therefore, teff grass is
palatable to horses, but forage maturity and rain damage are more important factors affecting palatability than forage species.

Acknowledgements

Thank you to Ashley Young for assistance with laboratory analyses, and thanks to Mandi Goodall and Kelsey Rogers for helping with general horse care during the project. I would like to thank the University of Arkansas Honors College and the Dale Bumpers College of Agricultural, Food and Life Sciences for financial assistance on this project. I would also like to thank the D.E. King Equine Program for the use of their facilities and horses.

Literature Cited


Table 1. Weather data during the growing period for teff grass and bermudagrass in 2013

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
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<tr>
<td>Avg. Temp. Max., °C</td>
<td>22.7</td>
<td>29.5</td>
<td>30.8</td>
<td>29.8</td>
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</tr>
<tr>
<td>Rainfall, cm</td>
<td>26.7</td>
<td>3.6</td>
<td>8.7</td>
<td>15.5</td>
<td></td>
</tr>
</tbody>
</table>

**30-year avg.**

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
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<tr>
<td>Avg. Temp. Min., °C</td>
<td>13.3</td>
<td>18.3</td>
<td>20.6</td>
<td>20.0</td>
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<tr>
<td>Avg. Temp. Max., °C</td>
<td>24.4</td>
<td>28.9</td>
<td>31.7</td>
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<tr>
<td>Rainfall, in.</td>
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<td>12.1</td>
<td>8.2</td>
<td>7.7</td>
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</table>
Table 2. Harvest dates and forage quality measurements of forages offered to horses in a palatability study.  

<table>
<thead>
<tr>
<th>Item</th>
<th>BLV</th>
<th>TLV</th>
<th>TLBR</th>
<th>BMB</th>
<th>TES</th>
<th>TSD</th>
<th>SEM</th>
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</thead>
<tbody>
<tr>
<td>Date baled</td>
<td>1-July</td>
<td>28-June</td>
<td>18-Aug.</td>
<td>2-Aug.</td>
<td>24-Aug.</td>
<td>24-Aug.</td>
<td></td>
</tr>
<tr>
<td>NDF, %</td>
<td>67.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>73.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86</td>
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<tr>
<td>ADF, %</td>
<td>28.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.60</td>
</tr>
<tr>
<td>Hemicellulose, %</td>
<td>39.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.68</td>
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<tr>
<td>Lignin, %</td>
<td>2.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.33</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means within a row without a common superscript letter differ (P < 0.05).  
<sup>2</sup>BLV = bermudagrass late vegetative; TLV = teff grass late vegetative, TLBR = teff grass late bloom with rain damage, BMB = bermudagrass mid-bloom, TES = teff grass with caryopsis visible, TSD = teff grass soft dough stage  
<sup>3</sup>NDF = neutral detergent fiber; ADF = acid detergent fiber  
<sup>4</sup>SEM = standard error of mean
Table 3. Pearson correlation coefficients relating forage quality measurements to palatability by horses across different forages

<table>
<thead>
<tr>
<th>Item 1</th>
<th>DM consumption per forage, kg/d</th>
<th>DM consumption, % of offer</th>
<th>DM consumption, % of total DMI</th>
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<tr>
<td>NDF, %</td>
<td>-0.73</td>
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<td>-0.72</td>
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<tr>
<td>p-value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<td>ADF, %</td>
<td>-0.75</td>
<td>-0.76</td>
<td>-0.74</td>
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<td>p-value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Hemicellulose, %</td>
<td>0.13</td>
<td>0.15</td>
<td>0.13</td>
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<tr>
<td>p-value</td>
<td>0.14</td>
<td>0.11</td>
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<tr>
<td>Lignin, %</td>
<td>-0.55</td>
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<td>-0.55</td>
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<tr>
<td>p-value</td>
<td>&lt;0.01</td>
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1NDF = neutral detergent fiber; ADF = acid detergent fiber
<table>
<thead>
<tr>
<th>Day</th>
<th>Stall 1</th>
<th>Stall 2</th>
<th>Stall 3</th>
<th>Stall 4</th>
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<tr>
<td>Monday 1</td>
<td>Petal</td>
<td>Sport</td>
<td>Des</td>
<td>Dailey</td>
<td>Pride</td>
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<td>Dailey</td>
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<td>Sport</td>
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<td>Des</td>
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<td>B</td>
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16
Forage Intake, kg/d

Forages and Growth Stages

BLV  TLV  TLBR  BMB  TES  TSD

BLV: Forage Intake, kg/d
TLV: Forage Intake, kg/d
TLBR: Forage Intake, kg/d
BMB: Forage Intake, kg/d
TES: Forage Intake, kg/d
TSD: Forage Intake, kg/d
Forage intake, % of DM offered

Forages and Growth Stages

BLV  TLV  TLBR  BMB  TES  TSD

Note: Different letters indicate significant differences.
Forage Intake, % of total DM intake

Forages and Growth Stages

- BLV
- TLV
- TLBR
- BMB
- TES
- TSD
Figure 1. Stall and corner layout for a study to evaluate the palatability of teff grass and bermudagrass harvested at different maturities. Forages were A – teff grass with the caryopsis visible; B – teff grass harvested at soft dough; C – teff grass harvested at late bloom that received 33 mm of rainfall; D – bermudagrass harvested at mid-bloom; E – bermudagrass harvested at the late vegetative stage; F – teff grass harvested at the late vegetative stage. Each horse’s name is in the center cell of each block.

Figure 2. Intake (kg/d) of teff grass and bermudagrass harvested under different conditions and offered to horses in combinations of 4 different forages each day for 6 days. Forages offered were bermudagrass late vegetative (BLV), teff grass late vegetative (TLV), teff grass late bloom with rain damage (TLBR), bermudagrass mid-bloom (BMB), teff grass with caryopsis visible (TES), and teff grass soft dough stage (TSD). Bars without a common superscript are different ($P < 0.05$).

Figure 3. Intake of teff grass and bermudagrass harvested under different conditions and offered to horses in combinations of 4 different forages each day for 6 days. Intake is expressed as a percentage of a particular forage offered. Forages offered were bermudagrass late vegetative (BLV), teff grass late vegetative (TLV), teff grass late bloom with rain damage (TLBR), bermudagrass mid-bloom (BMB), teff grass with caryopsis visible (TES), and teff grass soft dough stage (TSD). Bars without a common superscript are different ($P < 0.05$).

Figure 4. Intake of teff grass and bermudagrass harvested under different conditions and offered to horses in combinations of 4 different forages each day for 6 days. Intake was expressed as a percentage of the total daily dry matter offered. Forages offered were bermudagrass late vegetative (BLV), teff grass late vegetative (TLV), teff grass late bloom with rain damage (TLBR), bermudagrass mid-bloom (BMB), teff grass with caryopsis visible (TES), and teff grass soft dough stage (TSD). Bars without a common superscript are different ($P < 0.05$).