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Evaluation of Nutrient Content of Water-Soaked Southeastern Grass Hays as a Means to Limit Carbohydrates Consumed In Equine Diets

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• Hay; Carbohydrates; Laminitis

Abstract

Objective: The objective of this study was the evaluation of a time effect for soaking warm-season grass hays on the decrease of the soluble carbohydrates content, as well as on other nutrients. Often, hay is soaked to remove soluble carbohydrates to make a safer feed source for horses. This study specifically evaluated warm season grass hays grown in the southeastern United States.

Samples: Twenty separate Bermuda grass hay samples.

Procedures: The hay samples were divided into three groups: two treatment groups (A and B) and a control group (C). Group C was not soaked, Group A was soaked in water for 12 hours. Group B was soaked in water for 30 minutes. All groups were dehydrated and sent to Dairy One Laboratory to be analyzed for nutrient content utilizing Near Infrared Reflectance spectroscopy.Statistical analysis of the data was performed with a p value less or equal to 0.05 was considered significant.

Results: A significant difference was found when between group C (control group) and the treatment groups A and B for the following nutrients: DE, CP, lysine, NDF, WSC, ESC, ash, P, Mg, K, Zn, Mn and RVF. Soaking decreased the WSC content 41.5% and 26.9% and the ESC by 65.8% and 56.5% for samples in groups A and B, respectively.

Conclusions: Soaking hay for either 30 min or 12 hours is able to had a significantly reduced the amount of CP, NDF, WSC, ESC, ash, P, Mg, K, Zn, Mn, DE and RVF but did not significantly reduce the amount of lignin, ADF, starch, NFC, CF, Ca, Na, Fe, Cu, and Mo.

Clinical relevance: Soaking southeastern grass hays for 30 min or 12 hours significantly reduces WSC and ESC content.

INTRODUCTION

Forage is the basis of livestock diets. Although grains and lush pasture are traditionally blamed for laminitis and its associated diseases, hays can contribute as well. Hays which are high in water-soluble carbohydrates (WSC) and starch can contribute to obesity, laminitis, insulin resistance, and metabolic syndrome in horses [1-4]. The WSCs in hay are mostly ethanol-soluble carbohydrates (ESC) and fructans (poly and oligosucrosyl fructose). The simple sugars: glucose, fructose, and sucrose are all ESCs. Reducing the sugar intake of metabolically sensitive animals can be accomplished by hay soaking or through other means such as mowing, appropriate fertilization of soil, irrigation, hay harvesting conditions, controlled grazing and weed control [2,3].

In order to remove some of the WSCs from hay before feeding, owners have soaked hay in water [4,5]. Hay soaking has also been conducted to reduce airborne dust during feeding and to simulate the effects of rainfall on grasses [5,6]. Several studies have been conducted in order to find the effects hay soaking has on carbohydrate removal [5-9]. There are multiple methods described for water temperature and soaking time [5-9]. In a study by Martinson et al,it was reported that the temperature of the water had little to no effect on the removal of carbohydrates from alfalfa hays but did have an effect on Orchard grass hays.[8] There is less of a consensus on the appropriate soaking time among these studies [5-9]. Short soaking times such as; 15, 30, 60 min, remove less WSCs than longer soaking times such as 12 hours [6-8]. Longer soaking timescan also reduce the dry matter (DM) content of the hay considerably reducing its value [6-8]. These previous studies did not analyze grass hays produced in the southeastern United States and only contain a few varieties of grass [6-8]. Hence, our goal was to determine if soaking southeastern hays in water for 30 min or 12 hours would have a significant effect on nutrient content, especially soluble carbohydrates, WSC and ESC. We hypothesized that there would be a significant decrease in the amount of WSC along with other nutrients when soaking hay for 30 min and for 12 hours and that the 12 hours soak would result in a greater decrease in WSC, ESC, and nutrient content when compared to the 30 min soak.

MATERIALS AND METHODS

Experimental design

Twenty hay samples were harvested via a Best Harvest BHP550Chay probe sampler (Best Harvest; Largo, FL)[a] from farms in the southeastern United States were collected at a hay auction. The twenty samples consisted of 14 mixes of Bermuda grasses, three Coastal Bermuda grasses, one Timothy/ Orchardgrass mix, one Bahia/Coastal Bermuda mix, and one Russell Bermuda grass sample. The half-pound hay samples were divided into three aliquots: two treatment groups and a control group. The hay samples were placed into labelled, gallon sized ziplock bags where they remained for the duration of the study. The three groups in the study were designated as follows: Control (C), Treatment group A (A), Treatment group B (B). The Control group did not undergo any soaking. Group A was soaked in ambient temperature 65-72°F water for 12 hours. Group B was soaked in ambient temperature at 65-72 °F water for 30 minutes. Following soaking, All Groups A, B, and C were dried in a dehydrator (Model 778, L.E. M. Products, Harrison, OH)[b]and then sent to Dairy One Laboratory (Ithac, NY)[c] to by analyzed. Dairy One Laboratory[b] analyzed the samples utilizing Near Infrared Reflectance spectroscopy (NIR) (Foss Model 6500 Full Scanning Monochromator, Silver Springs, MD)[d]. Comparisons of the individual components of the hay in groups A, B, and C before and after soaking were made and recorded. A statistical analysis of the data was performed utilizing a repeated measures analysis of variance (ANOVA)(PROC MIXED, SAS, version 9.3, SAS Institute Inc, Cary NC)[e]along with a Tukey's pairwise comparison test to analyze the effect of different soaking times on specific nutrient content in the hays. A p-value was calculated for each nutrient analyzed and a p-value less than or equal to 0.05 was considered significant and the null hypothesis was rejected.

Soaking

The hay sampleswere stored in a controlled environment at a temperature of 68-72° F until treatment. The samples in group A were soaked in three liters of tap water with an approximate temperature of 55°F for 12 hrs. The samples in treatment group, B, were soaked in three liters of tap water with a temperature of approximately 55°F for 30 min. Three liters of water were added to the gallon ziplock bags to ensure the hay was completely submerged. Both soaks occurred during the evening, with the 12-hour soak lasting overnight. The samples in group C (control group) were not soaked.

Dehydration

Following the soaking procedure, the samples in groups A and Bwere dried in the dehydrator[a] for 5.5 hours at 120ºF and submitted to the Dairy One Laboratory[c] (Ithac NY) for nutrient analysis. The samples in group C were not soaked but dehydrated with a dehydrator[a] for 5.5 hours at 120ºF. The samples were then submitted to the Dairy One Laboratory[c] (Ithac, NY) for nutrient analysis.

Near infrared reflectance spectroscopy

Dairy One Laboratory[c] analyzed the samples for nutrient content utilizing near infrared reflectance spectroscopy[d] (NIR). This is a sophisticated technique for rapidly analyzing common forages and grains. Near infrared reflectance spectroscopy[d] is the official method of analysis by the Association of Official Analytical Chemists (AOAC). This technique analyzes the feed, in this case the hays, by utilizing spectral properties of the multiple nutrients in the feed simultaneously. This type of spectroscopy is calibration dependent and the calibration was built for each sample. Correlations of the chemical information contained in the samples were then compared to known spectral properties contained in similar samples. Dairy One Laboratory (Ithac, NY) [c] utilizes LOCAL® calibration software developed by Infrasoft International LLC(Port Matilda, PA, USA)[f]. The nutrients in the hay analyzed by NIR included; digestible energy (DE), crude protein (CP), lysine, neutral detergent fiber (NDF), WSC, ESC, ash, phosphorus (P), magnesium (Mg), potassium (K), zinc (Zn), manganese (Mn), relative feed value (RFV), lignin, acid detergent fiber (ADF), starch, non-fiber carbohydrates (NFC), crude fat (CF), calcium (Ca), sodium (Na), iron (Fe), copper (Cu), and molybdenum (Mo).

Statistical analysis

This was a prospective trial with the soaking times being the independent variables and the dependent variables being the amount of the analyzed nutrients in hay prior to soaking (group C) and following soaking (Groups A and B). In this project, the within-subject repeated measures ANOVA[d] along with the Tukey's pairwise comparison test was applied to see the effect of soaking time on specific nutrient content in the hays analyzed. Tukey's method considers all possible pairwise differences of means at the same time. There were two treatment groups, A (12 hr soak) and B (30 min soak), with one control group (C) in this study. There were 20 hay samples in each group. Samples in the control group were not soaked (0 soaking time). A p-value was calculated for each nutrient analyzed within the treatment groups. A p-value less or equal to 0.05 was considered significant. The null hypothesis for this study was: that all the groups A, B and C have the same mean value, or specifically, there is no significant effect of soaking on the nutrient content of the hays of the different groups examined. The alternative hypothesis was that at least one of the groups has a different mean value, or specifically, the effect of soaking in water for 30 min or 12 hours in tap water does have a significant effect on the nutrient content of hays.

RESULTS

A significant difference was found between the amount of nutrients found in the samples in group C (control group) and the samples in the treatment groups, A and B, for the following

Table 1: The table below lists the nutrients analyzed in the hay and the range of the quantities of these nutrients in the Control group, and the treated groups following soaking; A (12 hour soak), B (30 min soak). The amounts of the nutrients bolded where found to be significantly different following soaking.

unit	Minerals	Control; mean (range)	A (12 hr): mean (range)	B (30 min): mean (range)
Mcal/kg	Digestible Energy	2.31 (1.81-4.25)	1.90 (1.8-2.29)	1.93 (1.83-2.11)
g/kg	Crude Protein	111.26 (58.2-295.42)	76.96 (46.7-116)	82.95 (52.8-122)
g/kg	Lysine	1.85 (0.9-4.7)	2.67 (1.6-4)	2.88 (1.8-4.2)
g/kg	Neutral Detergent Fiber	897.73 (700.63-1714.1)	735.645 (597.5-796)	718.38 (625.3-766.6)
g/kg	Ash	78.34 (41-190.92)	50.09 (34-75.9)	52.96 (34.7-72.9)
g/kg	Phosphorus	1.74 (0.79-4.89)	0.88 (0.5-2.08)	1.2 (0.55-1.95)
g/kg	Magnesium	3.76 (1.85-8.27)	1.69 (1.01-2.37)	2.20 (1.18-3.34)
g/kg	Potassium	15.54 (5.56-37.28)	4.26 (2.14-7.31)	6.07 (1.85-12.2)
g/kg	Zinc	33.40 (13.23-57.32)	26.05 (13-52)	25.2 (11-40)
g/kg	Manganese (Mn)	225.2 (63.93-582.02)	127.45 (47-192)	145.65 (46-262)
no unit	Relative Feed Value	74.35 (65-82)	71.5 (65-96)	74.2 (65-89)
g/kg	Lignin	62.6 (36.38-132.28)	82.44 (41-422.4)	60.22 (45.9-86.7)
g/kg	Acid Detergent Fiber	493.53 (351.19-985.46)	400.73 (63-452.9)	407.62 (361.6-455.1)
g/kg	Crude Fat	23.35 (9.26-44.53)	22.17 (15.7-33.3)	21.25 (14.8-33.7)
g/kg	Calcium	4.65 (2.47-8.73)	3.79 (2.77-5.42)	3.95 (2.57-5.35)
g/kg	Sodium (Na)	0.12 (0.03-0.35)	0.13 (0.07-0.62)	0.12 (0.07-0.22)
g/kg	Iron (Fe)	178.13 (63.93-429.9)	183.20(80-577)	160.6 (75-407)
g/kg	Copper (Co)	10.25 (4.41-19.84)	9.25 (6-18)	9 (6-12)
g/kg	Molybdenum(Mo)	0.58 (0-1.54)	0.39 (0-4.2)	0.34 (0-3.8)
%	Water Soluble Carbohydrates	8.99 (4.9-13.8)	5.56 (1.7-10.5)	8.3 (5.3-12.2)
%	Ether Soluble Carbohydrates	5.84 (3.4-9.8)	2.43 (0.2-4.8)	3.1 (0.2-7.4)
%	Starch	1.76 (0.2-4.8)	2.3 (0.2-10.1)	1.77 (0.2-7.3)
%	Non-Fiber Carbohydrates	10.77 (5.6-14.8)	11.53 (4-23.4)	12.47 (6.6-19.6)
%	NSC	10.74 (6.8-15.8)	7.86 (3.6-18.4)	10.06 (5.5-15.8)

Table 2: The table below shows the summary of the result for the within subject repeated measures ANOVA analysis for the nutrients significantly affected by soaking and those nutrients that were not significantly affected by soaking in water.

Nutrient significantly affected by soaking time	Nutrient not significantly affected by soaking time with Group p value
Digestible Energy; p = 0.0352	Lignin; p = 0.256
Crude Protein; p = 0.00475	Acid Detergent Fiber; p = 0.0636
Lysine; p = 0.0001	Starch; p = 0.561
Neutral Detergent Fiber; p = 0.0144	Non-Fiber Carbohydrates; p = 0.467
Water Soluble Carbohydrates; p = 0.0001	Crude Fat; p = 0.61
Ether Soluble Carbohydrates; p = 0.0001	Calcium; p = 0.0631
Ash; p = 0.0005	Sodium; p = 0.843
Phosphorus; p = 0.000189	Iron; p = 0.413
Magnesium; p = 0.0001	Copper; p = 0.377
Potassium; p = 0.0001	Molybdenum; p = 0.293
Zinc; p = 0.0108	
Manganese; p = 0.0001	
Relative Feed Value; p = 0.035	

nutrients: DE, CP, lysine, NDF, WSC, ESC, ash, P, Mg, K, Zn, Mn, and RFV. These results are highlighted in Tables 1-3. The range and the median values of the samples are reported in Table 1. There was a significant difference in RFV between the A group and C group but not between B group and C group (Table 2&3.). The analysis of the amount of DE was significantly different using the repeated measures ANOVA when comparing the three groups together (Table 2). Soaking hay decreased the WSC in the hay by 42.9% and 7.7%for groups A and B, respectively. The effect of soaking on ESC was more profound with a decrease of 58.4% and 46.9%of ESC in the hay for groups A and B, respectively.

There was no significant difference found between nutrient content between A and B groups for CP, lysine, NDF, WSC, ESC, ash, P, Mg, K, Zn, and Mn. Additionally, there was no significant difference found between group C and the treatment groups, A and B, or between groups A and B for the following nutrients; lignin, ADF, starch, NFC, CF, Ca, Na, Fe, Cu, and Mo. The null hypothesis should be rejected and the alternated hypothesis accepted for DE, CP, lysine, NDF, WSC, ESC, ash, Ph, Mg, K, Zn, Mn, and RVF (Table 2&3). The null hypothesis should be accepted and the alternate

Table 3: The table below shows the summary of Tukey's pairwise comparison test involving the nutrients affected by soaking time. The mean value of the group C (control) and group A (12 hour soaking time) and group B (30 minutes soaking time) differ from each other.

Nutrients significantly affected by soaking time	The groups which have different mean value from each other
Digestible Energy	(Only Control vs Group is significant; p =0.0352)
Crude Protein	(Control vs A; p = 0.00301), (Control vs B; p = 0.01823)
Lysine	(Control vs A; p = 0.0001), (Control vs B; p = 0.001)
Neutral Detergent Fiber	(Control vs A; p = 0.0311), (Control vs B; p = 0.0143)
Water Soluble Carbohydrates	(Control vs A; p = 0.001), (Control vs B; p =0.00126)
Ether Soluble Carbohydrates	(Control vs A; p = 0.0001), (Control vs B; p = 0.0001)
Ash	(Control vs A; p = 0.001), (Control vs B; p = 0.0123)
Phosphorus	(Control vs A; p = 0.0001), (Control vs B; p = 0.0107)
Magnesium	(Control vs A; p = 0.0001), (Control vs B; p = 0.0001)
Potassium	(Control vs A; p = 0.0001), (Control vs B; p = 0.0001)
Zinc	(Control vs A; p = 0.0249), (Control vs B; p = 0.0101)
Manganese (Mn)	(Control vs A; p = 0.0001), (Control vs B; p = 0.0043)
Relative Feed Value	(Control vs A; p = 0.0426), (Control vs B; p = 0.9912)

hypothesis rejected for lignin, ADF, starch, NFC, CF, Ca, Na, Fe, Cu, and Mo (Table 2). Even though a significant difference was not found when evaluating starch, a soak of 30 min decreased the starch content in the hay by 9.4%. The NSC content in hays were found to be decreased by26.8% and 6.3% for groups A and B, respectively. The range of NSC of the hays evaluated was 6.8% to 15.8% and the average NSC was 10.0% on a DM basis prior to soaking. After soaking, the NSC of the hays were on average 7.9% and 10.1% for groups A and B, respectively.

DISCUSSION

For horses, it is very important to prevent excessive intake of highly digestible carbohydrates. This paper reinforces that soaking hay in water can aid in the removal of WSC and ESC. Starch and WSCs together comprise NSCs which affect the glycemic index of the forage [10]. Ruminant nutritionists often analyze NFC. Non-fiber carbohydrates include NSCs, pectic substances, and organic acids. Since organic acids and peptic substances have minimal impact on the glycemic index, they are of less concern to the equine nutritionist [11]. Therefore, the emphasis is to measure NSC, or more specifically WSC and starch, when analyzing forages to feed horses [4,7].

Watts and Chatterton detailed the factors affecting NSC accumulation in common forage grasses and the differences in both cool season and warm season grasses [4]. Cool season grasses (C3 plants) tend to store fructan while warm season grasses (C4 plants) tend to store starch. The cool season grasses accumulate more NSC, especially in the stem bases with cooler temperatures, drought conditions, and low nitrogen or phosphorus levels [10]. Cool season grasses are able to readily metabolize fructan into transportable simple sugars. Cool season grasses tend to pose a greater threat of induction of laminitis than warm season grasses because the sugars they contain are more bio available. The warm season grasses store starch, which is less readily converted into transportable simple sugars. However, in time starch will be converted to sugars and so hays with increased starch content can add to the glycemic index. Therefore, warm season grasses have potential to result in laminitis as well.

The WSCs in hay are mostly ethanol-soluble carbohydrates (ESC) and fructans (poly and oligosucrosyl fructose). The simple sugars: glucose, fructose, and sucrose are all ESCs. Reducing the sugar intake of metabolically sensitive animals can be accomplished by hay soaking or through other means such as controlled grazing, mowing, appropriate fertilization of soil, irrigation, harvesting conditions, and weed control [2,4].

In this study, soaking hay in water for 30 min or 12 hours did significantly reduce WSC and ESC but not starch. The grass hays analyzed in this study were warm season hays. One could conclude that soaking cool season hays in water may have more benefit than soaking warm season hays due to the greater amount of starch in warm season hays. The average range of WSC and ESC in the hays evaluated in this study was 4.9-13.8% and 3.4-9.8%, respectively (Table 1). Soaking warm season grass hays can have a beneficial effect by significantly reducing WSC, and ESC. Decreasing the amount of sugar and bio available carbohydrates can help reduce the hay's negative effects on the glycemic index. [7,9] Thereby preventing problems such as obesity, laminitis, insulin resistance and/or metabolic syndrome (EMS) [1-4,9,13]. Horses that have been diagnosed with EMS and/or having laminitic episodes should be fed hay with a WSC of 10% (100g) or less or if possible a NSC to be 100g or less [1]. The ESC and the starch together should also be 10% (100g) or less [1,13]. In the current study, the soaking of the hay decreased the WSC by about 43% and 8% for treatment A and B, respectively. The amount of ESC was decreased by 58.4% and 46.9% for treatment A and B, respectively. The differences seen in the decrease of WSC and ESC point to the solubility of fructan found in the WSC portion. Certain nutrients found in water may significantly affect the rate of the solubility of substances within the hay such as fructan.

The data in Table 1 were reported as dry matter (DM). The sampled hays were treated or not treated and then dehydrated and assessed for nutrients following dehydration. All reported nutrient values of the hay refer to their contents in DM. In this case WSC in control samples may ranges from 50-100 g/kg DM and from 20-105 and 50 to 120 g/kg for treatment A and B, respectively, which implies that following soaking some hays will not attain safe levels of WSC. Additionally, there is a possibility of southeastern hays to contain ESC of 9.8% (98g) even following a 12 hr soak, as was found in this study. If this level of ESC was combined with starch having a percentage of 0.2 to 10.1 then the combination could also result in a level of 100g or above. Thus, leading one to conclude that soaking does not always result in hays that are safe to feed. If the hay is still greater than 10% (100g) NSC following soaking then additionally steaming may be necessary to lower the soluble carbohydrates to a more acceptable level [13]. Additionally, a recent study reported that soaking can lead to increases in microbial population of the hay leading to a more hasty decomposition of hay and possible alteration of gut micro flora [18]. This study found that soaking increased Colony Forming Units (CFU) of bacteria per g of DM from 6.0×10^4 in dry hay up to 3.5×10^5in soaked hay [13,8].

The approach to reducing sugar and starch content of grass hays can be maximized not only through soaking but by implementing appropriate management of growth, fertilization, harvesting and testing of forages [2,4,9]. A multifactorial approach is best when managing the feeding of metabolically sensitive animals [2,4,9].

Additionally, it is important to test forages to manage feeds correctly especially those that have high levels of starch and sugar. Many of these grass hays were less than 10% NSC and might not pose a problem but some were as high as 15.8% NSC with a mean of 10.74% so testing warm season grass hays is important when feeding metabolically sensitive animals. For the most accurate analysis of forage, the lab should measure mono and disaccharides and fructans, which composes the sugar fraction. A separate enzymatic digestion should be utilized to quantify the starch [4]. It isvitally important to understand that NFC and NSC are not synonymous . Non-fiber carbohydrates are the portion of a diet or feed that is made up of different amounts of starch, simple sugars, beta-glucans, galatans, organic acids and pectins. However, NSC is the measure of only the starches, sugars, and fructans in the feed or forage. The beta-glucan, galactans, organic acids, and pectins are not included in the NSC measurement and represent the difference in these two measurements. Citrus pulp and Beet pulp are good examples of a feeds to illustrate the difference between NFC and NSC. The NFC of citrus pulp and beet pulp is 30.08 and 64.7 and the NSC is 35.9 and 19, respectively, making beet pulp a low glycemic index fiber source [12,14]. Than citrus pulp. There are a large amount of beta-glucans and galactans and pectins in citrus pulp and beet pulp increasing the percentage of NFC in these feedstuffs [15]. Acid detergent fiber is a measure of lignin and cellulose and is a predictor of digestibility in rations [14]. Lignin is the non-digestible portion of the plant that forms the outer cell wall and functions to channel water into the plant, which would explain why ADF and lignin did not decrease significantly following a 30 min or 12 hour soak. Besides lignin, ADF, starch, and NFC, CF, Ca, Na, Fe, Cu, and Mo were not significantly decreased by soaking for either length of time. There are minerals, Ca, Na, Fe, Cu and Mo as well as others, in the tap water utilized to soak the hay [16-17]. Therefore, thisis potentially the reason that there was not a significant decrease in the amount of these minerals seen in the hay samples post-soaking. Crude Fat is not soluble in water. So understandably, CF did not decrease following soaking.

In this study, CP was decreased by soaking at 30 min or 12 hours. These results are in contrast to a recent study in the United Kingdom (UK) which performed 16 hour soaks with and without agitation in warm and cold water [18]. They reported no significant losses of CP with any of the hays soaked [18]. They also stated that soaking in warm water plus agitation versus cold water, plus or minus agitation, had a greater reduction in the amount of WSC in the hays soaked. It may be that the UK hays are structured differently than grass hays in the US that the CP is not easily lost in the soaking process. Additionally, the tap water in the US may play a bigger factor on the CP in the hays than originally thought. The reduction in CP ultimately has an effect on the percentage of NFC. This study did not show a significant decrease in NFC but if a greater number of samples were evaluated then a significant effect on NFC might possibly have been detected.

Soaking for 12 hours did significantly decrease RFV but not soaking for 30 min. The RFV is a prediction of feeding value that combines estimated intake, NDF, and estimated digestibility, ADF, into a single index. The index of RFV is utilized in evaluation of hay and is used as a measure of the quality of the hay especially when evaluating legume hays. There was no significant decrease in ADF following soaking which most likely had an impact on why RVF was not significantly decreased following a 30 min soak. A decrease in RVF is not considered a desirable effect of soaking hay in water, nor is a decrease in DE, CP, P, Mg, K, Zn and Mn. However, a decrease in K might be beneficial when feeding horses with hyperkalemic periodic paralysis (HYPP) and anionic rations to pre-partum dairy cattle to reduce hypocalcemia. Alfalfa is high in K. In areas where alfalfa is abundant and fed to dairy cattle, soaking alfalfa could decrease the K and allow for a decrease in the feeding of anionic salts. Anionic salts are unpalatable. Hence, a decrease in anionic salts would result in a more palatable ration, better compliance by the cows and a decrease in hypocalcemia post-partum. For horses with HYPP or genetically predisposed for HYPP, limit feeding alfalfa and feeding grass hays is suggested [9]. Grass hays are lower in K but soaking can further lower the K resulting in safer forage to feed to HYPP horses [9].

The DE was significantly lowered by soaking as well, which could be desirable in horses that are on a calorie restricted diet to manage obesity. A 30 min soak would be indicated rather than the 12 hour soak if maintaining RFV, DE, P, Mg, K, Zn and Mn is desired. It would have been interesting to see if a 15 min soak would have been adequate to significantly decrease WSC and ESC without negatively affecting the amount of DE, P, Mg, K, Zn and Mn in the hay.

The Tukey's pair wise comparison test was utilized in addition to the repeated measures ANOVA because all observations were independent among the groups, the groups associated with each mean where normally distributed and there was a homogeneity of variance across the groups. Tukey's test was primarily implemented due to the multiple comparisons being made to prevent a Type I error.

SUMMARY

Soaking hay in water for 30 min or 12 hours does not differ significantly in effect accept for RVF where soaking 12 hours is necessary to produce a significant affect in southeastern grass hays. Soaking hay for 30 min seems to provide the most beneficial effect to reduce carbohydrate levels (WSC, ESC) in southeastern hays while minimizing other detrimental effects of soaking such as decreased nutrient content and the potential for increased microbial population with prolonged soaking protocols. However, soaked grass hays can still contain 100g of WSC that are above the suggested content. Hence, hays should be accurately analyzed prior to feeding, soaked and steamed if deemed necessary to reduce the soluble sugar content in grass hays to help prevent laminitic episodes in susceptible horses.

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FOOTNOTES

a. Best Harvest BHP550Chay probe sampler, Best Harvest; Largo, FL

b. Dehydrator, Model 778, L.E. M. Products, Harrison, OH

c. Dairy One Laboratory, Ithaca, NY

d. Near Infrared Reflectance spectroscopy, Foss Model 6500 Full Scanning Monochromator, Silver Springs, MD

e. PROC MIXED, SAS, version 9.3, SAS Institute Inc, Cary NC

f. LOCAL® calibration software developed by Infrasoft International LLC, Port Matilda, PA

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