Effect of Diet on Cortisol Concentrations in Response to Feeding Stress in Horses

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ABSTRACT

Stress can be detrimental to animal welfare and to the people reliant upon the animal because of the ability of stress to negatively affect feed up-take, growth, and reproduction. It is important to identify and understand the sources of persistent stress for both the benefit of the animal and people who are dependent upon its wellbeing. If a change in cortisol, the major corticosterone in horses and the physiological indicator of stress, is observed around feeding, it could have an impact on the way horses are fed and lend further insight into behavioral tendencies of the horse. In addition, the nature of the diet may be a contributing factor to the physiological stress experienced during these feedings. In this study, six mature Quarter Horses were used in a 6x6 Latin square design incorporating six 7-d feeding periods. The horses received commercial diets that varied in sugar/starch and fat/fiber content. Horses also received 2% of their body weight in grass hay each day and had ad libitum access to water. Jugular catheters were placed prior to sampling on day 7 of each feeding period. Blood samples were taken at -30, 0, 30, and 60 min before and after the morning meal for the determination of serum cortisol concentrations. There were no correlations between the diet fed and the amount of stress induced. However, there was a difference in cortisol concentrations in relation to time before or after the morning meal. The horses were showed an increase in cortisol concentrations 30 minutes prior to the morning meal in comparison to 30 minutes after the morning meal ($p < 0.05$).

INTRODUCTION

Stress can negatively affect animal welfare. The physiological role of stress is to induce a fight-or-flight response under the most extreme circumstances, specifically by increasing energy availability. A series of rapid changes in the nervous, cardiovascular, immune, and endocrine systems are precipitated by this bodily reaction. The cardiovascular system is induced to produce increased heart rates and stroke volume. The immune system is invigorated to prepare for the possibility of impending injury and, other systems which do not contribute to the fight-or-flight response, are shut down. Feed in-take, growth, and reproduction are among the bodily systems which are impaired during a time of stress (Evans et al., 1977).

When stressors are acute, the body is likely to return to a normal and productive state. However, a persistent state of stress can be detrimental to the animal and thus, to the people reliant upon the functioning of that animal. It is important to identify and understand the sources of persistent stress for both the benefit of the animal and those people who are dependent upon its wellbeing. Once the sources are identified, steps can be taken to eliminate or control these stressors.

It is appropriate that cortisol is an indicator of stress as it has been identified as the major corticosteroid in horse plasma, versus other hormonal indicators of stress such as cortisone, corticosterone and deoxycorticosterone which are present in

reduced amounts. (Evans et al., 1977). When analyzing cortisol concentrations as an indicator of stress, it is first important to understand the natural fluctuation and activity of cortisol concentrations within the horse. Cortisol levels adhere to a circadian rhythm with the highest values occurring in the morning from 6:00AM to noon, and lowest values reaching a minimum from 4:00PM to midnight (Evans et al., 1974, Hoffsis et al., 1970, Larsson et al., 1979, Bottoms et al., 1972). However, these values may be based on the seasonal and geographical light-dark rhythms (Stull, 1988).

Another factor concerning cortisol which is particularly relevant to this study is its role in digestion. Cortisol plays a role in initiating gluconeogensis, energy metabolism and mobilizing amino acids and fatty acids in tissue. (Stull, 1988). Perhaps changing the nutrient composition of the feed itself will contribute to a change in cortisol levels.

This study researches cortisol concentrations around set feeding times, taking into account the natural rise and fall in concentrations as well as cortisol's mechanism of action. If a change in cortisol is observed around feeding, it could have an impact on the way horses are fed and lend further insight into behavioral tendencies of the horse. In addition, the nature of the diet may be a contributing factor to the physiological stress experienced during these feedings, potentially impacting the nutritional realm of the horse industry.

The objective of this study was to determine the effect of diet on cortisol concentrations of six mature Quarter Horses.

MATERIALS AND METHODS

Animals and Treatments

Six mature Quarter Horses of varying ages, three mares and three stallions, were used in this study. The horses were housed in box stalls and allowed to acclimate to their new environment over a period of 7 days. Weather permitting, daily turnout was allocated. Both the stall and turnout environment were monitored to ensure no outside feedstuffs were obtainable.

The six horses were randomly assigned six diets which varied in sugar/starch and fat/fiber composition (Table 1). The amount of concentrate and forage fed to each horse was determined by the horse's body weight. At the beginning of each feed period, the horses were weighed to accurately determine their body weight and calculate the amount of feed to be given. Day one was also the day the horses were introduced to their new diet through half the amount of the prescribed feed mixed with the previous feed. On days two through six, the horses were kept on a strict diet of the randomly assigned feed, fed 2% of their body weight in hay, and had ad libitum access to water. On day six, a jugular catheter was inserted for blood sample collections the following day. On day seven, hay intake was restricted and blood samples were collected. This seven day period concluded one

individual feeding trial and the set-up was repeated five more times until all six horses had consumed each of the six commercial diets. Throughout the experiment, feedings took place at at 8:00 AM and 4:00 PM.

Diet	Energy (MJ/kg)	% Fiber	% Fat	% Protein	% Starch
1		5.385	4.134	12.597	6.156
2		15.689	8.001	15.689	9.998
3	8.4	20		10	10
4		12.572	7.99	12.648	15.107
5		5.301	4.99	32	7.076
6	8.8	25		8	4

Table 1: Fiber and starch formulation of the six commercial diets.

Sample Collection

On the morning of day seven, blood was collected at 30 minutes prior to feeding (-0.5), at feeding (0), 30 min after feeding (0.5) and 60 min after feeding (1.0) to determine cortisol levels. Blood was collected via the jugular vein into 10 mL Vacutainer™ tubes. The samples were centrifuged within 15 min of collection and the serum collected and stored at -80°C until laboratory analysis.

Determination of Cortisol Concentrations

Serum cortisol concentrations were measured by radioimmunoassay (RIA; Figure 1), Coat-A-Count® cortisol assay kit (Siemens Medical Solutions, Los Angeles, CA). This assay has been proven to be highly reliable and consistent (Panzani et al., 2009).

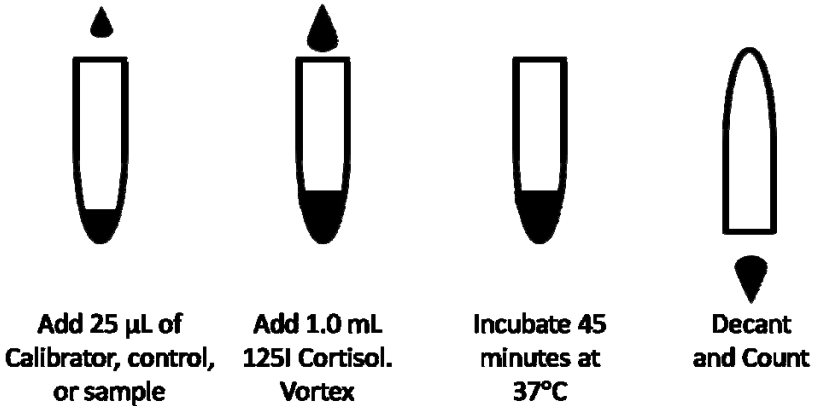

Figure 1. RIA procedure for serum samples. Adapted from Coat-A-Count® Cortisol (Siemens Medical Solutions, Los Angeles, CA).

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Statistical Analysis

When developing the standard curve for analysis, three points were excluded from the curve. This is acceptable as the vast majority of our data did not fall within the limits of the points where the exclusion was necessary. Rather, the almost all of the data lies within the standard curve which is strongly substantiated. Cortisol concentrations in correspondence to time and diet were analyzed by SAS (SAS Institute Inc., Cary, N.C.). Statistical significance was considered to be $P < 0.05$.

RESULTS

The six diets in this study had no impact on cortisol concentration levels in reference to the morning meal (Figure 2). However, the average cortisol concentrations for each of the diets were significantly different throughout the study (Figure 3).

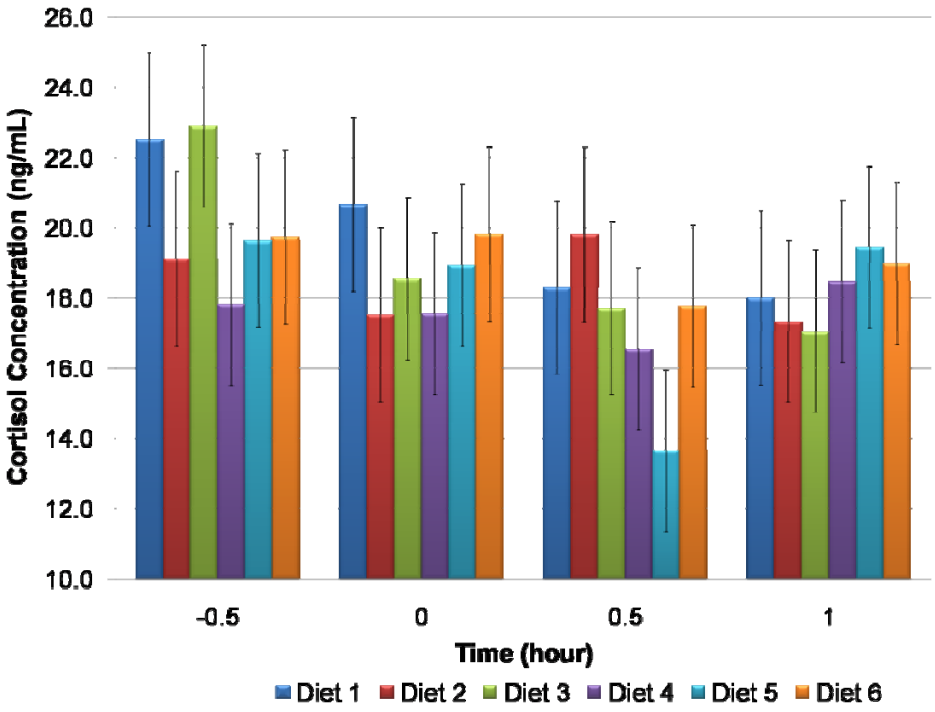

Figure 2: Cortisol concentrations according to diet before and after AM feedings. Concentrations compiled over the six week trial and averaged among six different horses. No statistical differences were observed.

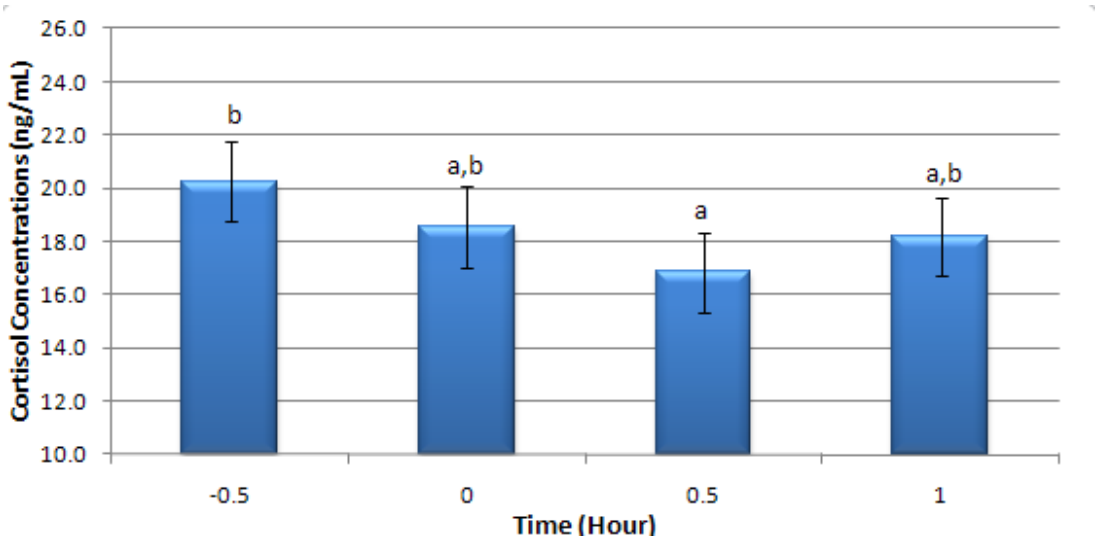

Figure 3: Cortisol concentrations according to time before and after AM feedings. Different letters indicate that levels differed among time points. Two or more letters indicate the overlap of data. Time point -0.5 and 0.5 were statistically different.

DISCUSSION

The aim of this study was to determine if the different diet formulations, specifically if the amount of starch/sugar, would affect the amount of cortisol circulating in the blood. Variances in cortisol were widely ranging because of the tendency of the different horses to become more easily stressed. Yet, there was no significant difference in cortisol concentrations due to diet in this study. These findings are supported by previous research which reported no correlation between cortisol concentrations and diet (Stull, 1988).

Cortisol concentrations did, however, increase immediately prior to and after the morning meal. Elevated cortisol levels prior to feeding could be due to the anticipation of receiving the morning meal after a period of several hours with no grain or hay or becoming acclimated to the daily feeding routine. This increase in

stress, as noted by the increase in cortisol concentrations, is challenging to substantiate as previous research studies involving cortisol levels did not involve experimental designs where the horses were kept on a routine schedule. Thus, little data is available as to if anticipation is a proponent of stress. However, extrapolating data which demonstrated decreased levels of cortisol and ACTH concentrations after a set number of kilometers in transport, one may be able to assume that the initial rise in cortisol levels at the onset of transport was due to anticipation of the transport and the drop in concentrations was indicative of the actual fulfillment of the anticipated event (Fazio et al., 2008). Perhaps the next step would be to design more studies to allow investigation to determine if anticipation of events is indeed a legitimate stressor in horses. In addition, it would be beneficial to study if *how* the horse is fed has any correlation with cortisol. Possibly, feeding three times a day when compared with once a day might influence the amount of stress displayed. In addition, free access to hay when compared with hay given only once a day could also impact cortisol levels as well as other feeding regimens.

This finding of increased stress before feeding can have implication in the way a horse is handled. In times where it is important to be aware of the negative impact of stress, such as times of sickness or breeding in which stress can impair both the immune system and reproduction, care should be taken to avoid other circumstances which would intensify the stress already experienced a half hour before feeding. For instance, a horse in a breeding program or one that is ill might

not be exercised before feeding as that will only augment the cortisol levels associated with morning feeding. Another future study might investigate if the same pattern of cortisol levels was consistent with PM feeding.

CONCLUSION

The different diets did not affect the amount of stress experienced by horses around morning feedings. However, a higher amount of stress, as indicated by increased serum cortisol concentrations, was observed 30 minutes prior to feeding the morning meal when compared to 30 minutes after the meal was consumed. Further research that results in more extreme variances in sugar/starch content in diets is needed to determine the effects, if any, on cortisol levels.

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