RESEARCH ON THE NUTRITION OF AGING HORSES

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ABSTRACT

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With advances in nutrition and dental care, the population of aged horses is growing. However, little investigation of digestibility differences in healthy aged compared to healthy adult horses has been performed. This study was designed to compare digestibility of various feedstuffs between two age groups and three different diets, further, the effect of a moderate fat diet on nutrient digestibility. Seventeen mares, 8 adult and 9 aged were assigned to diets: hay only (HAY), hay plus a carbohydrate-rich concentrate (CHO), and hay plus a fat and fiber-rich concentrate (FF). Three 6-wk diet periods of 3 wk outdoor group feeding and 2 wk stalled individual feeding were followed by a 72-h digestibility trial measuring feed intake and included collection of all feces and urine. To determine digestibility, feed, fecal, and urine samples were analyzed for minerals and CP. Feed and fecal samples were analyzed for energy, NDF, and fat; blood samples for vitamin C concentrations. No age differences were seen, however aged horses had higher plasma vitamin C concentrations than adult horses. The diets differed in intake for all minerals, fecal CP, and NDF. Diet differences were not seen for percent NDF digested or gross energy intake; however, differences were present for all other NDF and energy parameters. All fat parameters differed between diets. No diet differences were seen for blood vitamin C concentrations. The HAY diet consistently had a lower digestibility. It therefore appears that aged animals are as able to digest various feedstuffs as adult animals, that horses on a hay only diet may require supplementation, and that a diet with a moderate amount of fat does not appear to have a negative effect on the digestibility of other nutrients.

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CHAPTER 1

REVIEW OF LITERATURE

Nutrition and Digestion in the Adult Horse

Despite the lack of exploration into the nutritional needs of the healthy aged horse, there is general agreement about the needs of the healthy adult horse (5 to 20 yr), although opinions of what constitutes those nutritional needs vary between disciplines and decades (Harris, 1998). The National Research Council (NRC) is often cited as the main reference for understanding the needs of the adult animal (National Research Council, 2007). However, many of the nutritional recommendations are based on data from other species, and more research is needed in this area.

Digestibility of different feeds in the adult horse has been a longtime topic of interest for researchers. As a grazing animal, the horse breaks down (or digests) and absorbs nutrients in the stomach and small intestine and fermentation of fiber and other complex carbohydrates occurs in the cecum and large colon (Ralston, 2007; Hintz et al., 1978). Studies have examined a wide range of parameters associated with digestibility, including, but not limited to, differences between concentrates and forages (Rosenfeld et al., 2006; Kienzle et al., 2002; Hintz et al., 1971), 1 versus 2 compartment digesta passages for the equine gastrointestinal tract (Rosenfeld et al., 2006), and frequency of feeding times (Van Weyenberg et al., 2007). Most studies have concluded that the horse can digest food efficiently under a variety of conditions. However, differences, such as increased time required for digestion and increased apparent digestibility have been observed between rations containing similar amounts of dissimilar concentrates as well as between similar amounts of different forages (Kienzle et al., 2002). Further, it is been suggested that there are potential differences between aged and adult horse digestibility which are centered on a decreasing digestive and absorptive ability with increasing age. These findings

have implications for meeting the nutritional requirements of horses with challenges such as hyperadrenocorticism, or Cushings disease, which is common in aged horses (Jarvis, 2009; Siciliano, 2002).

Clinical Problems Associated with Aging

Weight loss is one of the major concerns in the health of aged horses (Jarvis, 2009; Dickinson et al., 2002). Some of the clinical problems associated with weight loss are poor dental health, arthritis-associated pain, renal and hepatic disease, pituitary dysfunction, decreased immune function, and intestinal scarring from parasite damage. Management factors such as the inability to compete for feed in a herd setting, changes in weather, and inadequate feed volume or poor feed quality are often problems with aged horses, as well as adult horses. These factors directly and indirectly impact the ability of the older animal to maintain body condition, which is often used to evaluate digestible energy requirements, along with affecting nutritional needs (Siciliano, 2002).

Dental disorders, which are more common in aged horses compared to adult horses, can cause pain during food mastication, which can result in decreased overall consumption (Jarvis, 2009; Dickinson et al., 2002). Dental problems can also increase the risk of impaction colic and choke as the horse is less likely to chew food properly. However, minor dental abnormalities have not been shown to create weight loss or decrease digestibility of food (Carmalt et al., 2008; Ralston et al., 2001). While regular dental care is recommended for optimal intake and digestion, other methods can be employed to ease chewing, such as providing complete feeds or soaking feed. Pain associated with chronic arthritis, which is also common to aged animals, may discourage the horse from shifting more weight to its front legs, an action integral to the horse lowering its head to graze or eat from the ground (Jarvis, 2009).

Renal and hepatic diseases also often cause a decrease in appetite. Uremia with kidney disease, can lead to hepatic and bladder stones, and death. To mitigate, a diet low in protein should be considered to reduce the magnitude of azstemia (Jarvis, 2009). As the liver is responsible for processing fat and protein and performing gluconeogenesis, the main problems associated with liver failure include a reduced ability to process energy sources, mainly fat and protein. A decrease in energy is seen as a side effect of this reduced ability, in addition to reduced appetite and weight loss. Therefore an animal with liver disease should be fed a diet rich in simple carbohydrates, containing little fat and little protein (Dickinson et al., 2002).

Pituitary dysfunction (often known as Cushing's disease) is one of the more common problems in aged equids and can be accompanied by insulin resistance and laminitis. Feeding Cushing's horses a diet low in non-structural carbohydrates is advisable because of the association of pituitary dysfunction with insulin resistance and laminitis, both of which are exacerbated by a diet high in those non-structural carbohydrates. Some studies suggest feeding a high percentage of fat to make up for the caloric loss when the amount of carbohydrates in the feed is reduced (Olsman et al., 2004).

Cumulative damage from parasites is thought to have a detrimental effect on the digestive capacity of aged animals, causing a decreased ability to digest or absorb certain nutrients. Therefore, the importance of using anthelmintics cannot be overlooked. More precisely, the use of anthelmintics should be encouraged throughout the animal's lifetime, with a frequency in adult horses of at least twice per yr (Nielsen et al., 2010; Von Samson-Himmelstjerna et al., 2009).

Another potential problem concerning aging in the horse which may have an impact on feeding recommendations is immune function. Research has indicated similar negative age-

related changes in the horse compared to changes seen in human studies (Fermaglich et al., 2002). These changes suggest a decreased efficacy of vaccinations, which can reportedly equate to a failure rate of up to 50% in aged humans (Gravenstein et al., 1989). Aged horses have also been documented to have increased numbers of lymphocytes in response to exercise (Williams et al., 2008). These data imply that aged animals are immune-compromised during exercise, although the authors admit that further investigation must be done to confirm or refute this hypothesis.

Possible Nutritional Changes Due to Aging

The landmark nutritional study that compared feedstuff digestibility in aged horses to that of adult horses concluded that aged animals (>20 yr) may experience decreased digestion of protein and phosphorus and a trend for decreased digestion of fiber (Ralston, 1989). However, the results of this study were later called into question by its authors due to concerns that differences found may have been a consequence of parasite damage which could have caused scarring of the large intestine, as the digestibility of the aged animals was similar to what is seen in horses that have undergone large colon resection (Ralston, 2008; Bertone et al., 1990; Bertone et al., 1989).

Studies of aging in other species (Swanson et al., 2004) have also suggested a decrease in digestive efficiency in dogs, cats, and humans. Dogs and cats are reported to develop problems with their immune systems, kidneys and other organs with age (Laflamme, 2005). Similar to humans and rats, aging dogs have a decrease in energy requirements, potentially due to a decrease in physical activity, whereas cats tend to have a decreased ability to digest fat and protein (Laflamme, 2005).

The National Academy of Sciences (2004) have reported a decreased requirement for aged humans of energy, sodium, chloride, and water. However, this may be due in large part to a decrease in physical activity, nevertheless aged people are often reported to not consume enough energy to meet their requirements (Morley, 2010; Risonar et al., 2009; Thomas, 2004), which can lead to inadequate intake of other nutrients. This decreased intake can be caused by various factors, including delayed gastric emptying, increased anal stretch, dehydration, altered hormonal responses, and altered sense of taste and smell (Morley, 2010; Thomas, 2004). Human studies have also found the potential need to supplement Se (Olivieri et al., 1994) and vitamin D to those who do not receive adequate sunlight (National Academy of Sciences, 2004).

Some data have been reported on hematological and biochemical values and plasma corticotrophin concentrations in older horses, which may have implications on their nutritional needs. Differences have been seen in mean red blood cell volume, mean cell hemoglobin concentrations, and absolute lymphocyte count (McFarlane et al., 1998). These values suggest a decreased ability of the aged horse to adapt to environmental factors.

Exercise-Related Changes to Aging

Some of the work done to date regarding the aged horse has examined the equine response to exercise. Findings indicate that, while aged horses do experience some changes in exercise capacity - mostly related to a decreased aerobic ability, the aged horse maintains an extraordinary athletic ability compared to aged animals in other species (McKeever et al., 1999; McKeever, 2002). A study evaluating apoptosis and antioxidant stress relating to exercise training in aged horses (Williams et al., 2008) found no differences between the adult and aged test groups in conjunction with antioxidant stress during exercise; however, the authors stipulated that the exercise intensity may not have been sufficient to see a difference between the

age groups. They did, however, find data to support a possible decrease in immune function in the aged group following due to the increased percentage of white blood cell apoptosis in those animals.

Endocrine responses to exercise have been evaluated as well, with results showing some differences between the aged and adult age groups with the aged horses having higher values compared to the adult horses at speeds producing VO2 max for plasma rennin activity, aldosterone, and plasma total protein concentrations. Aged horses had lower values compared to the adult horses at speeds producing VO2 max for atrial natruretic peptide and arginine vasopressin. These differences were nevertheless within normal parameters for horses exercising at maximum capacity (McKeever et al., 1999). The studies examining the ability of the aged horse to perform athletic tasks differ in several different experimental parameters that do need to be considered. While most of the aged animals used were in good health, their individual fitness levels were not taken into account. Therefore, results might merely reflect how aged animals, whom are otherwise healthy but out of shape, respond to exercise. Data are nevertheless acknowledged to be limited in this area and appropriate training and experimental protocols have not yet been established for the aged horse.

Knowing that the aged animal may have differences from the adult animal in respect to immune function, antioxidant status, and response to exercise raises questions about possible ways to compensate for these differences through specialized feeding practices. However, until the basic nutritional needs of the aged horse are established, such investigation is premature.

Conclusion

Despite research into the nutritional needs of the adult horse, the needs of the aged animal have been largely ignored. Much work is required on the subject of aged horse nutrition as it has

many implications on other areas of care. Aged horses, even those who do not struggle with health concerns, may therefore need a supplemental or altered feeding regimen. Especially bearing in mind that as many horses age they can experience weight loss, frequently stemming from a loss of muscle mass, it is important to examine the nutritional needs of the aged population as well as the impact of different common feeding practices. If the baseline nutritional needs of the healthy aged horse can be established, then more research can be initiated to address the needs of the aged animal in areas such as immune function, inflammation, exercise, muscle wasting, etc. Considering the growing population of these animals and their increasing life expectancy, demand for this work will continue to expand.

CHAPTER 2

EFFECT OF AGE ON DIGESTIBILITY OF FEEDSTUFFS IN HORSES

Abstract

The population of aged horses (>20 yr) is growing with advances in complete feeds and dental care. However, there has been little investigation of potential differences in digestibility of feedstuffs in healthy aged compared to healthy adult horses. The objective of this study was to compare digestibility of various feedstuffs between aged and adult horses. Seventeen healthy horses, 8 adult (5 to 12 yr) and 9 aged (19 to 28 yr) were studied. Horses were randomly assigned to diets: hay only (HAY), hay plus a carbohydrate-rich concentrate (CHO), and hay plus a fat and fiber-rich concentrate (FF). Three 6-wk diet periods consisted of 3 wk of outdoor group feeding and 2 wk of indoor stalled individual feeding, followed by a 72-h digestibility trial measuring feed intake and including collection of all feces and urine. Feed, fecal, and urine samples were analyzed for macro and micro minerals and CP to determine retention, percent retention, and percent apparent digestibility. Feed and fecal samples were also analyzed for energy, NDF, and fat to determine retention, percent apparent digestibility and, in the case of NDF, percent digestibility. Blood samples were analyzed to determine vitamin C concentrations. Weight and BCS were evaluated regularly, and while aged horses weighed on average less than adult horses ($P = 0.02$), no differences were observed between the two age groups for BCS ($P = 0.20$). No age differences in digestibility, apparent digestibility, or apparent retention were seen for any of the parameters measured. Aged horses had higher plasma vitamin C concentrations than adult horses ($P = 0.03$). Based on the results of this study, it appears that aged horses are as able to digest various feedstuffs as adult horses.

Introduction

Advances in the care of horses are continually being made. As a result, the population of aged horses (> 20 yr) is growing; it is becoming increasingly important to address potential nutritional needs of these animals as they may differ from the adult horse. Research is available regarding the treatment of the clinical problems associated with age; such as loss of body condition, osteoarthritis, hepatic dysfunction, and pituitary dysfunction (Jarvis, 2009; Dickinson et al., 2002). A good deal of research has also been conducted on the nutritional requirements of the healthy adult horse (National Research Council, 2007; Van Weyenberg et al., 2007; Olsman et al., 2004); however, little has been initiated on the behalf of the healthy aged horse. Previous research has indicated possible differences with aged horses having a lower absorption of P, lower fiber digestibility, and a trend for lower CP digestibility (Ralston, 1989). However, a subsequent study by the same author did not report lower CP, P, or fiber digestibility in aged horses, and it was speculated that intestinal damage associated with parasite infestation may have confounded the results of the original study (Ralston et al., 2007). Some information is available for the healthy aged population of other species, but again tends to center around animals with health problems (Laflamme, 2005). Human studies, as well, have documented different nutritional requirements for the aged such as an increased requirement for vitamin D (Morley, 2010), Se (Olivieri et al., 1994), and a decreased energy need (National Academy of Sciences, 2004). Nutritional requirements are yet again often based on those aged humans with specific health challenges, such as anorexia (Morley, 2010; Thomas, 2004). Despite limited data, popular opinion is that aged horses have increased nutritional requirements due to a decreased ability to absorb nutrients. This study was intended to examine the possible differences between the two age groups in the equine model to determine if any such nutritional variations exist. Our

hypothesis was that there would be no differences in the digestibility of various feedstuffs between aged and adult horses.

Materials and Methods

Animals. Seventeen healthy mares were utilized, 12 of which originated from a Canadian facility and shared the same background as pregnant mare urine (PMU) mares. All the mares originating from Canada had been on a similar management protocol with similar feeding, vaccination, and anthelmintic schedules. The remaining 5 mares were obtained from outside sources to ensure all mares fell within the proper age groups and were chosen for similar body type, size, and BCS (Henneke et al., 1983). Two main groups were established, one with 9 adult horses (ages 5 to 12 yr, 500 ± 13 kg) and the other with 8 aged horses (ages 19 to 28 yr, 455 ± 12 kg). Within each age group, horses were striated by age and BCS and then randomly assigned to one of three test groups, each containing 3 adult and 3 elderly horses. All materials and methods were approved by Michigan State University's (MSU) Animal Care and Use Committee (approval #11/09-174-00).

The horses had an acclimation period of at least 6 wk (6 outside mares having a range of 2 to 6 wk adaptation time) to adjust to the change in climate as well as their new surroundings before beginning their first diet period. During this time they were kept in pastures at MSU's College of Veterinary Medicine Bennett Farm and were supplemented once daily with free choice hay and had access to a trace mineralized salt block and free choice water. All animals were immunized shortly after arrival for eastern and western equine encephalomyelitis, influenza**,** rhinopneumonitis, strangles, tetanus, and rabies. All horses were vaccinated and dewormed with Ivermectin at least 2 wk prior to the start of their first diet period, with the exception of the two outside mares that were vaccinated and dewormed upon arrival. The horses underwent a dental examination under sedation to visually inspect the horses' mouths with the aid of a head stand and a speculum for head and mouth positioning. Those horses deemed in

need of dental work had their teeth floated by a licensed veterinarian. Fasting blood samples were taken for serum chemistry on d 40, 54, or 70 of period one. Records of vaccinations, dental work, blood work, and health certificates were housed at MSU's Research and Instructional Building.

Experimental Design. Following the acclimation period, horses were separated into their test groups and randomly assigned to pairs of one aged and one adult horse, within those groups. Pairs were then randomly assigned to a diet of hay alone (HAY), hay with a high fiber, high oil, low cereal starch concentrate (FF), and hay with a lower oil, high cereal starch concentrate (CHO). When beginning a diet, the horses were weighed, and fed 1.6% of body weight (BW) divided between two daily feedings. Horses on the CHO and FF diets had 0.6% BW of their diet composed of concentrate (representing about 50% of daily digestible energy) and 1.0% BW composed of hay. Horses on the HAY diet had all 1.6% of their BW fed as hay. On d 22 and 41 of each period, the horses were weighed and BCS ascertained by three trained individuals. It was determined that should the horses' BCS increase or decrease by one point during the study, the percentage DM fed would be adjusted up or down proportionally by 15% for hay and concentrate. Exact concentrate and hay amounts required were calculated and weighed out for each individual animal. Thirty-one days into the study it was determined that the amount fed of 1.6% BW was insufficient and was subsequently increased to 1.84% BW, as stated above. Horses on the CHO and FF diets had 0.69% BW of their diet composed of concentrate and 1.15% BW composed of hay. Horses on the HAY diet had all 1.84% of their BW fed as hay. Following this increase, it was concluded that one animal was still deficient, and her amount fed was increased by another 15%. To accommodate for the increase, this amount was split into three feedings versus two. The HAY and FF rations were obtained from one batch and the CHO

rations obtained from two batches. HAY, FF and CHO rations were stored in facilities at MSU's Veterinary Research Farm. The horses remained on their appointed diet for a period of 6 wk before starting on a different diet utilizing a modified Latin square design so that each horse received every diet. Upon commencement of a new diet treatment, horses transferring to the HAY diet were fed their full portion of hay. Horses moving to the FF and CHO diets were gradually adjusted to their new feed over 4 d. The horses were allowed ad libitum access to water.

Trace mineral blocks were removed at the beginning of the first diet period. Pairs were housed in a specific paddock for three wk of outdoor feeding, and each pair was fed the same diet. As the study began on the first d of January 2010, and was conducted during the pastures' dormant season, minimal grass was presumably consumed during this time. However, to avoid the possibility that horses may have had access to grass immediately prior to total collections, horses were moved to MSU's Veterinary Teaching Hospital two wk prior to total collections and housed in 2.4 x 3 m box stalls. The temperature inside the facility was kept at 15 +/- 7° C. Indoor and outdoor temperatures, as well as outdoor weather conditions, were recorded daily throughout the study. The amount of feed and water consumed, as well as orts, was recorded. At least three d per wk while stalled, the horses were allowed turn-out in a dry lot pen for a minimum of 1 h. Following two wk of individual stalled feeding, a period of three d was used in which to carry out total collections of urine and feces (Lindenberg et al., 2006). Twenty-four hours prior to, as well as during, the total collection periods, feeding was performed every 12 h at 0800 and 2000.

Total Collections. A total collection of urine for the first period was accomplished utilizing a collection apparatus provided by the Canadian horses' previous owner. The collection apparatus was composed of a heavy plastic tube which was laid against the horses' buttocks and

to the top of which was attached bungee cords that ran up to a suspension system about the horses stalls. The bottom of the tube was fitted with a long rubber hose which ran between the horses legs and drained into a sealed container. For the remainder of the collection periods, the horses were fitted with a flexible plastic urine collection device which was attached by velcro on the point of the buttock on each side of the animal and acted as a funnel for the urine. Both collection devices were designed to prevent fecal contamination of urine, which drained via plastic tubing into sealed plastic containers. The animals were tied in their stalls, and monitored continuously for the 72-h collection period with the amount of feed and water consumed and orts from each feeding recorded. Feces were gathered, and stored in a closed plastic bag as soon as possible after voided. Feces and urine were compiled every 8 h at 0800, 1600, and 2400. Total weight feces and total urine volume were recorded, and a 10% composite of each was saved, and frozen at -20° C for later analysis.

Concentrate and hay samples were collected weekly and composited for each of the three periods. They were tested for DM, Ca, P, Mg, Na, K, S, Ca, Cu, Co, Fe, Mn, Se, Zn, energy, NDF, CP, and fat. Concentrate and hay samples were also sent to Equi-analytical (Equi-analytical, Ithaca, New York) for analysis of ethanol soluble carbohydrates, water soluble carbohydrates, starch, and lignin. Likewise, water was sampled during each period, and tested for all aforementioned minerals. Blood was drawn from the jugular vein on d 40 of each period, and collected into 3-ml EDTA tubes for later analysis of plasma vitamin C concentrations.

After total collections, each animal was switched to a different diet and placed back at the beginning of a diet period until the animal was being tested on all diets. Following the conclusion of the study, horses were either placed in homes or utilized in different research studies.

Sample Preparation and Dry Matter Digestibility. All frozen fecal samples were thawed and oven dried at 105° C (Thelco, Precision Scientific, Winchester, VA) for 24 h. Separate fecal samples were also freeze-dried. Feed samples were passed through a Wiley mill with a 2-mm screen. Ground feed and dried fecal samples were passed through a Cyclone mill with a 1-mm screen (Cyclotec 1093 Sample Mill, Foss, Eden Prairie, MN). All samples were analyzed in duplicate with accepted CVs set at 10% except for NDF, CP, which were set at 5%.

Energy. Feed and fecal energy content was determined using an oxygen bomb calorimeter (Parr Instrument Company Inc., Moline, IL; Appendix A). Heat of combustion was calculated to determine Mcal content of the feed and feces, which was used to evaluate daily Mcal intake, fecal energy, apparent digestible energy and percent apparent digestible energy. All values were then analyzed on a BW basis.

Calculations

$$\text{Heat of combustion (cal/g)} = \frac{tW - (\text{calories of wire burned})}{\text{g of pellet sample}}$$

t = temperature final – temperature initial

W = energy equivalent of the bomb used

Apparent digestible energy:

Gross energy intake-fecal energy

Percent apparent digestible energy:

$$\frac{\text{Gross energy intake-fecal energy}}{\text{Gross energy intake}} \times 100$$

NDF. Neutral detergent fiber was determined by the method created by Goering and VanSoest (Goering and VanSoest, 1970; Robertson and VanSoest, 1977). See Appendix A for a detailed description of laboratory analysis. Neutral detergent fiber content in the feed and feces were used to calculate daily NDF intake, fecal NDF, NDF digested, and percent NDF digestibility. All values were then analyzed on a BW basis.

Calculations

$$\text{NDF (g)} = \frac{\text{(crucible and NDF residue weight) – (crucible and ash residue weight)}}{\text{g of sample dry matter}}$$

NDF digested:

NDF intake-fecal NDF

Percent NDF digested:

$$\frac{\text{NDF intake-fecal NDF}}{\text{NDF intake}} \times 100$$

Crude Protein. Concentrate, hay, and fecal samples were analyzed for CP according to the micro-Kjeldahl procedure (Ma and Zuazaga, 1942; Appendix A) and read by a spectrophotometer at 460 nm (Gomori, 1942). Crude protein was determined by multiplying nitrogen values by 6.25 and was used to determine daily average g of CP intake in the concentrate and hay as well as average g of CP excreted in the feces, urine, and the combination of both feces and urine. These data were used to calculate CP retained, percent retained, and percent apparent digestibility. Data were then adjusted for the animals' BW for analysis on a g per kg BW basis.

CP

CP retained:

CP intake-CP output (feces and urine)

Percent retained:

$$\frac{\text{CP intake-CP output (feces and urine)}}{\text{CP intake}} \times 100$$

Percent apparent digestibility:

$$\frac{\text{CP intake-fecal CP}}{} \times 100$$

CP intake

Fat. Samples of feed and feces were ether extracted (Horowitz, 2000; Appendix A) to calculate fat disappearance. Fat disappearance from the feed and feces was used to calculate g/d fat intake, g/d fecal fat, g/d amount digested, and percent apparent digestibility. All values were then analyzed on a BW basis.

Fat

Amount digested was calculated:

$$\text{Fat intake-fecal fat}$$

Percent apparent digestibility was calculated:

$$\frac{\text{Fat intake-fecal fat}}{\text{Fat intake}} \times 100$$

Vitamin C. Blood samples were prepared immediately following collection by adding 800 µL metaphosphoric acid (1:5 dilution with DI water) to 200 mL plasma. They were then vortexed into solution and frozen at -80 °C for later HPLC analysis by MSU's Diagnostic Center for Population and Animal Health.

Macro and Microminerals. Feed and fecal samples were microwave digested for inductively coupled plasma mass spectrometry (ICP-MS) analysis according to the method determined by Shaw et al. (2002). For a more detailed description, see Appendix A.

Urine samples were not digested, but were vortexed thoroughly prior to being analyzed. Digested feed and fecal samples were run in duplicate with bovine liver standard (1577b; NIST, Gaithersburg, MD) as a control, and analyzed for P, Mg, Na, K, S, Co, Cu, Fe, Mn, Se, and Zn by ICP-MS. Non-acidified urine samples were run in duplicate, and analyzed for Na, K, S, Co, Cu, Fe, Mn, Se, and Zn by ICP-MS and for P by colorimetric assay. ICP-MS analysis was completed by MSU's Diagnostic Center for Population and Animal Health. The urine was prepared for P analysis (Appendix A), and concentrations determined by colorimetric assay

(Gomori, 1942). Urine Ca concentrations were also determined via atomic absorption spectrometry (IL Atomic Absorption Application Laboratory, 1972; Unicam 989, Thermo Electron Corp., Franklin, MA); samples were acidified with 600 μl of 12 M HCl and diluted 500X with 1 percent lanthanum chloride prior to running on the spectrometer. Concentrations of minerals were used to determine average g of mineral per d intake from the concentrate and hay as well as average g of mineral per d excreted in the feces, urine, and the combination of both feces and urine. Data were then adjusted for the animals' BW to determine mineral averages on a g/kg BW/d basis. Mineral balance was then calculated. Percent retention and percent digestion of Na, Fe, and Se were non-estimable due to lower than detectible concentrations in the hay.

Calculations

Mineral retained:

$$\text{Mineral intake-mineral output (in both feces and urine)}$$

Mineral percent retained:

$$\frac{\text{Mineral intake-mineral output (both feces and urine)}}{\text{Mineral intake}} \times 100$$

Mineral percent apparent digested:

$$\frac{\text{Mineral intake-mineral output (feces only)}}{\text{Mineral intake}} \times 100$$

Statistical Analysis. Data were analyzed using SAS software (Version 9.2, SAS Inst., Inc., Cary, NC). The PROC mixed function was used with group set as a random factor and period as the repeated measure with the subject of horse. Results are reported as least square means (± SEM) and are on a DM basis. Significance was declared at $P<0.05$ and trends at $P<0.10$. A power test was also performed and it was determined that a sample size of 9-12 horses was sufficient to detect differences at 80 percent power.

Results

Animals remained clinically healthy throughout the study, and no age by diet differences were detected ($P \leq 0.05$). When comparing weights for the duration of the study, aged horses weighed less (455 ± 12 kg) than adult horses (500 ± 13 kg; $P = 0.02$). There were, however, no differences in BCS between the two age groups ($P = 0.20$), nor was there any interaction concerning weight or BCS between age and diet or between age and period.

DM Digestibility. No differences in daily concentrate ($P = 0.73$) or hay intake ($P = 0.25$) were seen between the 2 age groups on a g/kg BW basis. Likewise, there were no differences in fecal ($P = 0.29$) or urine output ($P = 0.29$). Results are presented in Table 1:1.

Energy, NDF, CP, fat, and vitamin C. There was no effect of age on energy, NDF digestibility, CP, or fat apparent digestibility, nor was there an effect of age on CP retention (Table 1:2). No interactions were observed for any of the variables. However, aged horses had higher plasma concentrations of vitamin C in contrast to adult horses (Table 1.2; $P = 0.03$). No interactions were observed for any of the variables.

Macrominerals. Results are presented by macromineral in Table 1:3. No age differences were seen for any of the macrominerals. There was, however, a trend for aged horses to excrete less fecal S compared to adult horses ($P = 0.06$). An age by diet by period interaction was also observed for fecal S ($P = 0.04$). There were no other interactions with age.

Microminerals. Table 1:4 presents the results by micromineral. Similar to macromineral results, neither age differences nor trends for differences were seen for any of the microminerals. An age by period interaction was observed for Se retention ($P = 0.03$) with aged horses during dietary period three having a greater Se retention compared to aged horses during dietary periods one and two. A trend for a difference in Se % digestion (P = 0.06) was also observed with the

aged horses exhibiting a lower % digestion compared to the adult horses. There were no other observed age trends or interactions.

Table 1:1. Dry matter intake, output, and weight data for aged versus adult horses

	Aged	Adult	P-value
Intake and output (g/kg BW/d)			
Avg Wt (kg)	455[a] ± 12	500[b] ± 13	*0.02*
Median BCS	4.8 ± 0.2	5.1 ± 0.2	*0.20*
Avg Concentrate Intake	4.98 ± 0.61	4.75 ± 0.64	*0.73*
Avg Hay Intake	12.50 ± 0.59	13.57 ± 0.64	*0.25*
Avg Fecal Output	7.08 ± 0.31	7.60 ± 0.34	*0.29*
Avg Urine Output (*ml/kg BW/d)*	13.64 ± 0.92	12.06 ± 1.00	*0.29*

[ab] *Means within rows with differing superscripts differ*

Table 1:2. Energy, neutral detergent fiber, crude protein, fat digestibility, and blood vitamin C concentrations for aged versus adult horses

	Aged	Adult	P-value
Energy (Mcal/kg BW/d)			
Intake	0.079 ± 0.003	0.082 ± 0.003	*0.39*
Fecal	0.034 ± 0.002	0.037 ± 0.002	*0.30*
Apparent Digestible Energy	0.045 ± 0.002	0.046 ± 0.002	*0.68*
% Apparent Digestible Energy	56.64 ± 1.24	55.53 ±1.36	*0.55*
NDF (g/kg BW/d)			
Intake	9.52 ± 0.53	10.11 ± 0.56	*0.35*
Fecal	4.55 ± 0.24	5.01 ± 0.26	*0.22*
NDF Digested	4.91 ± 0.40	5.08 ± 0.42	*0.65*
% NDF Digestibility	51.51 ± 1.69	49.79 ± 1.83	*0.41*
CP (g/kg BW/d)			
Intake	2.55 ± 0.10	2.66 ± 0.11	*0.38*
Fecal	0.61 ± 0.04	0.65 ± 0.04	*0.46*
Urinary	0.39 ± 0.03	0.38 ± 0.03	*0.86*
Retained	1.54 ± 0.07	1.62 ± 0.08	*0.46*
% Retained	60.05 ± 1.55	60.90 ± 1.69	*0.71*
% Apparent Digested	75.88 ± 0.93	75.88 ± 1.01	*1.00*
Fat (g/kg BW/d)			
Intake	0.78 ± 0.04	0.81 ± 0.04	*0.47*
Fecal	0.40 ± 0.03	0.39 ± 0.03	*0.69*
Amount digested	0.38 ± 0.02	0.42 ± 0.03	*0.25*
% Apparent Digestibility	46.27 ± 2.28	50.66 ± 2.50	*0.20*
Vitamin C blood concentrations (mg/dl)			
Vitamin C	1.20^{b} ± 0.04	1.07^{a} ± 0.05	*0.03*

[ab] *Means within rows with differing superscripts differ*

Table 1:3. Macromineral digestibility for aged versus adult horses

	Aged	Adult	P-value
Calcium (g/kg BW/d)			
Intake	0.16 ± 0.01	0.17 ± 0.01	*0.30*
Fecal	0.088 ± 0.005	0.095 ± 0.006	*0.39*
Urinary	0.024 ± 0.003	0.019 ± 0.003	*0.21*
Retained	0.053 ± 0.007	0.059 ± 0.008	*0.44*
% Retained	32.14 ± 3.04	32.70 ± 3.37	*0.90*
% Apparent Digested	47.01 ± 2.31	43.99 ± 2.53	*0.38*
Phosphorus (g/kg BW/d)			
Intake	0.052 ± 0.004	0.053 ± 0.004	*0.92*
Fecal	0.047 ± 0.003	0.046 ± 0.004	*0.74*
Urinary	0.0060 ± 0.0005	0.0056 ± 0.0006	*0.61*
Retained	-0.001 ± 0.002	0.001 ± 0.002	*0.40*
% Retained	-10.76 ± 5.74	-3.55 ± 6.03	*0.21*
% Apparent Digested	4.21 ± 4.26	9.56 ± 4.53	*0.26*
Magnesium (g/kg BW/d)			
Intake	0.038 ± 0.003	0.039 ± 0.003	*0.63*
Fecal	0.025 ± 0.002	0.025 ± 0.002	*0.99*
Urinary	0.0052 ± 0.0004	0.0045 ± 0.0005	*0.28*
Retained	0.008 ± 0.002	0.010 ± 0.002	*0.19*
% Retained	17.80 ± 3.65	24.20 ± 3.95	*0.18*
% Apparent Digested	32.65 ± 3.00	35.02 ± 3.24	*0.52*
Sodium (g/kg BW/d)			
Intake	0.030 ± 0.002	0.030 ± 0.002	*0.99*
Fecal	0.016 ± 0.002	0.017 ± 0.002	*0.31*
Urinary	0.013 ± 0.001	0.011 ± 0.001	*0.15*
Retained	0.0004 ± 0.0012	0.0014 ± 0.0013	*0.60*
% Retained	-2.54 ± 5.86	5.91 ± 6.49	*0.35*
% Apparent Digested	45.44 ± 3.81	40.32 ± 4.20	*0.35*
Potassium (g/kg BW/d)			
Intake	0.27 ± 0.01	0.28 ± 0.01	*0.31*
Fecal	0.065 ± 0.004	0.074 ± 0.005	*0.21*
Urinary	0.14 ± 0.01	0.14 ± 0.02	*0.68*
Retained	0.07 ± 0.02	0.06 ± 0.02	*0.79*
% Retained	24.32 ± 5.80	21.92 ± 5.99	*0.60*
% Apparent Digested	75.56 ± 1.37	72.96 ± 1.48	*0.22*

Table 1:3. (cont'd)

Sulfur (g/kg BW/d)			
Intake	0.023 ± 0.002	0.024 ± 0.002	*0.44*
Fecal	0.0115 ± 0.0004	0.0124 ± 0.0004	*0.06*‡
Urinary	0.0096 ± 0.0006	0.0103 ± 0.0006	*0.39*
Retained	0.002 ± 0.002	0.001 ± 0.002	*0.44*
% Retained	2.30 ± 7.73	0.84 ± 7.97	*0.81*
% Apparent Digested	45.84 ± 4.01	45.20 ± 4.12	*0.83*

‡ *Age by diet by period interaction*

Table 1:4. Microminerals digestibility for aged versus adult horses

	Aged	Adult	P-value
Cobalt (mg/kg BW/d)			
Intake	0.0044 ± 0.0006	0.0040 ± 0.0006	*0.51*
Fecal	0.0042 ± 0.0006	0.0036 ± 0.0006	*0.36*
Urinary	0.00006 ± 0.00001	0.00005 ± 0.00002	*0.66*
Retained	0.0002 ± 0.0001	0.0004 ± 0.0001	*0.22*
% Retained	-42.33 ± 12.10	-25.94 ± 13.21	*0.31*
% Apparent Digested	-40.03 ± 11.97	-23.73 ± 13.03	*0.31*
Copper (mg/kg BW/d)			
Intake	0.30 ± 0.03	0.29 ± 0.03	*0.81*
Fecal	0.27 ± 0.03	0.25 ± 0.03	*0.46*
Urinary	0.00061 ± 0.00006	0.00062 ± 0.00007	*0.89*
Retained	0.023 ± 0.006	0.038 ± 0.007	*0.14*
% Retained	5.52 ± 3.04	10.06 ± 3.28	*0.23*
% Apparent Digested	5.92 ± 2.96	10.38 ± 3.20	*0.24*
Iron (mg/kg BW/d)			
Intake	2.08 ± 0.19	2.10 ± 0.20	*0.95*
Fecal	2.23 ± 0.23	2.02 ± 0.24	*0.34*
Urinary	0.0012 ± 0.0001	0.0010 ± 0.0002	*0.25*
Retained	-0.16 ± 0.10	0.07 ± 0.11	*0.16*
% Retained	-221.4 ± 23.3	-203.1 ± 24.1	*0.34*
% Apparent Digested	-19.1 ± 10.8	-1.5 ± 11.8	*0.29*
Manganese (mg/kg BW/d)			
Intake	0.67 ± 0.05	0.64 ± 0.05	*0.66*
Fecal	0.61 ± 0.05	0.57 ± 0.06	*0.52*
Urinary	0.00010 ± 0.00001	0.00007 ± 0.00001	*0.17*
Retained	0.05 ± 0.02	0.07 ± 0.03	*0.64*
% Retained	1.87 ± 5.05	6.37 ± 5.53	*0.55*
% Apparent Digested	1.87 ± 5.05	6.37 ± 5.53	*0.55*
Selenium (mg/kg BW/d)			
Intake	0.006 ± 0.001	0.005 ± 0.001	*0.64*
Fecal	0.0028 ± 0.0003	0.0026 ± 0.0003	*0.42*
Urinary	0.0020 ± 0.0002	0.0018 ± 0.0003	*0.55*
Retained	0.0009 ± 0.0002	0.0010 ± 0.0002	*0.44*†
% Retained	-0.48 ± 0.05	-0.43 ± 0.05	*0.42*
% Apparent Digested	40.0 ± 2.8	49.8 ± 3.2	*0.06*

Table 1:4. (cont'd)

Zinc (mg/kg BW/d)			
Intake	0.86 ± 0.09	0.84 ± 0.09	*0.78*
Fecal	0.83 ± 0.09	0.76 ± 0.09	*0.47*
Urinary	0.003 ±0.001	0.003 ± 0.001	*0.71*
Retained	0.03 ± 0.02	0.07 ± 0.02	*0.15*
% Retained	-5.46 ± 5.18	1.29 ± 5.56	*0.28*
% Apparent Digested	-4.05 ± 4.46	1.62 ± 4.80	*0.30*

† *Age by period interaction*

Discussion

With the exception of higher blood values of vitamin C for aged versus adult horses, this study found no differences between the digestibility of macro and microminerals, energy, NDF, CP, or fat between the two age groups. These results do not agree with the previous work done by Ralston (1989) that found a lower digestibility of P and CP and a trend for a lower digestibility of fiber in aged animals. However, as the author of that study later published (Ralston, 2007), the results may have been confounded by parasite scarring in the large intestine of the aged horses used. As the horse digests its food in the small intestine with the fermentation of its main energy sources, fiber, and other carbohydrates occurring in the cecum and large colon (Ralston, 2007; Hintz et al., 1978), this scarring could very well have been one of the reasons behind their results. Considering that horses with resection of the left and right colons have been shown to have a similar decrease in their ability digest P, CP, and fiber (Ralston 2007; Bertone et al., 1990; Bertone et al., 1989), it seems likely that the parasite damage to the aged horses in the original work by Ralston was a potential reason for the observed decrease in the digestibility of those nutrients. The horses used for this study, however, all received regular anthalmentic treatment and were clinically healthy for the duration of testing. Also, while this study compared adult (5 to 12 yr) to aged ($\geq$ 19 yr) horses, the Ralston study compared young (2 to 3 yr) to aged ($\geq$ 20 yr) horses. Many of those horses also had separate clinical problems that may have contributed to the discrepancy in results.

In other species such as humans, rats, and dogs, altered nutritional requirements associated with aging include a decrease in the amount of GE required; a decrease in the ability of cats to digest fat and protein and an increased requirement for protein in dogs (Laflamme, 2005; National Academy of Sciences, 2004; Thomas, 2004). Aged humans need more Se,

vitamin B6, and without adequate sunlight, vitamin D (Morley, 2010; National Academy of Sciences, 2004; Olivieri et al., 1994). They require less dietary Na, Cl, and water; and in females less Fe (National Academy of Sciences, 2004). One of the major problems associated with age and nutrition in people, dogs, cats, and horses is anorexia, which contributes to inadequate intake of nutrients and, often, subsequent health problems (Morley, 2010; Jarvis, 2009; Laflamme, 2005). This study did not observe the same differences in digestibility as seen in other species, with the exception of the trend for aged horses to have a lower percent digestibility of Se compared to the adult horses, likely because research has mainly been carried out on carnivores and omnivores whose digestive process is different from the horse (NRC, 2007). Horses have however been shown to exhibit an increased susceptibility to disease with age (Williams et al., 2008; Fermaglich et al., 2002), as well as different endocrine responses to exercise (McKeever et al., 1999) and a decrease in some parameters related to exercise capacity (McKeever et al., 2002).

Another problem aged horses often encounter relates to inadequate dentition which can cause the animal to experience pain while eating. This pain can lead to decreased feed intake and severe dental defects can lead to a decreased ability to digest their food; however minor dental abnormalities do not seem to have the same negative effect on digestibility (Carmalt et al., 2008; Ralston et al., 2001). Still, care was taken to ensure that all horses on this study were able to masticate properly. The animals were also allowed a long diet adaptation period of five wk for complete adjustment to each diet. This was deemed especially important by investigators considering that one of the diets tested was high in fat. Fat supplementation has been associated with positive effects on horses exercising at a low intensity after supplementing for at least five

wk, suggesting that the animal is able to utilize dietary fat after five wk of supplementation (Pagan et al., 2002).

Diets all fell within NRC (2007) recommended values for intake of Ca, P, Mg, K, CP, and energy. All diets were lower than NRC recommendations for S; the CHO and HAY diets fell below recommended intake for Na; and the HAY diet was deficient in all microminerals measured excluding Mn. This lower than recommended intake of S and Se may have been one of the reasons for an observed age by diet by period interaction seen for fecal S and an age by period interaction for Se retention. The increase in amount fed on d 31 and a higher concentration of S in the FF concentrate also likely contributed to the observed age by diet by period interaction. Sulfur, an important component of organosulfur compounds, does not have known requirements for intake in the horse. Adequate consumption of dietary protein is thought to meet the needs of the animal (NRC, 2007). Given that the S intake of the horses was below NRC recommended intake yet a slightly positive S retention was calculated seems to indicate that NRC recommended S intake may be an overestimation of the horses needs. However, it is possible that if provided more dietary S the horse may retain more and the requirement was in reality not met. Given these data, it is clear more research needs to be done in this area to determine the true S requirements of the horse.

Selenium is an important component of many of the body's antioxidant systems, has a role in thyroid metabolism, and has been linked to immune function in humans (NRC, 2007; Fermaglich et al., 2002; McClain et al., 2002). Recommendations for dietary Se are established in the horse, and were met by the CHO and FF, but not the HAY diets (NRC, 2007). The diet by period interaction for Se retention was significant for aged horses during period 3, resultant from higher Se retention during that period. There were no age differences for Se, with the exception

of the trend for aged horses to have a lower percent apparent digestion compared to adult horses. Considering that this was a trend that was not seen for retention or percent retention, it seems that aged horses, unlike humans, may not have a need for supplementation of Se (Olivieri et al., 1994).

Public opinion has long been that aged horses need vitamin C supplementation, and previous research has indicated that aged horses have lower vitamin C blood concentrations compared to young horses (Ralston, 1988). Contributing to antioxidant functions and various enzymes, vitamin C comes in the form of L-ascorbic acid or dehydro-L-ascorbic acid, and is thought to be synthesizable from glucose (NRC, 2007). The requirements for vitamin C are not known in the horse, but are presumed to be satisfied by endogenous synthesis (Stillions et al., 1971). Humans do not have a different requirement of vitamin C associated with aging (National Academy of Sciences, 2004). Surprisingly, aged horses on this study had higher plasma vitamin C values compared to adult horses. This discrepancy compared to previous findings may be explained due to the fact that the 1988 Ralston study compared young horses (< 5 yr) to aged horses (≥ 20 yr), whereas this study focused on the differences between adult (5 to 12 yr) and aged horses (≥ 19 yr). Also, as lower plasma concentrations of vitamin C are known to be associated with compromised immune function and hormonal shifts (Brook et al., 1968), which was a problem with several of the aged horses used for the Ralston study, the values seen may have been understandably lower compared to the healthy animals used in the current study. Another contributing factor may have been the difference in laboratory analysis methods. Although both studies measured vitamin C concentrations in plasma, the 1988 study used a detection method from the 1976 Fundamentals of Clinical Chemistry (Hipolito and Shaw, 1976) whereas the current study utilized the more sensitive HPLC method, which is a validated method

for human plasma analysis (Tessier et al., 1996), as well as the method of choice for recent equine plasma analysis (White et al., 2001). Still, further research should be initiated in this area.

The current study did however have several limitations. All of the minerals were run on the ICP, which has matrix matched standard reference materials (SRM). The SRM of the feed is run with feed, urine run with urine etc. and they are typically certified by National Institute of Standards and Technology (NIST). Results are not reported unless they match the NIST value within their stated uncertainty. Reference materials used for the NIST standards are well homogenized and there for are easily prepared for analysis. However, real world samples typically have problems, such as feed matrixes that do not go totally into solution, and crystals in urine samples. Also many minerals, such as Na and Fe are notoriously hard to sample due to leaching of mineral from the containers they are stored in (as is the case with Na) or from the ground (as is the case with Fe). To mitigate this as much as possible, numbers from the ICP-MS are composed of an average of three mass spectra that are collected from each sample preparation, CV of which must all be below 1.25%, except for S which is 2.5%. Also to avoid as much variation as possible from sample and preparation, duplicate preparations were done.

Another limitation of the study was the estimation in urine output, especially during the first dietary period when the urine collection devices were changed from those obtained from the PMU farm to the new design. Once the new devices were utilized, urine losses were uncommon. There also was also an observed period effect, likely due to the necessary increase in feed. This effect did not however create diet or age interaction for percent retention or percent apparent digestion of any of the nutrients measured and therefore did not likely have an appreciable effect on digestibility.

Implications

In comparison to previous work, this study evaluated a larger number of healthy horses all of which had received regular anthelmintic treatment and had normal dentition. They were tested on three different commonly fed formulated diets (high roughage, high fat and fiber, and high cereal) and underwent a long diet adaptation period of five wk. This study suggests that under most practical feeding situations, differences in digestive capacity are unlikely to be present. However, it is important to note that the horses studied were all healthy, and nutrient requirements of compromised aged horses and those with dental disorders may differ.

CHAPTER 3

DIFFERENCES IN THE DIGESTIBILITY OF THREE COMMONLY FED DIETS

Abstract

The objective of this study was to compare digestibility of various feedstuffs, in particular the effect of a moderate fat diet on the digestibility of other nutrients. Seventeen healthy horses, 8 adult (5 to 12 yr) and 9 aged (19 to 28 yr) were studied. Horses were randomly assigned to diets: hay only (HAY), hay plus a carbohydrate-rich concentrate (CHO), and hay plus a fat and fiber-rich concentrate (FF). Three 6-wk diet periods consisted of three wk of outdoor group feeding and two wk of indoor stalled individual feeding, followed by a 72-h digestibility trial measuring feed intake and including collection of all feces and urine. Feed, fecal, and urine samples were analyzed on a g or mg/kg BW/d DM basis for macro and microminerals, CP, NDF, energy, and fat to determine apparent digestibility and retention. Blood samples were analyzed to determine blood vitamin C concentrations. The diets differed ($P < 0.05$) on intake for all macro and microminerals with the HAY diet having the lowest digestibility for all but K. Diets differed for fecal CP, with the CHO diet having a greater CP fecal output ($P = 0.01$) than the other two diets. The CHO diet was different from the other two diets with a lower intake and digestibility of NDF compared to the FF and HAY diets ($P < 0.01$) and the CHO and FF diets having lower fecal NDF than the HAY diet ($P = 0.01$), although none of the diets differed for percent NDF digested ($P = 14$). Gross energy did not differ between diets for intake ($P = 0.20$), however diets were different for fecal energy ($P = 0.03$), apparent digestible energy ($P = 0.02$), and percent apparent digestible energy ($P = 0.01$). The HAY diet was lower than the other two diets for fat intake, amount digested, and percent apparent digestibility ($P < 0.01$). No differences were seen between diets for blood vitamin C

concentrations ($P = 0.73$). The HAY diet consistently had a lower, often negative digestibility compared to the other two diets which, with the exception of some intake and digestibility values, were typically not different ($P > 0.05$). Given these data, it appears that horses may require supplementation if on a hay-only diet, and that moderate fat diets do not necessarily have a negative effect on the digestibility of other nutrients.

Introduction

The equine digestive system evolved to process a diet consisting primarily of forage. However, horses are often supplemented with concentrate feeds to provide additional energy for increased physical activity or to complement roughages lacking in specific nutrients (Hussein et al., 2004; Lewis et al., 1996). A common practice for increasing the energy density of feed has been top-dressing with oils or adding fat as part of the manufacturing process. There has been discussion about the effects of fat-supplemented diets in horses, with some studies suggesting no negative effects from fat supplementation (Kronfeld et al., 2004; Bush et al., 2001), and others demonstrating decreased fiber digestibility (Jansen et al., 2000). It has also been speculated that a fat-supplemented diet may have a similar effect in horses as it does in ruminants by decreasing Ca digestibility (Coppock et al., 1991). This study is one of the first to use total collection of both feces and urine to examine the effect of added fat on the digestibility of the aged, non-working horse. The objectives of this study were to examine possible differences in digestibility between three commonly-fed diets, as well as the potential effects of a moderate fat diet on the digestibility of various nutrients. Our hypothesis was that the three diets would be equally well digested and that a moderate fat diet would have no adverse effect on the digestibility of the nutrients tested.

Materials and Methods

Animals. Seventeen healthy mares were utilized, 12 of which originated from a Canadian facility and shared the same background as pregnant mare urine (PMU) mares. All the mares originating from Canada had been on a similar management protocol with similar feeding, vaccination, and anthelmintic schedules. The remaining five mares were obtained from outside sources to ensure all mares fell within the proper age groups and were chosen for similar body type, size, and BCS (Henneke et al., 1983). Two main groups were established, one with 9 adult horses (ages 5 to 12 yr, 500 ± 13 kg) and the other with 8 aged horses (ages 19 to 28 yr, 455 ± 12 kg). Within each age group, horses were striated by age and BCS and then randomly assigned to one of three test groups, each containing 3 adult and 3 elderly horses. All materials and methods were approved by Michigan State University's (MSU) Animal Care and Use Committee (approval #11/09-174-00).

The horses had an acclimation period of at least 6 wk (6 outside mares having a range of 2-6 wk adaptation time) to adjust to the change in climate as well as their new surroundings before beginning their first diet period. During this time they were kept in pastures at MSU's College of Veterinary Medicine's Bennett Farm facility and were supplemented once daily with free choice hay and had access to a trace mineralized salt block and free choice water. All animals were immunized shortly after arrival for eastern and western equine encephalomyelitis, influenza**,** rhinopneumonitis, strangles, tetanus, and rabies. All horses were vaccinated and dewormed with Ivermectin at least 2 wk prior to the start of their first diet period, with the exception of the two outside mares that were vaccinated and dewormed upon arrival. The horses underwent a dental examination under sedation to visually inspect the horses' mouths with the aid of a head stand and a speculum for head and mouth positioning. Those horses deemed in

need of dental work had their teeth floated by a licensed veterinarian. Fasting blood samples were taken for serum chemistry on d 40, 54, or 70 of period one. Records of vaccinations, dental work, blood work, and health certificates were housed at MSU's Research and Instructional Building.

Experimental Design. Following the acclimation period, horses were separated into their test groups and randomly assigned to pairs of 1 aged and 1 adult horse, within those groups. Pairs were then randomly assigned to a diet of hay alone (HAY), hay with a high fiber, high oil, low cereal starch concentrate (FF), and hay with a lower oil, high cereal starch concentrate (CHO). When beginning a diet, the horses were weighed, and fed 1.6% of body weight (BW) divided between two daily feedings. Horses on the CHO and FF diets had 0.6% BW of their diet composed of concentrate and 1.0% BW composed of hay. Horses on the HAY diet had all 1.6% of their BW fed as hay. On d 22 and 41 of each period, the horses were weighed and BCS ascertained by 3 trained individuals. It was determined that should the horses' BCS increase or decrease by 1 point during the study, the percentage DM fed would be adjusted up or down proportionally by 15% for hay and concentrate. Exact concentrate and hay amounts required were calculated and weighed out for each individual animal. Thirty-one days into the study it was determined that the amount fed of 1.6% BW was insufficient and was subsequently increased to 1.84% BW, as stated above. Horses on the CHO and FF diets had 0.69% BW of their diet composed of concentrate and 1.15% BW composed of hay. Horses on the HAY diet had all 1.84% of their BW fed as hay. Following this increase, it was concluded that one animal was still deficient, and her amount fed was increased by another 15%. To accommodate for the increase, this amount was split into three feedings versus two. The HAY and FF rations were obtained from one batch and the CHO rations obtained from two batches. HAY, FF and CHO

rations were stored in facilities at MSU's Veterinary Research Farm. The horses remained on their appointed diet for a period of 6 wk before starting on a different diet utilizing a modified Latin square design so that each horse received every diet. Upon commencement of a new diet treatment, horses transferring to the HAY diet were fed their full portion of hay. Horses moving to the FF and CHO diets were gradually adjusted to their new feed over 4 d. The horses were allowed ad libitum access to water.

Trace mineral blocks were removed at the beginning of the first diet period. Pairs were housed in a specific paddock for 3 wk of outdoor feeding, and each pair was fed the same diet. As the study began on the first d of January 2010, and was conducted during the pastures' dormant season, minimal grass was presumably consumed during this time. However, to avoid the possibility that horses may have had access to grass, horses were moved to MSU's Veterinary Teaching Hospital 2 wk prior to total collections and housed in 2.4 x 3 m box stalls. The temperature inside the facility was kept at 15 +/- 7° C. Indoor and outdoor temperatures, as well as outdoor weather conditions, were recorded daily throughout the study. The amount of feed and water consumed, as well as orts, was recorded. At least 3 d per wk while stalled, the horses were allowed turn-out in a dry lot pen for a minimum of 1 h. Following 2 wk of individual stalled feeding, a period of 3 d was used in which to carry out total collections of urine and feces (Lindenberg et al., 2006). Twenty-four hours prior to, as well as during, the total collection periods, feeding was performed every 12 h at 0800 and 2000.

Total Collections. A total collection of urine for the first period was accomplished utilizing a collection apparatus provided by the Canadian horses' previous owner. The collection apparatus was composed of a heavy plastic boot which was laid against the horses' buttocks and to the top of which was attached bungee cords that ran up to a suspension system about the

horses stalls. The bottom of the tube was fitted with a long rubber hose which ran between the horses legs and drained into a sealed container. For the remainder of the collection periods, the horses were fitted with a flexible plastic urine collection device which was attached by velcro on the point of the buttock on each side of the animal and acted as a funnel for the urine. Both collection devices were designed to prevent fecal contamination of urine, which drained via plastic tubing into sealed plastic containers. The animals were tied in their stalls, and monitored continuously for the 72-h collection period with the amount of feed and water consumed, approximate time required for consumption, and orts from each feeding recorded. Feces were gathered, and stored in a closed plastic bag as soon as possible after voided. Feces and urine were compiled every 8 h at 0800, 1600, and 2400. Total weight feces and total urine volume were recorded, and a 10% composite of each was saved, and frozen at -20° C for later analysis.

Concentrate and hay samples were collected weekly and composited for each of the 3 periods. They were tested for DM, Ca, P, Mg, Na, K, S, Ca, Cu, Co, Fe, Mn, Se, Zn, energy, NDF, CP, fat, and vitamin C. Concentrate and hay samples were also sent to Equi-analytical (Equi-analytical, Ithaca, New York) for analysis of ethanol soluble carbohydrates, water soluble carbohydrates, starch, and lignin. Likewise, water was sampled during each period, and tested for all aforementioned minerals. Blood was drawn from the jugular vein on d 40 of each period, and collected into 3 ml EDTA tubes for later analysis of plasma vitamin C concentrations.

After total collections, each animal was switched to a different diet and placed back at the beginning of a diet period until the animal was being tested on all diets. Following the conclusion of the study, horses were either placed in homes or utilized in different research studies.

Sample Preparation and Dry Matter Digestibility. All frozen fecal samples were thawed and oven dried at 105 °C (Thelco, Precision Scientific, Winchester, VA) for 24 h. Separate fecal samples were also freeze-dried. Feed samples were passed through a Wiley mill with a 2-mm screen. Ground feed and dried fecal samples were passed through a Cyclone mill with a 1-mm screen (Cyclotec 1093 Sample Mill, Foss, Eden Prairie, MN). All samples were analyzed in duplicate with accepted CVs set at 10% except for NDF, CP, which were set at 5%.

Energy. Feed and fecal energy content was determined using an oxygen bomb calorimeter (Parr Instrument Company Inc., Moline, IL; Appendix A). Heat of combustion was calculated to determine Mcal content of the feed and feces, which was used to evaluate daily Mcal intake, fecal energy, apparent digestible energy and percent apparent digestible energy. All values were then analyzed on a BW basis.

Calculations

$$\text{Heat of combustion (cal/g)} = \frac{tW - (\text{calories of wire burned})}{\text{g of pellet sample}}$$

t = temperature final – temperature initial

W = energy equivalent of the bomb used

Apparent digestible energy:

Gross energy intake-fecal energy

Percent apparent digestible energy:

$$\frac{\text{Gross energy intake-fecal energy}}{\text{Gross energy intake}} \times 100$$

NDF. Neutral detergent fiber was determined by the method created by Goering and VanSoost (Goering and VanSoest, 1970; Robertson and VanSoest, 1977). See Appendix A for a detailed description of laboratory analysis. Neutral detergent fiber content in the feed and feces were used to calculate daily NDF intake, fecal NDF, NDF digested, and percent NDF digestibility. All values were then analyzed on a BW basis.

Calculations

$$\text{NDF (g)} = \frac{\text{(crucible and NDF residue weight) – (crucible and ash residue weight)}}{\text{g of sample dry matter}}$$

NDF digested:

NDF intake-fecal NDF

Percent NDF digested:

$$\frac{\text{NDF intake-fecal NDF}}{\text{NDF intake}} \times 100$$

Crude Protein. Concentrate, hay, and fecal samples were analyzed for CP according to the micro-Kjeldahl procedure (Ma and Zuazaga, 1942; Appendix A) and read by a spectrophotometer at 460 nm (Gomori, 1942). Crude protein was determined by multiplying nitrogen values by 6.25 and was used to determine daily average g of CP intake in the concentrate and hay as well as average g of CP excreted in the feces, urine, and the combination of both feces and urine. These data were used to calculate CP retained, percent retained, and percent apparent digestibility. Data were then adjusted for the animals' BW for analysis on a g per kg BW basis.

CP

CP retained:

CP intake-CP output (feces and urine)

Percent retained:

$$\frac{\text{CP intake-CP output (feces and urine)}}{\text{CP intake}} \times 100$$

Percent apparent digestibility:

$$\frac{\text{CP intake-fecal CP}}{} \times 100$$

CP intake

Fat. Samples of feed and feces were ether extracted (Horowitz, 2000; Appendix A) to calculate fat disappearance. Fat disappearance from the feed and feces was used to calculate g/d fat intake, g/d fecal fat, g/d amount digested, and percent apparent digestibility. All values were then analyzed on a BW basis.

Fat

Amount digested was calculated:

$$\text{Fat intake-fecal fat}$$

Percent apparent digestibility was calculated:

$$\frac{\text{Fat intake-fecal fat}}{\text{Fat intake}} \times 100$$

Vitamin C. Blood samples were prepared immediately following collection by adding 800 µL metaphosphoric acid (1:5 dilution with DI water) to 200 mL plasma. They were then vortexed into solution and frozen at -80 °C for later HPLC analysis by MSU's Diagnostic Center for Population and Animal Health.

Macro and Microminerals. Feed and fecal samples were microwave digested for inductively coupled plasma mass spectrometry (ICP-MS) analysis according to the method determined by Shaw et al. (2002). For a more detailed description, see Appendix A.

Urine samples were not digested, but were vortexed thoroughly prior to being analyzed. Digested feed and fecal samples were run in duplicate with bovine liver standard (1577b; NIST, Gaithersburg, MD) as a control, and analyzed for P, Mg, Na, K, S, Co, Cu, Fe, Mn, Se, and Zn by ICP-MS. Non-acidified urine samples were run in duplicate, and analyzed for Na, K, S, Co, Cu, Fe, Mn, Se, and Zn by ICP-MS and for P by colorimetric assay. ICP-MS analysis was completed by MSU's Diagnostic Center for Population and Animal Health. The urine was prepared for P analysis (Appendix A), and concentrations determined by colorimetric assay

(Gomori, 1942). Urine Ca concentrations were also determined via atomic absorption spectrometry (IL Atomic Absorption Application Laboratory, 1972; Unicam 989, Thermo Electron Corp., Franklin, MA); samples were acidified with 600 µl of 12 M HCl and diluted 500X with 1 percent lanthanum chloride prior to running on the spectrometer. Concentrations of minerals were used to determine average g of mineral per d intake from the concentrate and hay as well as average g of mineral per d excreted in the feces, urine, and the combination of both feces and urine. Data was then adjusted for the animals' BW to determine mineral averages on a g/kg BW/d basis. Mineral balance was then calculated. Percent retention and percent digestion of Na, Fe, and Se were non-estimable due to lower than detectible concentrations in the hay.

Calculations

Mineral retained:

$$\text{Mineral intake-mineral output (in both feces and urine)}$$

Mineral percent retained:

$$\frac{\text{Mineral intake-mineral output (both feces and urine)}}{\text{Mineral intake}} \times 100$$

Mineral percent apparent digested:

$$\frac{\text{Mineral intake-mineral output (feces only)}}{\text{Mineral intake}} \times 100$$

Statistical Analysis. Data were analyzed using SAS software (Version 9.2, SAS Inst., Inc., Cary, NC). The PROC mixed function was used with group set as a random factor and period as the repeated measure with the subject of horse. Results are reported as least square means (± SEM) and are on a DM basis. Significance was declared at $P<0.05$ and trends at $P<0.10$. A power test was also performed and it was determined that a sample size of 9-12 horses was sufficient to detect differences at 80 percent power.

Results

Animals remained clinically healthy throughout testing. When comparing weights for the duration of the study, average BW did not differ between treatments, and the horses had a mean BCS of 5.0 ± 0.6. There were likewise no weight interactions between diet and age or diet and period.

DM digestibility. Intake and output data did not differ between CHO and FF diets (P = 0.64), nor did hay intake between the CHO and FF diets (P = 0.17). As expected, hay intake was greater on the HAY diet (P < 0.01). Fecal output was similar between treatments (P = 0.09), however urine output was different, with the highest output seen for the FF diet (P < 0.01) and no differences between the CHO and FF diets (P = 0.20). Results are presented in Table 2.1.

Energy, NDF, CP, fat, and vitamin C. Intake of GE did not differ between diets. Differences in fecal energy, apparent digestible energy and percent apparent digestible energy, however, were observed (P < 0.05). The CHO diet had lower fecal energy compared to the FF (P = 0.02) and HAY diets (P = 0.01) and a subsequently higher amount digested compared to the HAY (P = 0.01) but not FF diet (P = 0.71). The HAY and FF diets did not differ for fecal energy (P = 0.77), however, there was a trend for a difference between the two for percent apparent digestible energy (P = 0.06), and the FF diet had a higher apparent digestible energy compared to the HAY diet (P = 0.02). The CHO and FF diets were again not different for percent apparent digestible energy (P = 0.17). Intake and digestibility of NDF and intake, amount digested, and percent apparent digestibility of fat were all different between diets (P < 0.01). For NDF intake and NDF digested, the FF and HAY diets were higher compared to the CHO diet (P < 0.01), and the HAY diet was higher for fecal NDF than the CHO (P < 0.01) and FF diets (P = 0.02) which were not different. The percent digestibility of NDF did not differ between diets (P = 0.14).

Similar to what was observed with the differences seen for GE digestibility, for fat digestibility the HAY diet was lower compared to the other two ($P < 0.01$). The CHO and FF diets were different for intake and amount digested ($P < 0.01$), and there was a trend for a difference in percent apparent digestibility ($P = 0.055$) with the FF diet having the highest fat content for all parameters. No diet differences were seen for fecal fat ($P = 0.33$). There were no diet differences for CP digestibility, with the exception of fecal CP ($P < 0.01$) which was highest on the FF diet ($P < 0.05$). Neither were blood vitamin C concentrations different between diets ($P = 0.73$). Results are presented in Table 2.2.

Macrominerals. Results are presented by macromineral in Table 2.3. Calcium had intake and fecal output ($P = 0.01$) diet differences which were also seen for retention ($P < 0.01$), but not for percent retention ($P = 0.13$), although there was a trend for percent apparent digestibility to differ between diets ($P = 0.09$). The HAY diet was lower compared to the other 2 diets for intake and fecal Ca, but was lower only than FF for retention ($P < 0.01$). The FF diet was higher than the other two diets in Ca intake and retention ($P < 0.05$), but did not differ from CHO for fecal Ca ($P = 0.99$). Intake values for P were different between diets ($P < 0.01$) and were present for all parameters measured, with the exception of urinary P. For P intake, fecal P, and retention, HAY was lower than FF which was lower than CHO ($P < 0.05$). Retention, percent retention, and percent apparent retention of P for the HAY diet only were all negative. Mg had intake and fecal output ($P = 0.01$) diet differences, as well as retention ($P < 0.01$), but not for percent retention ($P = 0.13$), although there was a trend for percent apparent digestibility to differ between diets ($P = 0.09$). There was also a difference for urine Mg ($P < 0.01$). Diet differences for Mg consistently showed HAY to have lower values ($P < 0.05$) than CHO, which had lower values compared to FF ($P < 0.05$), with the exception of Mg retention where a trend was seen for

differences between the CHO and FF diets ($P = 0.07$). Sodium intake, fecal, and urine output were different between diets ($P < 0.01$), but Na retention was not ($P = 0.41$). For Na intake HAY was lower than CHO which was lower than FF ($P < 0.01$). For fecal Na, HAY was lower than FF which was lower than CHO ($P < 0.05$). Urinary Na was not different for HAY compared to CHO ($P = 0.73$), which were both lower than FF ($P < 0.01$). Retention and percent retention were not different between diets for Na, however CHO was lower compared to the other two diets from Na percent apparent digestion (P=0.002). Potassium was the only macromineral that showed differences in intake ($P =0.02$), fecal and urine output ($P < 0.01$), and percent digestion ($P < 0.01$) but not for retention ($P = 0.52$) or percent retention ($P = 0.33$). For intake, urinary K, and K percent digestion, HAY and FF did not differ and were higher than CHO ($P < 0.05$). The CHO and HAY diets did not differ for fecal K ($P = 0.29$) and were higher than the FF diet ($P < 0.05$). Intake values for S were also different between diets ($P < 0.01$) and were present for all parameters measured. Similarly, for most S parameters measured, the HAY diet was lower than the other two diets ($P < 0.05$). The only exceptions were fecal S where HAY and CHO did not differ ($P = 0.65$), and S percent retention where there was a trend for the FF diet to differ from the HAY ($P = 0.07$). Retention and percent retention, but not percent apparent digestibility, of S with the HAY diet was negative. The CHO and FF diets were typically not different ($P > 0.05$), although for fecal S, the FF diet was higher than the CHO ($P < 0.01$), and for S retention and percent retention FF was higher than the CHO ($P < 0.05$). When analyzing Ca and S, intake and P, Mg, Na, and K fecal output, a diet by period interaction was observed ($P < 0.05$). A 3-way age by diet by period interaction was also seen for fecal output of S ($P < 0.04$).

Microminerals. Results are presented by micromineral in Table 2.4. Values for Co differed between diets and were present for all parameters measured ($P < 0.01$). For Co, the

HAY and FF diet were never different ($P > 0.05$), exhibited negative retention, percent retention, percent apparent digestibility, and were always lower compared to CHO ($P < 0.05$) which did not have a negative retention. Copper had differences for intake, fecal output, retention, and percent retention ($P < 0.05$), but no differences in urine output or percent digestion. Again, HAY was lower than the other two diets ($P < 0.05$). The CHO and FF diets were different ($P < 0.05$) for Cu intake and retention with CHO having the higher values, but were not different for fecal Cu. With Cu percent retention, the HAY diet was the lower than CHO ($P = 0.01$), but the FF diet was not different from either the CHO ($P = 0.11$) or the HAY diets ($P = 0.31$). Iron had differences for intake and fecal output ($P \leq 0.01$), but not urine output. For Fe intake and fecal Fe, all diets were different with the HAY diet having the lowest values, followed by CHO, which was followed by FF ($P < 0.05$). Retention and percent apparent digestion of Fe were not different between diets, however percent retention was different ($P < 0.01$). For Fe percent retention the HAY diet had the highest percent retention compared the CHO diet, which was higher compared to the FF diet. Manganese intake, fecal output, and retention were different between diets ($P < 0.01$), but no differences were observed for urine output ($P = 0.53$). The HAY diet always had the lowest values, as well as being the only diet to have a negative retention, percent retention and percent apparent digestibility. For intake of Mn, all diets were different, with CHO having the highest values ($P < 0.05$). With fecal Mn and retention, the CHO was still higher, but the FF and HAY diets were not different ($P > 0.05$). Values for Se differed between diets and were present for most parameters measured. Selenium intake and fecal and urinary Se exhibited differences between all diets ($P < 0.05$). For intake, the lowest values were observed for CHO, followed by HAY, and with FF having the highest values. For fecal Se, the lowest values were seen for HAY, followed by FF and then CHO. For Se retention, the FF diet was not different

compared to the HAY. The CHO diet was higher compared to both the FF and HAY diets. Selenium percent retention was different between diets, with HAY being the highest compared to FF, which was higher compared to CHO ($P < 0.01$). Percent Se apparent digestion was not different between diets. Zinc had differences for all parameters measured ($P \leq 0.01$), with the exception of urine output ($P > 0.05$). Zinc intake and fecal Zn had the same diet differences with HAY having the lowest values followed by FF and the CHO having the largest values ($P < 0.05$). However, for Zn retention, percent retention and percent apparent digestion, the FF and CHO diets were no longer different, although the HAY diet was negative and lower than the other two ($P < 0.05$), which were not negative. For Co and Fe retention, Cu intake, and Se fecal output a diet by period interaction was observed ($P < 0.05$).

Table 2.1: DM intake, output, and weight data for three different diets (CHO, FF, and HAY)

	CHO	FF	HAY	P-value
Intake and output (g/kg BW/d)				
Avg Concentrate Intake	$7.46^{b} \pm 0.69$	$7.15^{b} \pm 0.63$	$-0.01^{a} \pm 0.65$	*<0.001*
Avg Hay Intake	$10.49^{a} \pm 0.57$	$11.29^{a} \pm 0.51$	$17.31^{b} \pm 0.53$	*<0.001*
Avg Fecal Output	6.89 ± 0.32	7.45 ± 0.28	7.67 ± 0.29	*0.09*
Avg Urine Output (*ml/kg BW/d)*	$10.63^{a} \pm 0.91$	$16.04^{b} \pm 0.80$	$11.88^{a} \pm 0.83$	*<0.01*
Avg Wt (kg)	481 ± 9	477 ± 9	474 ± 9	*0.07*
Median BCS	4.9 ± 0.2	5.1 ± 0.2	4.8 ± 0.2	*0.26*

[ab] *Means within rows with differing superscripts differ*

Table 2.2: Energy, neutral detergent fiber, crude protein, fat digestibility, and blood vitamin C concentrations for 3 different diets

	CHO	FF	HAY	P-value
Energy (Mcal/kg BW/d)				
Intake	0.080 ± 0.004	0.084 ± 0.003	0.078 ± 0.003	*0.20*
Fecal	0.032^{a} ± 0.002	0.037^{b} ± 0.001	0.037^{b} ± 0.002	*0.03*
Apparent Digestible Energy	0.048^{b} ± 0.003	0.047^{b} ± 0.003	0.040^{a} ± 0.003	*0.02*
% Apparent Digestible Energy	59.65^{b} ± 1.72	$56.45^{a,b}$ ± 1.50	52.14^{a} ± 1.56	*0.01*
NDF (g/kg BW/d)				
Intake	8.37^{a} ± 0.54	10.12^{b} ± 0.50	10.95^{b} ± 0.51	*<0.01*
Fecal	4.30^{a} ± 0.23	4.75^{a} ± 0.21	5.29^{b} ± 0.21	*<0.01*
NDF Digested	4.00^{a} ± 0.45	5.37^{b} ± 0.42	5.61^{b} ± 0.43	*<0.01*
% NDF Digestibility	47.98 ± 2.18	53.21 ± 1.93	50.77 ± 2.01	*0.14*
CP (g/kg BW/d)				
Intake	2.53 ± 0.11	2.73 ± 0.10	2.55 ± 0.10	*0.12*
Fecal	0.61^{a} ± 0.04	0.67^{b} ± 0.03	0.58^{a} ± 0.04	*<0.01*
Urinary	0.40 ± 0.04	0.39 ± 0.03	0.36 ± 0.03	*0.68*
Retained	1.50 ± 0.09	1.64 ± 0.08	1.60 ± 0.08	*0.45*
% Retained	60.09 ± 2.14	59.80 ± 1.87	61.53 ± 1.94	*0.80*
% Apparent Digested	76.18 ± 1.03	74.53 ± 0.90	76.93 ± 0.93	*0.11*
Fat (g/kg BW/d)				
Intake	0.77^{b} ± 0.05	1.00^{c} ± 0.04	0.62^{a} ± 0.04	*<0.01*
Fecal	0.39 ± 0.03	0.42 ± 0.03	0.38 ± 0.03	*0.33*
Amount Digested	0.37^{b} ± 0.03	0.58^{c} ± 0.03	0.24^{a} ± 0.03	*<0.01*
% Apparent Digestibility	49.03^{b} ± 3.16	57.42^{b} ± 2.76	38.94^{a} ± 2.86	*<0.01*
Vitamin C blood concentrations (mg/dL)				
Vitamin C	1.15 ± 0.05	1.15 ± 0.04	1.12 ± 0.04	*0.73*

[ab] *Means within rows with differing superscripts differ*

Table 2.3: Macromineral digestibility for three different diets

	CHO	FF	HAY	P-value
Calcium (g/kg BW/d)				
Intake	$0.17^{b} \pm 0.01$	$0.20^{c} \pm 0.01$	$0.14^{a} \pm 0.01$	*<0.01**
Fecal	$0.100^{b} \pm 0.007$	$0.100^{b} \pm 0.006$	$0.074^{a} \pm 0.006$	*<0.01*
Urinary	0.020 ± 0.004	0.024 ± 0.003	0.020 ± 0.003	*0.58*
Retained	$0.047^{a} \pm 0.009$	$0.078^{b} \pm 0.008$	$0.042^{a} \pm 0.009$	*<0.01*
% Retained	29.21 ± 4.23	38.83 ± 3.64	29.23 ± 3.81	*0.13*
% Apparent Digested	41.20 ± 3.20	50.62 ± 2.80	44.69 ± 2.90	*0.09*
Phosphorus (g/kg BW/d)				
Intake	$0.073^{c} \pm 0.005$	$0.057^{b} \pm 0.004$	$0.028^{a} \pm 0.005$	*<0.01*
Fecal	$0.062^{c} \pm 0.004$	$0.050^{b} \pm 0.004$	$0.028^{a} \pm 0.004$	*<0.01**
Urinary	0.005 ± 0.001	0.007 ± 0.001	0.006 ± 0.001	*0.06*
Retained	$0.00617^{c} \pm 0.00253$	$0.00001^{b} \pm 0.00232$	$-0.00674^{a} \pm 0.00238$	*<0.01*
% Retained	$9.20^{b} \pm 6.83$	$-3.49^{b} \pm 6.28$	$-27.17^{a} \pm 6.44$	*<0.01*
% Apparent Digested	$15.85^{b} \pm 5.25$	$9.96^{b} \pm 4.75$	$-5.15^{a} \pm 4.90$	*<0.01*
Magnesium (g/kg BW/d)				
Intake	$0.041^{b} \pm 0.003$	$0.052^{c} \pm 0.003$	$0.023^{a} \pm 0.003$	*<0.01*
Fecal	$0.027^{b} \pm 0.002$	$0.033^{c} \pm 0.002$	$0.015^{a} \pm 0.002$	*<0.01**
Urinary	$0.0048^{b} \pm 0.0006$	$0.0064^{c} \pm 0.0005$	$0.0032^{a} \pm 0.0005$	*<0.01*
Retained	$0.009^{b} \pm 0.002$	$0.013^{b} \pm 0.002$	$0.004^{a} \pm 0.002$	*<0.01*
% Retained	23.29 ± 4.76	23.53 ± 4.20	16.19 ± 4.37	0.34
% Apparent Digested	34.99 ± 3.86	35.85 ± 3.42	30.65 ± 3.55	0.45
Sodium (g/kg BW/d)				
Intake	$0.027^{b} \pm 0.002$	$0.050^{c} \pm 0.002$	$0.013^{a} \pm 0.002$	*<0.01*
Fecal	$0.020^{b} \pm 0.002$	$0.024^{c} \pm 0.002$	$0.006^{a} \pm 0.002$	*<0.01**
Urinary	$0.006^{a} \pm 0.001$	$0.025^{b} \pm 0.001$	$0.006^{a} \pm 0.001$	*<0.01*
Retained	0.0016 ± 0.0021	0.0011 ± 0.0018	0.0001 ± 0.0019	*0.89*
% Retained	5.8 ± 10.6	0.8 ± 9.0	-1.5 ± 9.4	*0.88*
% Apparent Digested	$25.9^{a} \pm 5.4$	$53.0^{b} \pm 4.7$	$49.8^{b} \pm 4.9$	*<0.01*
Potassium (g/kg BW/d)				
Intake	$0.25^{a} \pm 0.01$	$0.29^{b} \pm 0.01$	$0.28^{b} \pm 0.01$	*0.02*
Fecal	$0.071^{b} \pm 0.004$	$0.064^{a} \pm 0.003$	$0.074^{b} \pm 0.004$	*<0.01**
Urinary	$0.11^{a} \pm 0.02$	$0.16^{b} \pm 0.01$	$0.15^{b} \pm 0.02$	*<0.01*
Retained	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	*0.52*
% Retained	27.75 ± 6.52	22.47 ± 6.15	19.13 ± 6.26	*0.33*
% Apparent Digested	$71.06^{a} \pm 1.42$	$78.07^{b} \pm 1.25$	$73.67^{b} \pm 1.30$	*<0.01*

Table 2:3. (cont'd)

Sulfur (g/kg BW/d)				
Intake	$0.027^{b} \pm 0.002$	$0.028^{b} \pm 0.002$	$0.016^{a} \pm 0.002$	*<0.01**
Fecal	$0.0113^{a} \pm 0.0005$	$0.0136^{b} \pm 0.0005$	$0.0110^{a} \pm 0.0005$	*<0.01‡*
Urinary	$0.0107^{b} \pm 0.0008$	$0.0127^{b} \pm 0.0007$	$0.0065^{a} \pm 0.0007$	*<0.01*
Retained	$0.004^{c} \pm 0.002$	$0.001^{b} \pm 0.002$	$-0.001^{a} \pm 0.002$	*<0.01*
% Retained	$16.13^{c} \pm 8.67$	$0.90^{b} \pm 8.19$	$-12.31^{a} \pm 8.33$	*<0.01*
% Apparent Digested	$56.63^{b} \pm 4.46$	$49.88^{b} \pm 4.23$	$30.06^{a} \pm 4.30$	*<0.01*

^{ab} *Means within rows with differing superscripts differ*

‡ *Age by diet by period interaction*

***** *Diet by period interaction*

Table 2.4: Micromineral digestibility for three different diets (CHO, FF, and HAY)

	CHO	FF	HAY	P-value
Cobalt (mg/kg BW/d)				
Intake	$0.0110^{b} \pm 0.0007$	$0.0013^{a} \pm 0.0006$	$0.0003^{a} \pm 0.0007$	*<0.01*
Fecal	$0.0095^{b} \pm 0.0007$	$0.0015^{a} \pm 0.0006$	$0.0006^{a} \pm 0.0007$	*<0.01*
Urinary	$0.00012^{b} \pm 0.00002$	$0.00003^{a} \pm 0.00002$	$0.00001^{a} \pm 0.00002$	*<0.01*
Retained	$0.0014^{b} \pm 0.0001$	$-0.0002^{a} \pm 0.0001$	$-0.0003^{a} \pm 0.0001$	*<0.01**
% Retained	$14.28^{b} \pm 16.10$	$-19.78^{a} \pm 14.10^{b}$	$-96.90^{a} \pm 14.70$	*<0.01*
% Apparent Digested	$15.32^{b} \pm 15.8239$	$-17.22^{a} \pm 13.90^{b}$	$-93.73^{a} \pm 14.47$	*<0.01*
Copper (mg/kg BW/d)				
Intake	$0.44^{c} \pm 0.03$	$0.35^{b} \pm 0.03$	$0.09^{a} \pm 0.03$	*<0.01**
Fecal	$0.38^{b} \pm 0.03$	$0.32^{b} \pm 0.03$	$0.09^{a} \pm 0.03$	*<0.01*
Urinary	0.00055 ± 0.00008	0.00074 ± 0.00007	0.00056 ± 0.00007	*0.1*
Retained	$0.059^{c} \pm 0.009$	$0.029^{b} \pm 0.008$	$0.004^{a} \pm 0.008$	*<0.01*
% Retained	$14.42^{b} \pm 3.92$	$6.75^{a,b} \pm 3.48$	$2.21^{a} \pm 3.61$	*0.05*
% Apparent Digested	14.53 ± 3.83	7.06 ± 3.40	2.87 ± 3.53	*0.06*
Iron (mg/kg BW/d)				
Intake	$2.3^{b} \pm 0.2$	$3.2^{c} \pm 0.2$	$0.7^{a} \pm 0.2$	*<0.01*
Fecal	$2.31^{b} \pm 0.27$	$3.21^{c} \pm 0.25$	$0.86^{a} \pm 0.26$	*<0.01*
Urinary	0.0009 ± 0.0002	0.0014 ± 0.0002	0.0010 ± 0.0002	*0.12*
Retained	0.01 ± 0.14	0.02 ± 0.12	-0.17 ± 0.13	*0.50*
% Retained	$-230.9^{b} \pm 28.4$	$-320.4^{a} \pm 26.4$	$-85.4^{c} \pm 27.0$	*<0.01**
% Apparent Digested	1.1 ± 14.5	-0.3 ± 12.6	-30.8 ± 13.1	*0.16*
Manganese (mg/kg BW/d)				
Intake	$1.12^{c} \pm 0.06$	$0.50^{b} \pm 0.05$	$0.35^{a} \pm 0.05$	*<0.01*
Fecal	$0.96^{b} \pm 0.07$	$0.46^{a} \pm 0.06$	$0.36^{a} \pm 0.06$	*<0.01*
Urinary	0.00008 ± 0.00002	0.00010 ± 0.00002	0.00007 ± 0.00002	*0.53*
Retained	$0.15^{b} \pm 0.03$	$0.03^{a} \pm 0.03$	$-0.01^{a} \pm 0.03$	*<0.01*
% Retained	15.23 ± 6.99	5.73 ± 6.11	-8.60 ± 6.34	*0.05*
% Apparent Digested	15.23 ± 6.99	5.73 ± 6.11	-8.60 ± 6.34	*0.05*
Selenium (mg/kg BW/d)				
Intake	$0.0112^{c} \pm 0.0007$	$0.0042^{b} \pm 0.0007$	$0.0012^{a} \pm 0.0007$	*<0.01*
Fecal	$0.0050^{c} \pm 0.0003$	$0.0024^{b} \pm 0.0003$	$0.0006^{a} \pm 0.0003$	*<0.01**
Urinary	$0.0037^{c} \pm 0.0003$	$0.0015^{b} \pm 0.0003$	$0.0005^{a} \pm 0.0003$	*<0.01*
Retained	$0.0023^{b} \pm 0.0002$	$0.0003^{a} \pm 0.0002$	$0.0001^{a} \pm 0.0002$	*<0.01*
% Retained	$-0.87^{a} \pm 0.06$	$-0.39^{b} \pm 0.05$	$-0.11^{c} \pm 0.06$	*<0.01*
% Apparent Digested	$55.2^{b} \pm 5.4$	$38.0^{a} \pm 4.6$	$41.4^{a} \pm 4.8$	*0.09**

Table 2:4. (cont'd)

Zinc (mg/kg BW/d)				
Intake	$1.37^{c} \pm 0.10$	$0.94^{b} \pm 0.09$	$0.24^{a} \pm 0.09$	*<0.01*
Fecal	$1.23^{c} \pm 0.11$	$0.87^{b} \pm 0.10$	$0.28^{a} \pm 0.10$	*<0.01*
Urinary	0.003 ± 0.002	0.002 ± 0.001	0.004 ± 0.001	*0.68*
Retained	$0.13^{b} \pm 0.03$	$0.06^{b} \pm 0.03$	$-0.04^{a} \pm 0.03$	*<0.01*
% Retained	$12.11^{b} \pm 6.58$	$3.51^{b} \pm 5.87$	$-21.87^{a} \pm 6.08$	*<0.01*
% Apparent Digested	$12.16^{b} \pm 5.72$	$3.87^{b} \pm 5.09$	$-19.68^{a} \pm 5.27$	*<0.01*

^{ab} *Means within rows with differing superscripts differ*

***** *Diet by period interaction*

Discussion

Several factors can influence nutrient digestibility including source of the nutrients and accompanying availability, as well as the amount of the nutrients available (Olsman et al., 2004; Kienzle et al., 2002; Hintz et al., 1971). As the nutrient amounts increase, digestibility can decrease. This typically occurs if the amount provided exceeds the horse's requirements, and absorption is decreased as a result (Glade, 1984). Alternatively, as the amount increases, the apparent digestibility can increase as a result of endogenous secretions constituting a smaller fraction of the nutrients found in the feces (NRC, 2007; Asano et al., 2002). Interactions between feedstuffs are also considered to be important, especially with low-digestible roughages (Kienzle et al., 2002). As addition of fat and oils, extruded feeds, pelleted feeds, mineral supplementation, and a better understanding of fiber and energy requirements have developed over the years, (Harris, 1998); there is still a significant amount of research to be explored for equine nutrition.

Values seen for apparent digestible energy were similar to what has been observed in previous work (Lindberg et al., 2001, VanWeyenberg et al., 2007). Although gross energy intake was similar between diets, the percent apparent energy digestibility was greater with the CHO diet compared to HAY. As with many of the macro and microminerals, as intake increased, so did digestibility, although the CHO and FF diets often did not differ in respect to percent retention or percent apparent digestibility. This suggests the source, instead of the quantity, influenced energy digestibility.

It has been seen that as NDF content increases, digestibility decreases (Glade, 1983). The NDF fraction contains cellulose, most hemicelluloses, and lignin and digestion of monosaccharides, for example starch and maltose, occurs in the small intestine and providing more energy than those carbohydrates digested by microbes (NRC, 2007). For this study, while

NDF intake was lower with the CHO diet, and NDF digested was greater for the FF and HAY diets, the percent digestibility did not differ between any of the diets. Considering that the horse is very efficient at digesting various sources of fiber (Staniar et al., 2010; Eckert et al., 2010; Oradakowke-Burk et al., 2006), these results are not surprising.

Although nutrient concentrations varied between diets, this did not always produce consistent differences in digestibility. As seen by Kronfeld et al. (2004) and Bush et al. (2001), the efficiency of fat digestion increased with greater dietary fat load. This study observed similar results with the FF diet having the greatest digestibility compared to the other two diets, although it was not greater than the CHO diet for percent amount digested. This increase in fat digestibility with an increase in dietary fat is not unexpected, as the horse seems to be able to digest fat very easily with up to 15% added fat tolerated (NRC, 2007). Previous research on the effect of supplemental fat has been mixed with some studies exhibiting a reduced fiber digestibility (Jansen et al., 2000), and some suggesting no negative effects from fat supplementation (Kronfeld et al., 2004; Bush et al., 2001). It has also been speculated that a fat-supplemented diet may have a similar effect in horses as it does in ruminants by decreasing Ca digestibility (Coppock et al., 1991). This study did not observe any negative effects of fat digestibility on NDF or Ca digestibility; in fact, as stated above, the FF diet seemed to have a positive effect on Ca. This study was unique in that the animals were also allowed a long diet adaptation period of five wk for complete adjustment to each diet. This was deemed especially important by investigators considering that one of the diets tested had a moderate fat content. Fat supplementation has been associated with positive effects on horses exercising at a low intensity after supplementing for at least 5 wk, suggesting that the animal is able to utilize dietary fat after five wk of supplementation (Pagan et al., 2002). Also, the horses used for this study, in contrast

to the previous work done by Kronfeld et al. (2004) and Jansen al. (2000), were not exercised. Also in contrast, Kronfeld et al. (2004), Bush et al. (2001), and Jansen al. (2000) only carried out collection of feces; this study utilized total collections of both feces and urine. Therefore, it appears for the non-working healthy adult horse there are no negative effects of fat supplementation with the moderate amount present in the FF concentrate used for this study.

For adequate CP digestion, which occurs in the forgut via stomach and small intestine enzymes, the horse must have adequate energy intake (NRC, 2007; Glade, 1983). As with fat, increasing CP concentrations in the diet increases CP digestibility, and the CP source does not seem to have as great of an impact on digestibility (NRC, 2007; Gibbs et al., 1988). The 2007 NRC recommends that the mature horse consume a minimum of 1.08 g/kg BW/d CP. For this study, all diets met this requirement. No differences were seen for CP digestibility, and results are similar to previous work (Eckert et al., 2010; Lindberg et al., 2006; Crozier et al., 1997), suggesting that the diets were all sufficient and met the needs of the animal.

Contributing to antioxidant functions and various enzymes, vitamin C comes in the form of L-ascorbic acid or dehydro-L-ascorbic acid, and is thought to be synthesizable from glucose (NRC, 2007). The requirements for vitamin C are not known in the horse, but are presumed to be satisfied by endogenous synthesis (Stillions et al., 1971). For this study, no diet differences for blood vitamin C values were observed.

In this study, all diets were within NRC (NRC, 2007) recommended values for intake for Ca, Mg, K, CP, and GE. All diets were lower than NRC recommendations for S; and the HAY diet was deficient in P, Na, and for all microminerals measured excluding Mn. In the case of P, S, Co, Fe, Mn, and Zn, the negative retention suggests that horses were not receiving adequate amounts of those minerals in their diet (NRC, 2007). Hence, it would appear that horses, on an

all-hay diet of similar composition and at similar amounts to which were fed in this study, may not be able to meet their requirements. It is important to note that if horses had been able to have ad libitium access to concentrate and hay, the observed negative retentions may not have been detected, considering that multiple studies have found that the horse has no major differences in the digestibility P, Na, Fe, Mn, and Zn when comparing different types of all hay diets (Staniar et al., 2010; Ordakowski-Burk et al., 2006). A study by Crozier et al. (1997) comparing alfalfa, tall fescue, and caucasian bluestem hays found that as long as the grass hays are eaten as 100% of the diet, diets came close to meeting NRC requirements for most minerals. Some supplementation with P, S, Cu, and crude protein may be required for caucasian bluestem hay, although it was the only hay tested that met the 2007 NRC Zn requirement. However, ramifications of negative retentions are unknown for several of the afore mentioned minerals due to the limited research on S and many microminerals in horses (NRC, 2007; Siciliano, 2002). At least for the other macrominerals reported on in this study, adequate amounts appeared to be present for the mature horse.

The 2007 NRC provides recommendations for daily intake of all of the macro and microminerals examined for this study. The minimum amount of Ca recommended is 0.04 g/kg BW/d. Calcium is said to be 50% absorbed in the small intestine, and digestibility is positively affected by increases in dietary Ca, and competes with P for absorption (NRC, 2007; Nielsen et al., 1997). It has roles in bone and in cell membranes active in blood clotting and enzyme regulation (NRC, 2007). For this study, Ca had diet differences for intake and fecal output which continued for retention, but not for percent retention, although there was a trend for percent apparent digestibility ($P = 0.09$) to differ between diets. Diets lower in Ca intake tended to have lower Ca digestibility, similar to previous work (Nielsen et al., 1997). However, the FF diet did

not appear to have a negative influence on Ca absorption. This effect is contrary to what has been previously seen in cattle, where increasing the fat content of the feed had a negative effect on Ca absorption (Coppock et al., 1991), although similar results have not been seen in horses (Meyer et al., 1997). This is a very interesting finding as it indicates that Ca absorption is not negatively affected by a moderate fat diet in horses.

Phosphorus is recommended at an intake of 0.028 g/kg BW/d and is 30-55% absorbed in small intestine of the horse (NRC, 2007). Its absorption is negatively affected by increasing dietary Ca and positively affected by increasing dietary P (Cymbaluk, 1990). An integral component of bone, P also has roles in energy transfer reactions and synthesis of phospholipids, nucleic acids, and phosphor proteins (NRC, 2007). Differences were present for all parameters measured of P digestibility, with the exception of urinary P, and negative values were seen for retention, percent retention, and percent apparent retention of P for the HAY diet. The high Ca:P ratio of almost 5:1 for intake compared to the ideal ratio of 2:1 (NRC, 2007) for the HAY diet and competition between Ca and P may have been the reason for the depressed P retention seen for that diet. Negative percent retentions and sometimes negative percent digestions that appear contrary to positive retentions, were due to the nature of the equations used to determine them; as dividing by such small intake numbers, in particular those that were frequently seen for the HAY diet, inflated the percent values observed. This inflation did not appear to have an unexpected effect on significance of any of the percent retentions or percent apparent digestions in that their corresponding standard errors were high and they followed an expected pattern for the data.

Absorbed 40 to 60% mostly in the small intestine with some absorption in the large intestine (NRC, 2007), Mg is an important macromineral in the equine skeleton, muscle, and various enzymes. Its absorption is negatively affected by increased P and is recommended at a

intake of 0.015 g/kg BW/d (NRC, 2007; Hintz et al., 1972). Differences in Mg intake were seen for retention, but not for percent retention, or percent apparent digestibility. Differences for Mg consistently showed the HAY diet having lower values. The observed absorption of Mg was slightly lower than the NRC (2007) and Hintz et al. (1972) reported values for low P diet which were similar to the high P diet. However, the low P diet consisted of 0.04 g/kg BW which was similar to the intake on this study and the high P diet of 0.20 g/kg BW. Considering these data, it seems that the lower absorption of Mg seen was not due to the P content of the diet which was on the low end of the 2007 NRC recommended range. The reason for this lower digestibility is therefore not known.

Functioning in the central nervous system, cross membrane transport, acid/base balance, osmotic regulation, and as an extracellular cation, approximately 48 to 84% of Na is absorbed in large intestine (NRC, 2007; Schryver et al., 1987). The NRC recommends at least 0.02 g/kg BW/d in the animals' diet. Sodium intake was different between diets, with the FF diet having the highest intake; this did not carry over to a difference in Na retention. This was due to the high Na urinary output which also was the probable cause of the higher urine output of the horses on the FF diet. High Na intake has not been seen to have adverse effects on mineral metabolism (Schryver et al., 1987). The HAY diet fell below NRC recommended intake values.

Potassium absorption is influenced by time, exercise, concentration of K in feed and is absorbed 61 to 75% (NRC, 2007). An important cation with roles in the horse's acid/base balance, osmotic pressure, and neuromuscular excitability, the 2007 NRC recommends intake of at least 0.05 g/kg BW/d. Potassium showed differences in intake and percent digestion, but not for retention or percent retention. The lack of differences was due to differences in fecal and urinary K. For intake, urinary K, and K percent digestion, HAY and FF tended to be higher than

CHO, although the CHO and HAY diets did not differ for fecal K and were higher than the FF diet. All values were within expected ranges and were similar to previous work (Staniar et al., 2010; NRC, 2007).

While the Co requirements are not known in the horse, the 2007 NRC recommends an intake of 0.001 mg/kg BW/d. The main function of Co is to synthesize vitamin B_{12} (Salmien, 1975). Values for Co differed between diets and were present for all parameters measured. The HAY and FF diet did not differ and exhibited negative retention, percent retention, and percent apparent digestibility. They were always lower compared to CHO ($P < 0.05$) which did not have a negative retention. The HAY diet was the only diet to fall below 2007 NRC recommended values, but the FF diet, which also had a negative retention, did not. This may suggest that the recommendations for Co may need to be raised.

Several microminerals influence the absorption of Cu; normally 24 to 40%. Cadimum, Mo, Zn, Se, Ag, Fe, and Pb can all interact with Cu, which has roles in forming enzymes, synthesizing and maintaining connective tissue, mobilizing Fe stores, preserving the integrity of mitochondria, helping with melanin synthesis, and aids in the detoxification of superoxides (NRC, 2007; Young et al., 1987; Cymbaluk et al., 1986). Copper is recommended at 0.2 mg/kg BW/d in the diet (NRC, 2007). Copper had differences for intake that translated to differences in retention and percent retention, but no differences in percent digestion. Similar to many of the other micro and macrominerals, the HAY diet was lower than the other two diets. Retentions were positive, close to 0, and similar to previous work (Cymbaluk et al., 1981) for all three diets, suggesting that the dietary concentrations were sufficient.

Iron is typically poorly absorbed by the horse, reportedly 15% or less (NRC, 2007). Its absorption can be affected by the concentration of Fe, Cd, Co, Cu, Zn, and Mn. Present in

hemoglobin, myglobin, cytochromes, Fe also has a role in many enzyme systems. Endogenous losses are not known, but dietary Fe is recommended at 0.8 mg/kg BW/d (NRC, 2007). For the current study, differences were seen for most parameters measured. The HAY diet typically had the lowest values, followed by CHO which was followed by FF. The HAY diet fell below NRC recommended values and retention of Fe was negative for the HAY diet. This was likely due to confounding affects from other microminerals.

Requirements of Mn in the horse are not known but, dependant on type and source of Mn, an estimated 13 to 16% is absorbed (NRC, 2007). The main roles of Mn include CHO and lipid metabolism and synthesis of condroitin sulfate: recommended intake of Mn is 0.8 mg/kg BW/d (NRC, 2007). Manganese intake diet differences were also seen for retention, but not for percent retention or percent apparent digestion. The HAY diet always had the lowest values, and although both the HAY and FF diets fell below NRC recommended intake level, the HAY diet was the only diet to have a negative retention, percent retention and percent apparent digestibility. Considering that only the CHO diet was absorbed in the expected range of 13 to 16%, these values were somewhat anticipated.

Zinc absorption ranges from 5 to 15%, and can be affected by exercise and Zn concentrations (NRC, 2007). An important component of many enzymes, it has especially high concentrations in the eye, and has a recommended intake of 0.8 mg/kg BW/d (NRC, 2007). In this study, Zn had differences for all parameters measured with the exception of urine output. Zinc intake was lowest on the HAY diet, followed by FF and CHO. For Zn retention, percent retention and percent apparent digestion, the HAY diet was negative and lower than the other two diets which were not negative. Considering that the HAY diet was the only diet to fall below 2007 NRC recommended values, these data and results were within expected parameters.

Lower than recommended intake of S and Se may have been one of the reasons for the observed age by diet by period interaction. The increase in amount fed beginning on d 31, due to the observed weight loss in the horses, and a higher concentration of S in the FF diet also likely contributed to the observed age by diet by period interaction. Diet differences for S were also present for all parameters measured and the HAY diet was typically lower than the other two diets. The HAY diet also was negative for retention and percent retention, but not percent apparent digestibility. Sulfur, which is absorbed in the hind-gut of the horse by microbial protein from dietary S and is an important component of organosulfur compounds, does not have known requirements for intake in the horse (NRC, 2007). Adequate consumption of dietary protein is thought to meet the needs of the animal (NRC, 2007). Given that the S intake of the horses was below NRC recommended intake, and a slightly positive S retention was calculated, it seems to indicate that NRC recommended S intake may be an overestimation of the horses' needs. However, ramifications of either excess or inadequate dietary S are unknown due to the limited research on S in horses. At least for the other macrominerals reported on in this study, adequate amounts appeared to be present for the mature horse. However, more research needs to be done in this area to determine the true S requirements of the horse.

Selenium is an important component of many of the body's antioxidant systems, has a role in thyroid metabolism, and has been linked to immune function in humans (NRC, 2007; Fermaglich et al., 2002; McClain et al., 2002). It is approximately 50% absorbed, and absorption is dependent on the type and source of Se. Recommendations for dietary Se are available in the horse, are set at 0.002 mg/kg BW/d (NRC, 2007), and were met by the CHO and FF, but not the HAY diets. Values for Se differed between diets and were present for most parameters measured.

Selenium was at its lowest values for HAY followed by FF and then CHO diets. However, for Se retention, the FF diet was the lowest compared to HAY.

The current study did however have several limitations. All of the minerals were run on the ICP, which has matrix matched standard reference materials (SRM). The SRM of the feed is run with feed, urine run with urine etc. and they are typically certified by National Institute of Standards and Technology (NIST). Results are not reported unless they match the NIST value within their stated uncertainty. Reference materials used for the NIST standards are well homogenized and there for are easily prepared for analysis. However, real world samples typically have problems, such as feed matrixes that do not go totally into solution, and crystals in urine samples. Also many minerals, such as Na and Fe are notoriously hard to sample due to leaching of mineral from the containers they are stored in (as is the case with Na) or from the ground (as is the case with Fe). To mitigate this as much as possible, numbers from the ICP-MS are composed of an average of three mass spectra that are collected from each sample preparation, CV of which must all be below 1.25%, except for S which is 2.5%. Also to avoid as much variation as possible from sample and preparation, duplicate preparations were done.

Another limitation of the study was the estimation in urine output, especially during the first dietary period when the urine collection devices were changed from those obtained from the PMU farm to the new design. Once the new devices were utilized, urine losses were uncommon. There also was also an observed period effect, likely due to the necessary increase in feed. This effect did not however create diet or age interaction for percent retention or percent apparent digestion of any of the nutrients measured and therefore did not likely have an appreciable effect on digestibility.

Implications

This study suggests that at least in respect to formulated, isocaloric diets in non-working, mature horses, there are no negative effects connected with moderate fat-supplementation for the macronutrients measured. However, in most cases the HAY diet had a lower, often negative digestibility in many of the macro and microminerals versus the other two diets. This may suggest that, depending on the quality of the forage, supplementation of the animal may, in some circumstances, be advisable.

Conclusions and Further Discussion

This study is unique compared to previous work considering that it evaluated a larger number of healthy horses, all of which had received regular anthelmintic treatment and had normal dentition. All horses were tested on three different commonly fed formulated diets (high roughage, high fat and fiber, and high cereal) for a long diet adaptation period of five wk. Results from this study suggest that under current practical feeding situations, differences in digestive capacity between healthy aged and healthy adult horses are unlikely to be present. However, nutrient requirements of aged horses in poor health and those with dental disorders may differ.

Implications from this study also include that moderate fat-supplementation has no apparent negative effect on the macronutrients measured, at least in respect to formulated, isocaloric diets in non-working, mature horses. However, for many of the nutrients examined, especially macro and microminerals, the HAY diet had a lower, often negative digestibility compared to the other two diets. Considering these results it could be that, depending on the type or quality of the forage, supplementation of the animal may be warranted.

This research opens the door to examine many different parameters associated with the aging horse as the aged animal seems to have similar nutritional needs as that of the adult animal. Avenues of research concerning the use of moderate fat diets to supplement energy requirements can also be explored for potential benefits, as moderate levels of added fat do not seem to have a negative effect on nutrient digestibility. Research to mediate the inflammation that often occurs with aging as well as with exercise by adding dietary omega three fatty acids may be of particular interest. As it stands, further work in the area of the aged horse is called for, and many new opportunities are available for researchers to examine this not yet well known topic.

APPENDICES

APPENDIX A

SAMPLE PREPARATION

Energy

Prepared samples (0.9 ± 0.28 g) were pelleted then placed into a combustion capsule in contact with a 10 cm fuse wire, which was attached to the capsule head. The capsule was screwed closed, and oxygen added to bring the pressure to 32 atm. A calorimetry bucket was filled with 2 L distilled water, and the capsule was placed into the bucket, which was then lowered into the combustion chamber. The terminal sockets of the capsule were attached to two ignition wires, the calorimeter was closed, and an initial temperature was taken. The sample was ignited, and at least 6 min later the temperature was again taken, the bucket removed from the calorimeter, and the capsule removed from the bucket. Any unburned wire was removed, and its length recorded. The 2 bombs used had energy equivalents of 2,400 cal/° C (bomb A) and 2,398 cal/° C (bomb B).

NDF

Dried and ground feed and fecal samples were weighed (0.5 ± 0.01 g) into 600 ml Berzelius beakers. A heat stable amalyse solution was prepared and 2 ml added, along with 100 ml neutral-detergent solution and 0.5 g sodium sulfite. The samples were then placed on a reflux apparatus, brought to a boil, and refluxed for 1 h. Beakers were removed from the apparatus and an additional 2 ml of heat stable amalyse added. Contents of the beakers were washed with hot water (90 to 100° C) into 5 ml sand bottom Gooch crucibles and vacuumed dry. Crucibles were then rinsed with acetone, air dried for at least 5 min, and placed in a forced air oven at 105° C overnight, after which they were hot weighed. Hot weights were recorded, and crucibles were placed in a muffle oven, and ignited at 500° C for 5 h. After cooling, they were placed in a

forced air oven at 105° C overnight and again hot weighed. The weights were recorded in order to calculate NDF content.

CP

Samples of the FF and HAY diets, as well as fecal samples were weighed (0.200 $\pm$ 0.010 g) as well as CHO samples (0.2500 $\pm$ 0.010 g) into 100-ml Digesdahl flasks. Urine samples were pipetted (0.50 ml) into flasks. Concentrated sulfuric acid (4 ml) was added and allowed to soak samples before the flasks were fitted with a condenser and capillary funnel and placed on a hot plate at 440° C for 6 min, following which 10 ml hydrogen peroxide (50%) was added to the capillary funnel. Once the hydrogen peroxide was completely drained into the flask, it was removed from the hot plate and allowed to cool. Samples were brought up to volume with DI water. An 800-µl aliquot of the digest was transferred to a centrifuge tube and 20 ml of a 0.1 g/L solution of polyvinyl alcohol added. The centrifuge tube was vortexed and a 160-µl aliquot pipetted into a microplate, after which 40 µl of Nessler's reagent solution and an additional 40 µl of polyvinyl alcohol was added. The plates were shaken for approximately 10 s and allowed to set for 5 min prior to reading on a spectrophotometer at 460 nm (Gomori, 1942).

Fat

Samples of feed and feces were weighed onto filter paper and made into packets. The packets were placed in ethyl ether and refluxed for 24 h. Following reflux, they were allowed to set in the hood for at least 1 h and then placed in a laboratory oven at 105° C and hot weighed. The weights were recorded in order to calculate fat disappearance.

Microwave digestion

Samples were weighed into a digestion vessel, and then 10 ml of 70% nitric acid was added. Samples were digested overnight at room temperature. Following the digest, vessels were transferred into the appropriate assembly and placed in the microwave accelerated reaction system (MARS-5, CEM Corp., Matthews, NC), which ran the samples at 1,200 W, 100% power, 200 PSI max, 190 °C. Then 2 ml of 30% hydrogen peroxide was added, and the samples were brought up to 25 ml with ddH_2O, and stored at room temperature. Any glassware, as well as the Teflon-lined digestions vessels, was washed in 30% nitric acid before being rinsed with ddH_2O.

Urine P

The urine was prepared for P assay by pipetting 25 µl urine and 25 µl DI water into well plates. Two solutions were made; a Molybdate-Sulfuric (MS) solution composed of 10 g Sodium Molybdate Dihydrate dissolved in 28 ml concentrated sulfuric acid and 2 L deionized water and a p-Methylaminophenol (Elon) solution composed of 6 g sodium bisulfate and 2 g Elon dissolved in 200 ml deionized water. 250 µl of MS solution and 25 µl of Elon solution was then added to the plated samples. They were shaken on a plate shaker for 45 min, after which they were and analyzed for P on a spectrophotometer at 700 nm. To determine urine Mg, samples were acidified with 20 ul of 12 M HCl.

APPENDIX B

DIET COMPOSITION

Table 3:1A. Lignin, water soluble carbohydrates (WSC), simple sugars (ESC), and starch content of three different diets (CHO, FF, and HAY)

	CHO	FF	HAY
Item (DM basis)			
Lignin *(g/kg)*	28.1	40.9	68.7
WCS *(g/kg)*	76.5	86.0	106.3
ESC *(g/kg)*	61.9	6.9	57.2
Starch *(g/kg)*	311.4	5.4	4.9

APPENDIX C

TEMPERATURE DATA

Table 3:3A. Temperature values for three different periods

	Period 1	Period 2	Period 3	P-value
Avg Outdoor Temperature (°C)	$-4.92^{a} \pm 1.04$	$2.32^{b} \pm 1.03$	$10.12^{c} \pm 1.03$	*<0.01*
Avg Outdoor Humidity	$76.47^{c} \pm 1.32$	$67.75^{b} \pm 1.26$	$59.94^{a} \pm 1.26$	*<0.01*
Avg Indoor Temperature (°C)	$64.16^{a} \pm 1.47$	$65.33^{a} \pm 1.44$	$69.28^{b} \pm 1.44$	*<0.01*
Avg Indoor Humidity	$39.43^{a} \pm 2.03$	$43.61^{b} \pm 1.99$	$57.69^{c} \pm 1.99$	*<0.01*

[ab] *Means within rows with differing superscripts differ*

APPENDIX D

PERIOD VALUES

Table 3:4A. Intake, output and weight data for three different periods

	Period 1	Period 2	Period 3	P-value
Intake and Output Values (g/kg BW/d)				
Avg Concentrate Intake	$3.68^{a} \pm 0.67$	$4.94^{a,b} \pm 0.65$	$5.97^{b} \pm 0.65$	*0.01*
Avg Hay Intake	$10.61^{a} \pm 0.56$	$13.78^{a,b} \pm 0.53$	$14.71^{b} \pm 0.53$	*<0.001*
Avg Fecal Output	$5.51^{a} \pm 0.31$	$7.91^{b} \pm 0.29$	$8.60^{c} \pm 0.29$	*<0.01*
Avg Urine Output (*ml/kg BW/d)*	12.21 ± 0.89	12.76 ± 0.83	13.57 ± 0.83	*0.35*
Avg Wt (kg)	$468^{a} \pm 9$	$476^{b} \pm 9$	$487^{c} \pm 9$	*<0.01*
Median BCS	$4.7^{a} \pm 0.2$	$4.9^{a} \pm 0.2$	$5.2^{b} \pm 0.2$	*<0.01*

[ab]*Means within rows with differing superscripts differ*

APPENDIX E

ENERGY, NEUTRAL DETERGENT FIBER, CRUDE PROTEIN AND FAT DIGESTIBILITY FOR THREE DIFFERENT PERIODS

Table 3:5A. Energy, NDF, CP, fat digestibility, and blood vitamin C concentrations for three different periods

	Period 1	Period 2	Period 3	P-value
Energy (Mcal/kg BW/d)				
Intake	$0.066^{a} \pm 0.003$	$0.085^{b} \pm 0.003$	$0.091^{c} \pm 0.003$	*<0.01*
Fecal	$0.026^{a} \pm 0.002$	$0.037^{b} \pm 0.002$	$0.042^{c} \pm 0.002$	*<0.01*
Apparent Digestible Energy	$0.039^{a} \pm 0.003$	$0.047^{b} \pm 0.003$	$0.049^{b} \pm 0.003$	*<0.01*
% Apparent Digestible Energy	58.84 ± 1.67	55.72 ± 1.56	53.68 ± 1.56	*0.09*
NDF (g/kg BW/d)				
Intake	$7.72^{a} \pm 0.53$	$9.85^{b} \pm 0.51$	$11.87^{c} \pm 0.51$	*<0.01*
Fecal	$3.65^{a} \pm 0.23$	$5.20^{b} \pm 0.21$	$5.49^{b} \pm 0.21$	*<0.01*
NDF Digested	$4.04^{a} \pm 0.44$	$4.61^{a} \pm 0.43$	$6.34^{b} \pm 0.43$	*<0.01*
% NDF Digestibility	$52.09^{a,b} \pm 2.11$	$46.91^{a} \pm 2.01$	$52.94^{b} \pm 2.01$	*0.05*
CP (g/kg BW/d)				
Intake	$2.14^{a} \pm 0.11$	$2.69^{b} \pm 0.10$	$2.98^{c} \pm 0.10$	*<0.01*
Fecal	$0.50^{a} \pm 0.04$	$0.67^{b} \pm 0.04$	$0.73^{b} \pm 0.04$	*<0.01*
Urinary	0.35 ± 0.04	0.42 ± 0.03	0.38 ± 0.03	*0.37*
Retained	$1.28^{a} \pm 0.09$	$1.60^{b} \pm 0.08$	$1.87^{c} \pm 0.08$	*<0.01*
% Retained	59.43 ± 2.07	59.12 ± 1.94	62.86 ± 1.94	*0.34*
% Apparent Digested	76.64 ± 1.00	75.28 ± 0.93	75.73 ± 0.93	*0.51*
Fat (g/kg BW/d)				
Intake	$0.64^{a} \pm 0.05$	$0.83^{b} \pm 0.04$	$0.92^{c} \pm 0.04$	*<0.01*
Fecal	$0.32^{a} \pm 0.03$	$0.41^{b} \pm 0.03$	$0.46^{b} \pm 0.03$	*<0.01*
Amount digested	$0.32^{a} \pm 0.03$	$0.42^{b} \pm 0.03$	$0.45^{b} \pm 0.03$	*<0.01*
% Apparent Digestibility	48.36 ± 3.06	49.21 ± 2.86	47.82 ± 2.86	*0.94*
Vitamin C blood concentrations (mg/dL)				
Vitamin C	1.14 ± 0.04	1.09 ± 0.04	1.18 ± 0.05	*0.17*

[ab] *Means within rows with differing superscripts differ*

APPENDIX F

MACROMINERAL DIGESTIBILITY FOR THREE DIFFERENT PERIODS

Table 3:6A. Macromineral digestibility for three different periods

	Period 1	Period 2	Period 3	P-value
Calcium (g/kg BW/d)				
Intake	$0.145^{a} \pm 0.01$	$0.19^{b} \pm 0.01$	$0.18^{b} \pm 0.01$	*<0.01**
Fecal	$0.073^{a} \pm 0.007$	$0.093^{b} \pm 0.006$	$0.109^{b} \pm 0.006$	*<0.01*
Urinary	$0.013^{a} \pm 0.003$	$0.026^{b} \pm 0.003$	$0.026^{b} \pm 0.003$	*0.01*
Retained	$0.059^{a,b} \pm 0.009$	$0.068^{b} \pm 0.009$	$0.041^{a} \pm 0.009$	*0.02*
% Retained	$39.43^{b} \pm 4.07$	$36.35^{b} \pm 3.81$	$21.49^{a} \pm 3.81$	*0.01*
% Apparent Digested	$48.87^{b} \pm 3.10$	$50.68^{b} \pm 2.90$	$36.95^{a} \pm 2.90$	*<0.01*
Phosphorus (g/kg BW/d)				
Intake	$0.045^{a} \pm 0.005$	$0.055^{b} \pm 0.005$	$0.058^{b} \pm 0.005$	*0.01*
Fecal	$0.039^{a} \pm 0.004$	$0.045^{a} \pm 0.004$	$0.056^{b} \pm 0.004$	*<0.01**
Urinary	0.006 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	*0.67*
Retained	$-0.001^{a,b} \pm 0.002$	$0.004^{b} \pm 0.002$	$-0.004^{a} \pm 0.002$	*0.01*
% Retained	$-11.12^{a} \pm 6.67$	$3.50^{b} \pm 6.44$	$-13.83^{a} \pm 6.44$	*0.03*
% Apparent Digested	$5.61^{a,b} \pm 5.11$	$15.36^{b} \pm 4.90$	$-0.31^{a} \pm 4.90$	*0.03*
Magnesium (g/kg BW/d)				
Intake	$0.032^{a} \pm 0.003$	$0.038^{b} \pm 0.003$	$0.045^{c} \pm 0.003$	*<0.01*
Fecal	$0.021^{a} \pm 0.002$	$0.026^{b} \pm 0.002$	$0.029^{b} \pm 0.002$	*<0.01**
Urinary	$0.0033^{a} \pm 0.0005$	$0.0055^{b} \pm 0.0005$	$0.0056^{b} \pm 0.0005$	*0.01*
Retained	0.008 ± 0.002	0.007 ± 0.002	0.011 ± 0.002	*0.06*
% Retained	23.00 ± 4.61	16.49 ± 4.37	23.51 ± 4.37	*0.38*
% Apparent Digested	33.90 ± 3.74	31.51 ± 3.55	36.08 ± 3.55	*0.58*
Sodium (g/kg BW/d)				
Intake	0.028 ± 0.002	0.030 ± 0.002	0.032 ± 0.002	*0.46*
Fecal	$0.013^{a} \pm 0.002$	$0.016^{a} \pm 0.002$	$0.020^{b} \pm 0.002$	*<0.01**
Urinary	0.011 ± 0.001	0.013 ± 0.001	0.014 ± 0.001	*0.30*
Retained	0.004 ± 0.002	0.001 ± 0.002	-0.003 ± 0.002	*0.11*
% Retained	15.6 ± 10.2	7.1 ± 9.4	-17.6 ± 9.4	*0.09*
% Apparent Digested	$52.2^{b} \pm 5.2$	$44.2^{a,b} \pm 4.9$	$32.2^{a} \pm 4.9$	*0.03*

Table 3:6A. (cont'd)

Potassium (g/kg BW/d)				
Intake	$0.23^{a} \pm 0.01$	$0.30^{b} \pm 0.01$	$0.30^{b} \pm 0.01$	*<0.01*
Fecal	$0.051^{a} \pm 0.004$	$0.076^{b} \pm 0.004$	$0.082^{c} \pm 0.004$	*<0.01**
Urinary	0.13 ± 0.02	0.15 ± 0.02	0.13 ± 0.02	*0.35*
Retained	0.05 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	*0.11*
% Retained	17.66 ± 6.42	24.05 ± 6.26	27.65 ± 6.26	*0.22*
% Apparent Digested	$76.63^{b} \pm 1.38$	$73.82^{a,b} \pm 1.30$	$72.34^{a} \pm 1.30$	*0.04*
Sulfur (g/kg BW/d)				
Intake	$0.021^{a} \pm 0.002$	$0.025^{b} \pm 0.002$	$0.025^{b} \pm 0.002$	*0.03**
Fecal	$0.0100^{a} \pm 0.0005$	$0.0124^{b} \pm 0.0005$	$0.0135^{b} \pm 0.0005$	*<0.01*‡
Urinary	0.0110 ± 0.0008	0.0092 ± 0.0007	0.0097 ± 0.0007	*0.25*
Retained	0.000 ± 0.002	0.003 ± 0.002	0.001 ± 0.002	*0.10*
% Retained	-6.47 ± 8.53	8.98 ± 8.33	2.21 ± 8.33	*0.13*
% Apparent Digested	48.95 ± 4.40	46.16 ± 4.30	41.46 ± 4.30	*0.14*

[ab] *Means within rows with differing superscripts differ*

‡ *Age by diet by period interaction*

***** *Diet by period interaction*

APPENDIX G

MICROMINERAL DIGESTIBILITY FOR THREE DIFFERENT PERIODS

Table 3:7A. Micromineral digestibility for three different periods

	Period 1	Period 2	Period 3	P-value
Cobalt (g/kg BW/d)				
Intake	0.0038 ± 0.0007	0.0037 ± 0.0007	0.0052 ± 0.0007	*0.13*
Fecal	0.0031 ± 0.0007	0.0037 ± 0.0007	0.0049 ± 0.0007	*0.07*
Urinary	0.00005 ± 0.00002	0.00005 ± 0.00002	0.00006 ± 0.00002	*0.93*
Retained	0.0007^{b} ± 0.0001	0.00001^{a} ± 0.0001	0.0002^{a} ± 0.0001	*<0.01**
% Retained	-35.23 ± 15.56	-48.03 ± 14.70	-19.14 ± 14.70	*0.33*
% Apparent Digested	-31.90 ± 15.30	-46.26 ± 14.47	-17.47 ± 14.47	*0.32*
Copper (g/kg BW/d)				
Intake	0.24^{a} ± 0.03	0.28^{a} ± 0.03	0.35^{b} ± 0.03	*0.01**
Fecal	0.20^{a} ± 0.03	0.25^{a} ± 0.03	0.32^{b} ± 0.03	*<0.01*
Urinary	0.0008^{b} ± 0.0001	0.0005^{a} ± 0.0001	0.0006^{a} ± 0.0001	*<0.01*
Retained	0.037 ± 0.008	0.030 ± 0.008	0.024 ± 0.008	*0.52*
% Retained	11.22 ± 3.80	10.35 ± 3.61	1.81 ± 3.61	*0.09*
% Apparent Digested	11.85 ± 3.71	10.58 ± 3.53	2.03 ± 3.53	*0.07*
Iron (g/kg BW/d)				
Intake	1.73^{a} ± 0.21	$2.10^{a,b}$ ± 0.20	2.43^{b} ± 0.20	*0.02*
Fecal	1.86 ± 0.27	2.18 ± 0.26	2.33 ± 0.26	*0.22*
Urinary	0.0017^{b} ± 0.0002	0.0008^{a} ± 0.0002	0.0009^{a} ± 0.0002	*<0.01*
Retained	-0.13 ± 0.14	-0.09 ± 0.13	0.08 ± 0.13	*0.45*
% Retained	-188.1 ± 27.9	-216.6 ± 27.0	-232.0 ± 27.0	*0.32**
% Apparent Digested	-22.1 ± 14.0	-5.4 ± 13.1	-3.5 ± 13.1	*0.57*
Manganese (g/kg BW/d)				
Intake	0.56^{a} ± 0.06	0.61^{a} ± 0.05	0.80^{b} ± 0.05	*<0.01*
Fecal	0.44^{a} ± 0.06	0.57^{a} ± 0.06	0.77^{b} ± 0.06	*<0.01*
Urinary	0.00012^{b} ± 0.00002	0.00007^{a} ± 0.00002	0.00007^{a} ± 0.00002	*0.04*
Retained	0.12 ± 0.03	0.04 ± 0.03	0.02 ± 0.03	*0.06*
% Retained	8.52 ± 6.78	0.82 ± 6.34	3.01 ± 6.34	*0.70*
% Apparent Digested	8.52 ± 6.78	0.82 ± 6.34	3.01 ± 6.34	*0.70*
Selenium (g/kg BW/d)				
Intake	0.0047 ± 0.0007	0.0052 ±0.0007	0.0066 ± 0.0007	*0.05*
Fecal	0.0025 ± 0.0003	0.0025 ± 0.0003	0.0031 ± 0.0003	*0.15**
Urinary	0.0016 ± 0.0003	0.0019 ± 0.0003	0.0022 ±0.0003	*0.43*
Retained	0.0005^{a} ± 0.0002	0.0008^{a} ± 0.0002	0.0014^{b} ± 0.0002	*<0.01†*
% Retained	-0.41 ± 0.06	-0.44 ±0.06	-0.52 ± 0.06	*0.28*
% Apparent Digested	28.2 ± 5.2	51.6 ± 4.8	54.8 ± 4.8	*<0.01**

Table 3:7A. (cont'd)

Zinc (g/kg BW/d)				
Intake	$0.71^{a} \pm 0.10$	$0.83^{a,b} \pm 0.09$	$1.00^{b} \pm 0.09$	*0.02*
Fecal	$0.64^{a} \pm 0.10$	$0.76^{a} \pm 0.10$	$0.99^{b} \pm .10$	*0.02*
Urinary	$0.006^{b} \pm 0.002$	$0.001^{a} \pm 0.001$	$0.001^{a} \pm 0.001$	*0.03*
Retained	0.07 ± 0.03	0.07 ± 0.03	0.01 ± 0.03	*0.23*
% Retained	-7.79 ± 6.38	2.45 ± 6.08	-0.91 ± 6.08	*0.41*
% Apparent Digested	-5.50 ± 5.54	2.63 ± 5.27	-0.77 ± 5.27	*0.49*

[ab] *Means within rows with differing superscripts differ*

† *Age by period interaction*

***** *Diet by period interaction*

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