A COMPARISON OF CHAFFHAYE AND ALFALFA HAY ON DIGESTIBILITY AND GLUCOSE METABOLISM IN MATURE STOCK-TYPE HORSES

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Abstract

The objectives of the study were to evaluate the effects of Chaffhaye and alfalfa hay on digestibility and glucose metabolism. Ten geldings (13.8 ± 8 yr, 553.2 ± 81 kg) were used in a cross-over design. Periods consisted of 21 d for adaptation, 1 d of blood collection and 4 d of fecal collection. Horses were fed at 1.9% of their BW/d (as fed) of either Chaffhaye (CHAF; 92% DM, 22.7% CP, 3.8% Crude Fat, 32.9% NDF and 25.35% ADF) or alfalfa hay (ALF; 42% DM, 15.5% CP, 1.7% Crude Fat, 48.1% NDF and 40.1% ADF). Blood collections were taken at 0, .5, 1, 2, 4, 6 h after a meal on day 22 and feces were collected every 6 h with a subsample frozen for analysis. Glucose (GLU) and insulin (INS) parameters were evaluated for area under the curve (AUC) and for the highest value (PEAK). Dry matter intake for horses receiving ALF was higher ($P < 0.01$) for OM, NDF, ADF, CP, NFC, WSC, and ESC. Starch and crude fat intake were higher ($P \leq 0.02$) for CHAF receiving horses. Water intake was insignificant ($P = .41$). There was a tendency for horses to have a lower insulin PEAK and AUC when fed CHAF ($P \leq .11$). Both GLU AUC and GLU PEAK were insignificant ($P \geq .55$). Wet fecal output per day was higher for horses consuming ALF ($P < .01$). Digestibility for ALF was higher for DM, OM, ADF and NDF ($P \leq .01$). Crude protein and crude fat digestibility was higher for CHAF ($P \leq .02$). NFC digestibility was not different ($P = .84$) between treatments. Therefore, ALF had higher digestibility in all parameters except CP and crude fat, and CHAF elicited a lower insulin response that could be helpful for metabolically-prone horses.

Keywords: Horse, Digestibility, Haylage, Metabolites

1. Introduction

Many feedstuffs were created to solve a problem in the industry, whether it is to make life easier for the horse owner, to increase nutrients to a horse in competition, to reduce gastric or metabolic disorders that occur in the horse, or to preserve forages while they are growing to be fed in the winter months of a northern climate. Fermented feeds have been seen in horses to have a significantly higher DM, ADF, NDF and CP digestibilities when compared to dried hay [1,2]. Further, the ensiling process has been shown to lower WSC concentrations in silage when compared to dried hay [3,4,2]. This study was conducted to compare the fermented feedstuff, Chaffhaye, and dried alfalfa hay in terms of digestibility when fed to horses and their subsequent effects on metabolites in the horse.

2. Materials and Methods

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee, and the study was conducted at the New Mexico State University Equine Education Center. Ten stock-type mature geldings were used in a crossover design where the two groups were stratified by age and weight. Prior to the study, horses were dewormed with Durvet Ivermectin Paste, 1.87 % (Durvet, Blue Springs, MO). Dentition was also checked before the study, and any abnormalities found were corrected. The geldings used in the study had an average age of 13.8 ± 8 y and an average weight of 553.2 ± 81 kg. Horses were fed at 1.9 % of BW on an as fed basis (to simulate the method of feeding commonly used by horse owners) and the diets were divided into equal portions to be fed in 12 h intervals (0700 and 1900). Horses were weighed utilizing Purina Weight Tape at the first day of each collection period to determine the diet level for each animal. Horses were housed individually in 8 x 4 m stalls with rubber mat floors. Horses were turned out in a sand arena for 2 h each day of the acclimation period. The forage type (treatment) was weighed each day and forage samples were taken by grab sample every week and combined into one sample per period. Orts were collected before each feeding and recorded. Horses were allowed ad libitum access to a trace mineral block (Champion's Choice Trace Mineral Salt 50 lb Block, Tractor Supply) and fresh water. Other than a mineral block, the forage treatment composed 100 % of the diet.

The two treatments were dried alfalfa hay (ALF) and haylage (CHAF) that were harvested from irrigated fields in Dell City, Texas, and provided by Chaffhaye, Incorporated. The dried hay was harvested with a field mower, sun-dried and then square-baled with a conventional baler. The haylage was harvested by field mower, then cane molasses and dried fermentation product of *Pediococcous pentosaceus* and *Propionibacterium freudenreichii* were added to the forage and sealed in a plastic bag [5]. Samples were collected every week of each period of the study and were combined to determine an average composition of the forage for that period. Forage samples analyzed by Dairy One Forage Testing Laboratory (Ithaca, NY). Forage samples were analyzed for DM, CP, crude fat, ash, pH (Methods 930.15, 990.03, 2003.05, 942.05, 973.41, respectively; AOAC, 1990), NDF, and ADF (ANKOM, Fairport, NY). Ethanol soluble carbohydrates and WSC were analyzed using a method developed by previous research [6]. Nutrient analyses for both forages are given in Table 1.

Table 1

Nutrient analysis of Chaffhaye[1] (CHAF) and alfalfa hay (ALF).

	Treatments			
	Period 1		Period 2	
Nutrient (%)	ALF	CHAF	ALF	CHAF

DM	92.5	39	91.5	45
OM	89.24	86.65	89.24	86.65
NDF	52.5	33	43.7	32.7
ADF	44.9	24.8	35.2	25.9
CP	14.3	23.8	16.6	21.6
Crude Fat*	1.7	3.8	1.7	3.8
Ash*	10.76	13.35	10.76	13.35
NFC[2]	20.74	26.05	27.24	28.55
Starch	0.6	2.7	1.1	2.6
WSC	8.3	4.8	8	5.2
ESC	5.6	2.4	7.6	3
TDN	56	63	58	63

[1]Alfalfa haylage with cane molasses and dried fermentation product of *Pediococcous pentosaceus* and *Propionibacterium freudenreichii* in a sealed bag, Dell City, Texas Chaffhaye, Incorporated.
[2]NFC= 100% - (CP % + Crude Fat % + Ash % + NDF %) (values on a DM basis).
*Forage analysis done on compiled forage sample from both periods

2.1. Experimental Collections

The study was conducted in two periods. Each period consisted of 26 d; horses were allowed a minimum of 21 d of acclimation to each treatment suggested by previous research [7]. The horses were subjected to a frequent sampling I.V. blood collection the day after the completion of the 21 d acclimation period. The geldings were fed 12 h prior to beginning the blood sampling. Catheters were inserted in the jugular vein of the horse 30 minutes before the first blood collection. Horses were fed their 0700 meal directly after a baseline (0) blood sample was taken. Venous blood samples were drawn at 30, 60, 120, 240, and 360 minutes after the meal was offered and were immediately deposited in sterile, 5.0 mL BD Vacutainer blood tubes (BD, Franklin Lakes, NJ). Samples were centrifuged at 1000 x *g* for 20 min. Serum was removed from samples directly after being centrifuged and frozen at -20°C until later analysis.

Horses were fitted with a fecal collection bag during the final 4 d of each period and feces were collected every 6 h. Feces from each horse were weighed individually and after mixing by hand, a 5 % subsample by weight was preserved in plastic freezer bags. All fecal samples were frozen at -20 °C immediately after collection for later analysis. Fecal samples were weighed and dried at 55 °C in a forced-air oven for 48 h, air-equilibrate and weighed again. The four fecal samples from each day from each horse were combined into a representative 24 h sample. Horses were not allowed any turnout during fecal collection.

Water intake (not considering water from diet) was recorded he final 4 d of each period. Tap water was provided to each horse in two 20-quart plastic buckets. Water intake as well as water refusals were recorded morning, afternoon, and evening directly after fecal collection. Buckets were emptied and refilled each morning to ensure horses had access to fresh water.

2.2. Laboratory Analyses

Forage and fecal samples were sent to be analyzed at a commercial laboratory (Dairy One Forage Testing Laboratory, Ithaca, NY). Forage and Fecal samples were analyzed for DM, CP, crude fat, ash, pH (Methods 930.15, 990.03, 2003.05, 942.05, 973.41, respectively; AOAC, 1990), NDF and ADF (ANKOM, Fairport, NY). ESC and WSC were analyzed using a method developed by previous research [6].

Glucose and insulin concentrations via serum samples were sent for analysis by North Dakota State University Animal Science Laboratory. Glucose concentrations were determined by colormetric assay which has been used in previous equine research [8]. Insulin concentrations were evaluated utilizing the Immulite 1000 (Siemens Healthcare Diagnositics, Tarrytown, NY) which utilizes assay-specific, antibody or antigen-coated plastic beads as a solid phase, alkaline phosphate-labeled reagent, and a chemiluminescent enzyme substrate. A photomultiplier tube detected light emission and printed reports for each sample were generated by the system computer. All analyses of serum insulin concentrations were performed in duplicate. This method of evaluating serum insulin level in horses has been utilized by previous research and in clinical setting [9,10]. Further, GLU and INS were analyzed using area under the curve (trapezoidal summation).

2.3. Statistical Analysis

Data was evaluated using the mixed procedure in SAS version 9.4 (SAS Inst. Inc., Cary, NC) where the fixed effects were treatment, period and horse. There was no random effect. Treatment effect was considered significant when $P < .05$. Tendencies were also considered from $.05 < \alpha \leq .15$.

3. Results

The moisture content of the CHAF (avg. of 42 % DM) resulted in a notable difference in average DM intake of nutrients when compared to horses fed ALF (avg. 92 % DM) Therefore, intake of DM (9.66 vs. 4.48 kg/d), OM (8.62 vs. 3.87 kg/d), NDF (4.65 vs. 1.47 kg/d), ADF (3.87 vs. 1.14 kg/d), CP (1.49 vs. 1.01 kg/d), NFC (2.32 vs. 1.23 kg/d), WSC (0.79 vs. 0.22 kg/d), ESC (0.64 vs. 0.12 kg/d), TDN (5.51 vs. 2.82 kg/d), and ASH (1.04 vs. 0.60 kg/d) was significantly higher for ALF than CHAF ($P < .0001$). Even with the lower absolute amount of nutrients that the horses on CHAF received, fed starch was significantly higher for horses fed CHAF when compared to ALF (0.12 vs. 0.08 kg/d; $P < .0001$) for the CHAF than the ALF. Similarly, fed crude fat was significantly higher (0.17 vs. 0.16 kg/d; $P = .02$) in CHAF than ALF (Table 2).

Table 2

Dietary intake of nutrients, fecal analysis and apparent digestibility of nutrients in mature, stock-type horses fed Chaffahaye[1] (CHAF) and alfalfa hay (ALF).

	Treatments			
Item	ALF	CHAF	SE[2]	*P*-value[3]
n	10	10	-	-
Intake, kg/d				
DM	9.660	4.4676	0.1465	<.0001
OM	8.621	3.871	0.1334	<.0001
NDF	4.649	1.467	0.0863	<.0001
ADF	3.871	1.135	0.0742	<.0001
CP	1.492	1.010	0.0165	<.0001
Crude Fat	0.164	0.170	0.0014	.0228
NFC[4]	2.316	1.225	0.0346	<.0001
WSC	0.788	0.224	0.0153	<.0001
ESC	0.637	0.122	0.0144	<.0001
Starch	0.082	0.118	0.0016	<.0001
TDN	5.506	2.815	0.0778	<.0001
Ash	1.040	0.597	0.0132	<.0001
Water Intake, L	51.532	48.352	2.7255	.4132
Fecal, kg/d				
DM	3.598	1.861	0.0679	<.0001
OM	3.269	1.609	0.0635	<.0001
NDF	2.112	0.929	0.0601	<.0001
ADF	1.626	0.718	0.0472	<.0001
CP	0.463	0.292	0.0060	<.0001
Crude Fat	0.149	0.101	0.0076	.002
NFC[4]	0.546	0.288	0.0240	<.0001
Ash	0.328	0.252	0.0101	.0007
pH	8.550	7.945	0.0648	.0002
Digestibility, %				
DM	62.672	58.034	0.5775	.0005
OM	62.003	58.239	0.5327	.0011
NDF	53.967	36.786	1.3818	<.0001
ADF	57.106	36.904	1.3484	<.0001
CP	68.719	71.056	0.5585	.0182
Crude Fat	9.367	39.881	4.7742	.002
NFC[4]	76.160	75.741	1.3905	.8369

[1]Alfalfa haylage with cane molasses and dried fermentation product of *Pediococcous pentosaceus* and *Propionibacterium freudenreichii* in a sealed bag, Dell City, Texas Chaffhaye, Incorporated.
[2]SE = (standard error) comparison within row.
[3]P– value = comparison within row.
[4]NFC= 100% - (CP % + Fat % + Ash % + NDF %) (values on a DM basis).

Glucose and INS parameters were evaluated in terms of area under the curve (AUC), base line (resting) and for the highest value recorded for each animal (PEAK) and results are displayed on Table 3. Mean intra-assay CVs were 7.29 for equine insulin and between 6.1 and 7.9 for the control inta assay CV. Insulin AUC had a tendency to be less (*P* = .08) for horses fed

CHAF than ALF. There was a tendency for horses to have a lower INS PEAK when fed CHAF vs. ALF (19.29 vs. 24.28 µlU/mL, respectively; *P* = .11). Both GLU AUC and GLU PEAK (ALF: 85.35, CHAF: 83.43 mg/dL) were not significant between the two treatments (*P* = .55 and *P* = .54, respectively). Furthermore, by analyzing the 0 time point of GLU and INS, a resting GLU and INS was measured. There was a tendency for horses fed CHAF to have a lower resting serum INS level than horses fed ALF (10.49 vs. 19.24 µlU/mL, respectively; *P* = .13). There was also a tendency for horses fed CHAF to have a lower resting GLU level then horses fed ALF (72.80 vs. 75.37 mg/dL, respectively; *P* = .10).

Table 3

Glucose (GLU) and insulin (INS) PEAK and baseline[6] in response to a Chaffhaye[1] (CHAF) and alfalfa hay (ALF) meal of all horses[5].

	Treatments			
Item	ALF	CHAF	SE[3]	*P*- value[4]
All Horses[5]				
GLU PEAK (mg/dL)	85.35	83.43	2.10	.54
INS PEAK (µlU/mL)	19.29	24.28	1.94	.11
Baseline GLU (mg/dL)	75.37	72.80	3.24	.13
Baseline INS (µlU/mL)	19.24	10.49	0.99	.10

[1]Alfalfa haylage with cane molasses and dried fermentation product of *Pediococcous pentosaceus* and *Propionibacterium freudenreichii* in a sealed bag, Dell City, Texas Chaffhaye, Incorporated.
[2]Generally accepted as insulin resistant according to previous research (Frank et al., 2006).
[3]SE = (standard error) comparison within row.
[4]*P*– value = comparison within row.
[5]All Horses = All horses that blood collection was performed on (n = 9)
[6]Baseline= Resting glucose and insulin concentration

Average daily fecal output as well as specific nutrient fecal outputs were calculated for each treatment. The average fecal output per day was significantly higher for ALF (*P* < .0001) than CHAF (16.253 vs. 6.915 kg per day, respectively; Table 4). Further, fecal DM (3.60 vs. 1.86

kg/d), OM (3.269 vs. 1.61 kg/d), NDF (2.11 vs. 0.93 kg/d), ADF (1.63 vs. 0.72 kg/d), CP (0.46 vs. 0.29 kg/d) and NFC (0.55 vs. 0.29 kg/d) output were significantly higher for ALF (*P* < .0001). Compared with CHAF, fecal crude fat (0.15 vs. 0.10 kg/d) and fecal pH (8.55 vs. 7.95 kg/d) was significantly higher for ALF than CHAF (*P* = .002) as well as fecal ash (*P* = .0007; Table 2).

Apparent total tract digestibility was determined for each nutrient. The percent total tract digestibility for ALF was significantly higher for DM (62. 67 vs. 58.03 %; *P* = .0005), OM (62.00 vs. 58.24 %; *P* = .0011), NDF (53.97 vs. 36.78 %, *P* < .0001) and ADF (57.12 vs. 36.90 %; *P* < .0001) when compared to CHAF. In contrast, CP (71.06 vs. 68.72 %) and crude fat (39.88 vs. 9.37 %) total tract digestibility was significantly higher for CHAF than ALF (*P* = .02, *P* = .0020, respectively). Lastly, NFC digestibility was not significant (ALF: 76.16 vs. CHAF: 75.74 %; *P* = .84) between treatments (Table 2).

Table 4

Average intake on an as fed basis and average wet fecal output of horses fed a diet of Chaffhaye[1] (CHAF) and alfalfa hay (ALF).

	Treatments			
Item	ALF	CHAF	SE[2]	*P*- value[3]
Average Intake (As Fed, kg)	10.5040	10.616	0.08265	.37
Average Wet Fecal Output (kg)	16.253	6.915	0.4627	<.0001

[1]Alfalfa haylage with cane molasses and dried fermentation product of *Pediococcous pentosaceus* and *Propionibacterium freudenreichii* in a sealed bag, Dell City, Texas Chaffhaye, Incorporated.
[2]SE = (standard error) comparison within row.
[3]*P*– value = comparison within row.

Water intake was recorded over the last 4 d of each period. There was no significant difference (*P* = .41) in water intake from the bucket between either treatment (*P* > .41). Regardless, the horses on CHAF had a numerically lower mean intake than the horses fed ALF

(48.35 vs. 51. 53 L/d, respectively; Table 2). Total daily water intake from the buckets as well as from the feed also had no difference between treatments (*P* = .55). Yet, horses fed ALF had a numerically lower mean intake than horses fed CHAF (52.37 vs. 54.63 L/d, respectively).

4. Discussion

The forage analysis revealed that even though both the CHAF and ALF were to be harvested from the same fields, the hay provided by the Chaffhaye company for the project contained a differing nutrient profile. There is support of the ensiling process altering the nutrient profiles of nearly identical forages [3,2,11]. The forage analysis for the project revealed that the CHAF had significantly lower (*P* < .0001) WSC than ALF. It has been shown that the ensiling process lowers WSC concentrations in silage when compared to dried hay [3]. Further, ensiling has also been shown to affect CP content of a feedstuff. Of silages and hay collected at the same time from the same field, there were still differences in crude protein content of the feedstuffs as hay had a lower crude protein content than the silage (155 and 167 g/kg DM, respectively) [2]. The average percent NDF and ADF found in the forage analysis of CHAF (32.85 % and 25.35, respectfully) is less than that of ALF (48.1 % and 40.05, respectfully). This may be due to the fermentation process. Neutral detergent fiber has been seen to have a higher concentration in hay than in silage when the same forages are cut simultaneously [11].

The horses being fed ALF received a significantly (*P* < .0001) larger average daily DM intake (9.6602 kg) when compared to the average daily DM intake of the CHAF (4.4676 kg; Table 2). Horses on CHAF received more crude fat and starch, but had a lower fed amount of the other nutrients analyzed. This could be advantageous to horse owners that want to feed a higher fat diet. The Chaffhaye, Inc.'s feeding instructions found on the bag states to feed 1.5 lbs of Chaffhaye per hunderedweight of the horse and did not specify if this was on a DM or as fed basis or if it should be fed in with any other feedstuffs. This would suggest that owners feed a 454.5 kg horse 6.82 kg per day on an as fed basis. This would further imply that Chaffhaye suggests feeding a 454.5 kg horse only 2.86 kg per day on a DM basis per day (42 % average DM % of CHAF). For this project, horses received a higher DMI per day; a 454.5 kg horse received 8.635 kg of feed on an as fed basis per day and 3.626 kg on a DM basis. This feeding level of CHAF did not meet the digestible energy for a horse at maintenance. During this time, the horses' weight and body condition score were monitored. No horses moved up or down a BCS while on either treatment, this is likely due to the horses' low daily activity level (only 2 h of turnout each day) and that horses were on each treatment (forage) for only 26 d.

It was found that serum GLU parameters were statistically insignificant between treatments. This could be due to the fact that insulin in the blood was effective at maintaining a consistent blood glucose level in the horses or even the low NSC concentrations of the feed.

Though CHAF had a higher starch content than ALF (Period 1: 2.7 and 0.6 %, respectively; Period 2: 2.6 and 1.1 kg, respectively) and horses on CHAF received minimally more kg of starch per meal than ALF (0.118 and 0.082 kg, respectively), there was a trend for a lower INS AUC and PEAK response for the CHAF (P = .07) and INS peak (P = .11). This could be a result of the higher WSC and ESC percent on a dry matter basis of ALF than CHAF (Period 1: 8.3 and 4.8 %; 5.6 and 2.4 %, respectively; Period 2: 8 and 5.2 %; 7.6 and 3 %, respectively). Horses on the study received a higher absolute amount (kg) of WSC and ESC on a diet of ALF than CHAF (0.7788 and 0.224 kg; 0.637 and 0.122 kg, respectively). High amounts of WSC and ESC can increase blood glucose levels and subsequently blood insulin levels that could eventually lead to insulin resistance (Storlien et al., 2000). Furthermore, this tendency for horses fed CHAF to have a lower INS AUC and PEAK could be due to the less DM horses on CHAF received.

There was a significantly reduced fecal output of horses fed CHAF, this is likely due to the highly reduced intake of animals consuming CHAF. This could also be due to the water absorbing capacity of fiber as ALF had a higher structural carbohydrate content adding to the weight of the fecal matter. Further investigation is needed to discern weather CHAF, fed at the same DM basis, would have a lower fecal output. Even if CHAF had slightly lower fecal output, it could result in hundreds of kg less of fecal output a year. Chaffhaye's reduced fecal output seen in this study could save horse owners time and money in cleaning manure and help reduce environmental effects of fecal matter.

The dry matter digestibility (DMD) of CHAF is significantly less (P = .0005) than ALF. The average DMD of ALF is 62.67 % when compared to CHAF at 58.03 %. Because of the multitude of differences in form, the treatments were presented in, differences in moisture, particle size, DM feeding level etc. It is impossible to ascertain with certainty the specific cause(s) of the lower DM and OM digestibility seen in the CHAF. It is possible, however, to speculate. The lower DM digestibility found in the CHAF could be a response of the high moisture content found in the CHAF (42 % DM). The high degree of moisture could have allowed the feedstuffs to move more rapidly though the digestive tract, resulting in a lower MRT and thus less time for nutrient absorption [12,13].

Additionally, as CHAF has been reduced in particle size though the process of chopping before being preserved, the smaller particle size could result in a quicker passage rate of the CHAF and thus, a reduced opportunity in digestion and absorption, ultimately result a lower DMD. A quicker MRT of a feedstuff in the GI tract has been seen to result in a lower DMD [14,15,16].

Both CP and crude fat are largely digested and absorbed in the foregut of the horse. Because CHAF is a fermented product, this could have allowed more availability of these nutrients in the foregut of the horse compared to ALF. Hypothetically, the ALF then had a longer MRT in the hindgut of the horse due to the larger particle size and lower moisture content allowing for more total digestion and absorption of the other nutrients [17,12,18,19]. Thus, leading to higher digestibilities of the fiber contents of the ALF feed.

The use of a marker may have elucidated a more clear and accurate theory behind the digestibility differences of the two feedstuffs [20]. Ideally, the use of a marker (such as Cr-EDTA, Co-EDTA, yeterbium chloride among others) would have been utilized to determine MRT and passage rate of particles in the study and would be warranted for future research on the subject.

Overall, there were differing advantages in feeding both treatments in terms of nutrient digestibility. These differing advantages in nutrient digestibility may be appropriate for certain equine facilities and certain horses. Furthermore, more research should be conducted to determine the metabolic effects of the feedstuffs. Through this study, horses had a tendency to have a lower AUC, baseline and PEAK insulin response to the CHAF. Therefore, CHAF may be a feedstuff that is more appropriate for horses that are prone to or currently suffering from metabolic diseases.

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