THE EFFECT OF DENTAL FLOATING ON NUTRIENT DIGESTION IN NON-PREGNANT QUARTER HORSE MARES

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by

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ABSTRACT

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Irregular wear patterns can cause poor feed digestion, weight loss, performance problems, and pain in the horse when wearing a halter or bridle. The objective of this study was to test the hypothesis that dental floating would increase crude protein and fiber digestion in non-pregnant stock-type mares. The study was conducted on 16 mares of mature age (4-17) that have not previously received dental care in the previous year. A diet consisting of *ad libitum Cynodon dactylon* hay and 1.36 kg of a balancer concentrate was fed daily prior to and during the study. Eight mares received dental work and eight served as untreated controls. Feces were collected for four days, before and 56 days after dental work, whereby acid insoluble ash (AIA) was used as a marker of digestibility. Feces and feed were analyzed for dry matter, AIA, crude protein, NDF, and ADF using standard wet chemistry laboratory techniques.

Estimated apparent neutral detergent fiber digestibility was not affected by the time by treatment interaction or treatment (P>0.1). However, digestibility was greater on day 56 (86.3±1.3%) than day 0 (67±1.3%). There was a tendency for an effect of the time by treatment interaction for estimated apparent acid detergent fiber digestibility (P=0.073), whereby digestibility was not different for groups on day 0 (50.3±0.8% vs. 50.3±0.8%; P > 0.9) but was greater (P = 0.014) in dental treated horses (59.5±0.8%) than controls (56.5+0.8) on day 56. There was a tendency for dentistry to improve the month by treatment interaction for estimated apparent crude protein digestibility (P=0.0931), whereby digestibility was not different for groups at day 0 (50.2±0.8% vs.

49.9±0.8%; $P > 0.9$) but was greater ($P = 0.0883$) in dental treated horses (59.5±0.8%) than controls (56.5+0.8) at day 56. The main finding of this study was that dental correction tended to improve fiber and protein digestion, which is possibly due to the increased amount of mechanical digestion of forages.

The second objective of this study was to determine whether the Computerized Horse Aging Program (CHAP) could accurately age horses within a 95% confidence interval and 10% margin of error. Photos were uploaded to the mobile app and compared to registered ages using Bland-Altman agreement analysis. The app accurately predicted age using upper incisors in horses between 8 and 13, while lower incisors were accurate between 8 and 10 years. CHAP can accurately predict age within 1 year, in horses aged 8-13.

KEY WORDS: ADF; Crude protein; Dentistry; Digestibility; Equine; NDF

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CHAPTER I

INTRODUCTION

Dental care is essential for a horse's oral health and ultimately, their overall health. Dental care has also been known to reduce equine mouth pain in horses suffering from severe lesions (Jeffrey, 2009). Correct equilibration allows for adequate digestion of nutrients from forages, enabling a horse to maintain a healthy body condition (Klugh, 2010). Equine dentistry first caught momentum in the late 1700's in Europe and rapidly progressed, especially during World War II when military members recognized that dental care improved their horse's temperament and performance (Jeffrey, 2009). Routine dentistry allows for good lateral movement of the horse's molar table where digestion of feeds begins. Lack of proper dentistry can result in dental abnormalities consisting of: malocclusions, wave complexes, ramps, hooks, wedges, excessive transverse ridges, and accentuated transverse ridges. These will be discussed in greater detail later in the text. Dental abnormalities may lead to the horse dropping feed material, known as quidding, not maintaining a healthy body condition, suffering from an impaction colic, or exhibiting behavioral vices (Klugh, 2010). If teeth are not maintained on a regular basis, these abnormalities will arise. When they become apparent, these abnormalities need to be removed in order to see full rotation of food traveling across the oral cavity during chewing. This enables horses to chew roughages for the duration of their lifetime. Horses chew in a circular rotation from side to side moving the lower mandible jaw against the upper maxilla (Jeffrey, 2009). A level molar table allows for horses to adequately use mechanical digestion to break down their feed in an unconstrained and painless manner.

By taking horses out of their natural grazing state, there is a higher responsibility placed on the owner to ensure quality oral health. This is due to the fact that the mandible is never allowed to properly function in its natural forward position. Horses evolved to eat with their heads down, and when horses' heads are lowered the arcades align and perform at optimum levels of mastication (Jeffrey, 2009). Feeding horses in elevated feeders causes excessive incisor table angles and upper front hooks and lower ramps in the rear of the mouth (Jeffrey, 2009). Horses that are placed in modified environments or fed higher concentrate diets create these malocclusions in the oral cavity. Therefore, dentistry becomes a priority for horses that are placed in stalls or un-natural eating environments. There are growing demands placed upon domestic horses, including athletic activity, bits placed in the mouth, diets consisting of more grains than forages, and feeding in an elevated position, all of which modify the way a horse uses their oral cavity. If we want horses to perform at elite levels, routine dentistry will need to be a priority in the future care of horses to aid in comfort and prevent these malocclusions from occurring.

The Computerized Horse Aging Program (CHAP) is a media application used to age horses based off of their teeth. The knowledge of aging horses along with artificial intelligence and the vision capabilities of a trained computer model was designed to age horses based on a photo of their incisor teeth. To train this model, a computer analyzed 18 features on the lower incisor teeth and 18 features on the upper incisor teeth on more than 1000 images collecting 36,000 points of data. The CHAP app was developed for use on a mobile platform and uses the camera on a mobile device to capture the occlusal surface of the incisor teeth. The mobile app was used to age horses in the digestion study as well as the age comparison study.

CHAPTER II

OBJECTIVE

The objective of this study was to test the hypothesis that dental floating will increase crude protein and fiber digestion in non-pregnant quarter horse mares. This research used sixteen Quarter horses that had not received dental care in the previous year. Eight of the horses received dental work and eight served as untreated controls.

CHAPTER III

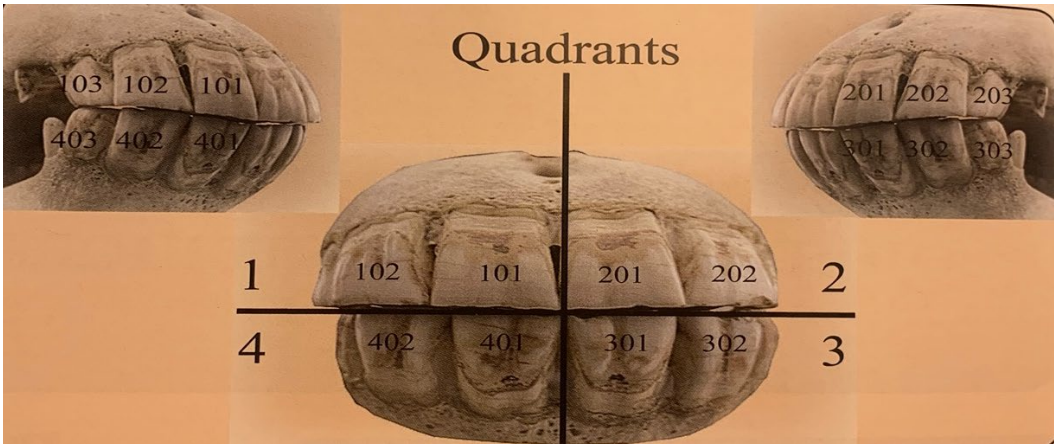
LITERATURE REVIEW

Anatomy of the Equine Paradigm

Mouth. The mouth is located terminally, rostral to the horse's head and is the site where taste and mastication occur. Equines have hypsodont teeth, meaning that teeth grow and develop prior to eruption around age five or six, and then continue to erupt at the same rate they wear. Wear rates are due to contact with the opposing teeth, and wear continues until the tooth expires. A horse's skull is outlined by the maxilla and mandible bone. The maxilla houses the upper cheek teeth along with nerves that communicate the five senses; smell, sight, hearing, taste, and touch (Jeffrey, 2009). The mandible bone moves in opposition to the maxilla bone during the mastication process. It includes the lower cheek teeth and is constructed of compact bone to endure the biomechanical movement of the head. Cheek teeth sit in a gompholic type joints found inside alveolar sockets that are surrounded by periodontal ligament fibers. These collagen fibers attach the outer cementum shell of the tooth with the bone, amplifying strength (Jeffrey, 2009).

Horses have four types of teeth in their oral cavity: incisors, canines, pre-molars, and molars (Peffers, 2016). The incisors are located proximal to the lips while the pre-molars and molars, otherwise known as cheek teeth, are located more distally from the opening of the oral cavity. There is a total of twelve lateral incisor teeth: six in the upper maxilla jaw and six in the lower mandibular jaw. There are also twenty-four cheek teeth when fully erupted, three premolars followed by three molars all laterally located.

The mouth is made up of four quadrants that split the mouth from dorsal to ventral and from left to right. Quadrant one is the dorsal left side of the mouth followed with quadrant two being the dorsal right. Quadrant three is found in the ventral left and the ventral right is quadrant four. There are three numbering systems used for horses: The Standard System, The Cheek Teeth System, and The Triadan System (Jeffrey, 2009).



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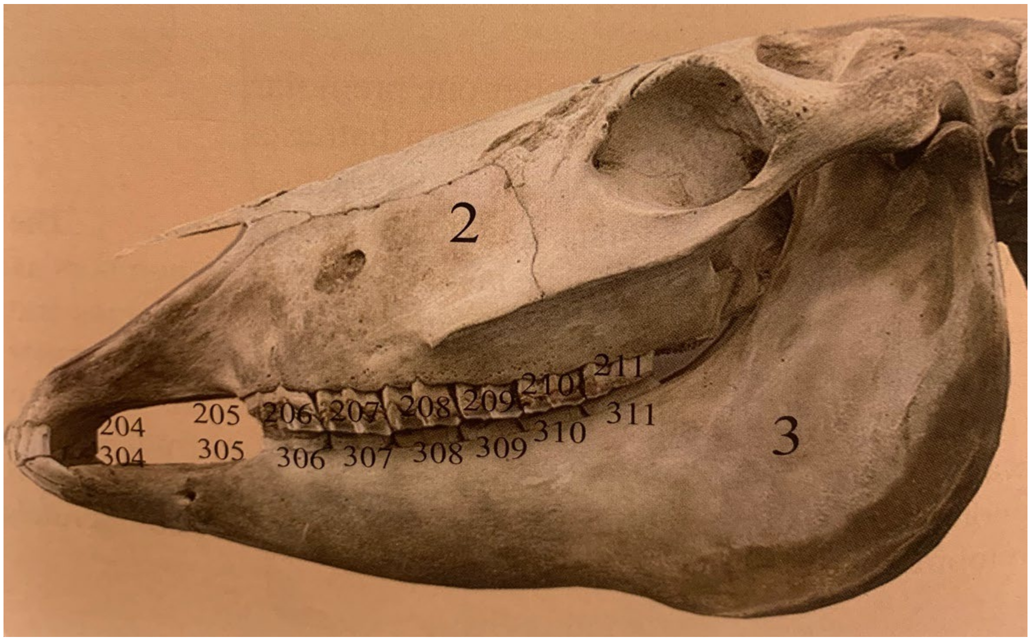

Figure 1. The Triadan System is the most universal method used to number teeth (Jeffrey, 2009)

These systems all identify the central incisors as one's, the middle incisors as two's, and the lateral, corner incisors as three's. The Standard System identifies the teeth by their name and position in sections based on category of incisor, premolars, and molars. The system works medially and moves distally in the mouth. The Cheek Teeth System recognizes the premolars and molars as numbers one through six from rostral to caudal. The Triadan numbering system is used to assign individual numbers to each tooth (Peffers, 2016). The numbering process begins at the rostral part of the mouth with one and proceeds to eleven the further back the teeth are located. Tooth number one is assigned to the first central incisor in each quadrant. Incisors are numbers one to three and allocated to their quadrant. For example, the 103 is the dorsal left corner incisor of quadrant one.

Foals are usually born without teeth and gain the first pair of deciduous, central incisors, at 6-8 days old, the second lateral pair at 6-8 weeks old, and the third lateral pair at 6-8 months old (Peffers, 2016). These incisor teeth erupt from the medial line of the oral cavity outwards. The central incisors are the center most medial tooth in the Equidae or family of horses. This is the eruption pattern for deciduous incisor teeth, which are later replaced with permanent teeth. Caps are the deciduous cheek teeth. The first three caps in the molar arcade can appear when a foal is born or shortly after (Peffers, 2016). The three caps begin to shed as the permanent molars start to erupt. Permanent central incisor teeth erupt at 2.5 years and include teeth 101, 201, 301, 401, 3.5 years for the teeth numbered 102, 202, 302, and 402. The 103, 203, 303, and 403 erupt at 4.5 years. The number three incisor teeth are in wear at age five (Jeffrey, 1989). Therefore, the corners would be considered in occlusion, with an absence of space gapping them open at five years of age.

There are four canines found in the interdental space between the incisors and cheek teeth of male horses. Female horses only have rudimentary canine teeth that occasionally break through the gums as tiny enamel pieces (Jeffrey, 2009). They become present at four and a half years of age and are considered the number four tooth. They are predominately closer to the incisor teeth, but not in occlusion with one another. The lower canine is seated closer to the front than the upper canine so they do not come in contact during the mastication process. These teeth are often recognized as tusks because they were used as fighting teeth (Peffers, 2016). They do not erupt continuously like cheek teeth.

Moving back in the oral cavity, the numbers proceed sequentially. The premolars come after the canines and are teeth number five through eight. Tooth number five is the wolf tooth. Studies suggest 20% of all horses have wolf teeth (Peffers, 2016). This tooth is commonly located on the inside of the upper maxillary jaw. It is less likely to be found on the lower inside of the mandibular jaw, but can sometimes be hidden or overlooked because of its small vestigial size. It can appear when the horse is six to eighteen months of age. By two and a half years the wolf tooth is typically no longer present as it is pushed to the surface and loosened as eruption takes place from the teeth erupting superior to it. After loosening, these teeth typically fall out. Removal of wolf teeth that did not naturally fall out, occurs during the horse's first dentistry appointment to provide comfort for riding with a bit. The last three teeth in the equine mouth are the molars, otherwise known as the cheek teeth (Jeffrey, 1989). They follow the premolars in eruption and are numbers nine through eleven. Once the horse is past five years of age, they have twenty-four permanent cheek teeth. This includes three premolars and three molars in all four quadrants of the oral cavity.

Permanent tooth number nine erupts first at age one. At two years of age, the number ten tooth begins to erupt followed by the number six permanent tooth erupting under the cap at age two and a half. Caps have shallow roots that allow them to shed on their own or be easily removed by an equine dentist if needed. If not removed a cap can be uncomfortable to the cheeks or tongue of the horse. At three years, the number seven permanent tooth will erupt pushing out the second cap in the molar arcade. The last molar finally erupts at three and a half and at four years the last cap falls out as the number eight tooth moves in. The shedding of the molar caps is a three-year process that results in permanent teeth all aligning almost always in a straight line down the horse's jaw (Peffers, 2016).

Digestion and Nutrition

Digestibility. Digestibility is the portion of total nutrients available in the horse's ration that are digested from feedstuffs and absorbed (Carmalt and Allen, 2008). Digestibility is calculated by subtracting nutrients contained in feces from the nutrients in the horse's diet; often reported as a percentage of the nutrient consumed. (Staff, 2017). This apparent difference provides an estimate as it does not include the loss of metabolic waste in urine, the nutrients excreted as methane gases, or byproducts from microbial fermentation. Apparent digestibility also does not estimate endogenous losses from digestive enzymes produced by the pancreas. The nutrients available for absorption provide for growth, reproduction, and performance. Lower feed digestibility lowers the amount of available energy to the animal. This may be beneficial for horses that are overweight or those with insulin resistance where their cells fail to respond to their bodies use of sugars as energy. The higher the feed digestibility, the more energy from nutrient sources is readily available for absorption (Staff, 2017). Diets with higher digestibility values are more ideal for horses needing high amounts of energy, such as performance horses or pregnant mares.

Mechanical Digestion. A horse's chewing cycle holds a consistent pattern like many other herbivores. This pattern includes the opening stroke, the closing stroke, and the power stroke (Peffers, 2016). In the course of the opening stroke, the mouth is slightly opened allowing for the lower mandible jaw to extend sideways the width of one- and one-half incisor tooth. The closing stroke occurs when the teeth of the upper maxilla come in contact with the teeth of the lower mandible. In doing so this forces an opening at the incisor teeth. The cheek teeth crush the food particles on the surface of the molar tables when colliding during the power stroke. The mandible extends outwards in order to sweep across the complete surface of the upper maxilla teeth. In doing so, an intense grinding of food particles occurs when contact is met. After the lower jaw is over-extended sideways during the power stroke it then has a small recovery stroke where it returns to its natural jaw alignment. Then the chewing cycle is started over. The horse's chewing cycle can occur twelve times in ten seconds, equivalent to seventy-two times per minute or 4,320 chewing cycles per hour (Peffers, 2016).

A horse's diet can make a difference in its chewing cycle. Crossley et al. (2003) discussed that horse's fed a hay-based diet experience more movement in the lower jaw than in horses eating a pellet-based diet. The forage diet allows for the jaw to extend sideways with a greater radius providing more of a grinding surface on the molar tables. Horses fed a pelleted diet saw smaller extensions occurring from the mandible jaw limiting the contact taking place on the chewing surface (Crossley, 2003). This limited rotation during the chewing cycle in horses fed a pelleted diet, may lead to irregular wear on the outside rim of the upper cheek teeth and the inside edge of the lower cheek teeth. It is possible that horses fed a pelleted diet may require more frequent dental work due to these differences. If a horse can fully extend its jaw sideways then it can slow down the irregular wear patterns caused by concentrates.

Chemical Digestion. Equines are naturally free-ranging herbivores that come from grassland habitats. Their digestive system can be split into two compartments: foregut and hindgut. The foregut consists of the stomach and small intestine, while the fermentative chambers of the hindgut are the cecum and colon. In the foregut of the horse, the stomach acts as a mixing tank for food and prepares it to enter the small intestine for further breakdown. Horses have an enlarged hindgut adaptation that allows them to obtain energy and nutrients from plant structural polysaccharides through microbial fermentation (Mura et al., 2019). Both compartments make up a large majority of the digestive tract and are home to bacteria, anaerobic fungi, methanogenic archaea and protozoa.

The horse begins its digestion when it grips ahold of a piece of forage or grain with the lips of its mouth. The food is broken down into smaller pieces with the help of its cheeks, tongue, and teeth. Papillae or protuberances on the dorsal surface of the horse's tongue allow for the tongue to grip ahold of the feed and move it across the mouth. Mechanical digestion involves the grinding of these feed particles into chemically digestible molecules that can then proceed into absorption (Carey et al., 2019). Once the feed is ground and mixed with the horse's saliva, it forms into a bolus of food that travels across the cheek teeth due to rhythmic muscle action of the tongue and is then pushed down the esophagus and into the non-glandular upper stomach. As food travels to the lower, glandular stomach, the pH drops to approximately 1 to 2 and chemical digestion begins. This digestion occurs in the form of hydrochloric acid and peptidases, which chemically denatures proteins, preparing them for digestion in the small intestine. From the lower stomach, partially digested food enters the 70-foot-long small intestine.

The horse's intestine is like other animals, in that it can only absorb monosaccharides, amino acids and di and tri-peptides, and volatile fatty acids. It must digest starch into glucose prior to absorption and proteins to amino acids and di and tri-peptides. Food particles begin chemical digestion in the duodenum as they work their way through the remaining sections of the small intestine, the jejunum and the ileum. From the small intestine, food particles enter the 3- to 4-foot-long cecum where chemical digestion is furthered via bacteria and then transported into the large colon, a 12-foot-long tri-folded passageway. What is left of the particles end up in the small colon and rectum, where water is absorbed and fecal balls are formed.

Starch. In the stomach, food particles are mixed with bacteria producing digestive enzymes that digest starches further and also produce lactic acid and propionic acid via fermentation. This process happens in an environment of pH 5 to 6 (Al Jassim et al., 2005). The small intestine can also absorb lactic and propionic acids produced from bacterial fermentation and starch digestion that occurred in the stomach. Particularly when fed in large amounts, some starch is found to escape during small intestine digestion due to the horse's limited production of pancreatic amylase (Williams et al., 2019). This resistant starch is fermented to lactic acid in the large intestine, which can be converted to propionic acid and both of these acids can then be absorbed. Starch fermentation allows large amounts of propionate and lactate to be produced and can saturate the buffer capacity, resulting in a decreased pH in the hindgut. High amounts of lactic acid can also lower the pH in the system, thereby negatively impacting the pH sensitive bacteria (cite). If large amounts of starch are fermented in the hindgut, energy production from cellulose and hemicellulose can decrease as a direct result of microbial imbalance.

Protein. When feeding a horse, it is important to supply the proper amount of protein that can provide sufficient amino acids (NRC, 2007). A good protein source is easily digested and supplies all of the essential amino acids. The horse's body utilizes these proteins to synthesize enzymes, hormones, and tissues. Protein digestion takes place in the foregut of horses during enzymatic digestion. The more efficient the foregut is at digesting these proteins, the more amino acids are absorbed into the bloodstream to supply the horse's body with nutrients.

Enzymatic digestion takes place in the stomach using pepsin to digest peptide bonds of acid-denatured proteins. Particularly, pepsin acts on aromatic amino acids, such as phenylalanine and tryptophan (McMeniman et al., 1987). Partially digested proteins are sent to the small intestine, which has pancreatic proteases that are secreted to breakdown proteins to absorbable dipeptides and amino acids. The dipeptides that are absorbed are then hydrolyzed into amino acids in the gut wall prior to being transferred to the blood. The digestion and transfer of amino acids to the blood can be affected by dry matter intake, the digestion site, protein intake, and the travel time through the digestive tract. The protein that is absorbed as amino acids makes up the metabolic amino acid pool. The protein that escapes during digestion finds its way to the hindgut where it is broken down into ammonia and is excreted in waste. The nutritional value of proteins is hard to measure in horses because the gastrointestinal tract limits the amount being absorbed in the intestine (McMeniman et al., 1987). There is an interconversion of one amino acid to another in the large intestine via microbial action, so the outcome in the feces does not accurately represent what was consumed in the mouth. Some plant proteins are wrapped in lignin, waxes, and other indigestible forages that horses cannot fully extract. (Leichliter et al., 2021) did a study on dietary protein digestion in rats and guinea pigs showed that plant and animal-based feeds were metabolized at different efficiencies with proper dentition. Tooth enamel reflects diet enrichments. Feed groups high in protein and nitrogen will be preserved over geological time for teeth enamel in these animals (Leichliter et al., 2021). If this is the case for enamel surfaces in the equine, then mechanical digestion of proteins can induce longevity of tooth structures in the oral cavity of a horse. Less wear on tooth surfaces allows for a greater enamel surface for digestion of protein and nutrients to take place.

Cellulose and Hemicellulose Digestion and Absorption of Volatile Fatty Acids. Horses obtain the majority of their energy from carbohydrates in feeds, grains, and grain byproducts. The largest group of carbohydrates that make up the horse's diet is polysaccharides. Of polysaccharides, starch and cellulose are the two predominant polysaccharides followed by pectin and hemicellulose. Cellulose coming from forages produces the most energy for horses during fermentation in the gastrointestinal tract. Polysaccharides do not all ferment at the same rate nor do they all produce the same amount of volatile fatty acids. Starch and hemicellulose can be rapidly fermented, while cellulose is slowly fermented. The horse's digestion site for these compounds hydrolyzes the α1-6 and the α1-4 linkages of starch and maltose taking place in the small intestine. Horses do not produce the enzyme needed to digest the β1-4 linkage found in cellulose or hemicellulose (Harris and Geor, 2009). The digestion of these two takes place due to microbial fermentation. The fiber movement in the hindgut is slow, allowing for volatile fatty acids to be absorbed passively via the gastrointestinal wall.

Effect of Mechanical Digestion on Nutrient Digestibility. Digestibility is greatly impacted by the physical makeup of the feed and the particle's size after grinding (Hymøller, 2012). The breakdown of food particles through enzymatic digestion depends on the surface area of food particles produced in the oral cavity. Meyer et al. (1986) found that horses fed grain with healthy and maintained teeth are more capable of grinding their feed, with up to half of the food particles swallowed being less than 0.1 mm in size. Diets made up primarily of grain produce a similar particle size once ground by the cheek teeth. Horses with poor and neglected teeth are not as efficient in grinding of food particles (Meyer et al., 1995). These horses chew feedstuff slower and produce larger particles that take longer to be digested. A horse with an efficient chewing cycle will be able to digest the fibers provided from feeds to meet their nutritional requirements (Karasov et al., 1986). The size of food particles secreted into the small intestine influences the amount of secreted pancreatic and intestinal enzymes, the travel time through the digestive tract, physical conditions in the small intestine, and the chemical and physical composition of the feedstuff. These factors all influence the horse's feed efficiency (Hymøller, 2012).

Measures of Nutritional Health and Digestibility

Body Condition Score. A horse's body condition score (BCS) is an indicator of whether a horse is too thin, fat, or moderate. The horse is graded on the amount of fat located in six different areas on its body: neck, withers, spinous processes, transverse processes, tailhead, ribs, and behind the shoulders (Henneke, 1983). A physical palpation of these areas is used along with a visual observance. A scale of one to nine is used with one being poor and nine being extremely overweight. Body condition scores of 1-3 are considered underweight, BCS of 4-6 are average or moderate, and horses with a BCS of 7-9 are found to be overweight or in extreme cases, obese (DeBoer et al., 2020). It is important to examine all body areas of a horse and be considerate of a horse's coat, pregnancy status, or breed as these factors may alter body physique judgement.

Body Weight. A horse's body weight depicts its overall health. Having an understanding for a horse's body weight can help determine if there is a health issue. A mature horse can gain or lose weight quickly due to a number of reasons. Gaining of body weight can be caused by an unbalanced diet, lack of exercise, over feeding, or genetics. A horse may lose weight because of dental discomforts, parasites, being overworked, under feeding, or genetics. Anecdotally, the average weight of an adult horse is 1,000 pounds, although individuals within a breed can vary based on individual genetics, feeding, and exercise. (DeBoer et al., 2020).

Fecal Particle Size. Fecal particle size is used as a tool to identify the effect of feedstuff processing on digestion. Horses reduce food particle size and excrete undigested particles in feces. Fecal particle size can be used to determine whether or not dental care can improve a horse's ability to digest feedstuffs. The smaller the fecal particle size the greater the digestion taking place. In horses consuming the same diet, larger fecal particle sizes indicate less effective grinding of feedstuffs taking place in the horse's oral cavity (Carmalt and Allen, 2006, Carmalt and Allen, 2008).

Digestibility Measurements. Digestibility is measured classically as what goes in minus what comes out. Traditionally, this is measured through total collection, where 100% of feed intake is measured and fecal output is collected over 4 days (Müller, C. E. (2012). This total collection allows determination of apparent digestibility, which does not account for alterations in digesta concentrations in the cecum and large colon (Müller and Udén, 2007, Müller et al., 2008). For instance, microbial production of amino acids that would increase the amount of an amino acid in feces as compared to what was present in digesta at the end of the ileum. However, total collection is much more feasible compared to the management of cecally cannulated horses. Total collection requires the use of equine nappies or collection stalls with solid floors that enable frequent removal of fecal material. On the other hand, field-based studies looking to perform digestibility measurements often rely on the use of markers that do not require collection of the entire fecal output, but instead a subset, with the marker being something indigestible. Therefore, concentrations of the marker in feces compared to marker concentrations in feed can be used to calculate dry matter digestibility as (1- Acid Insoluble Ash in hay/ Acid Insoluble Ash in feces). Dry matter digestibility can then be used to calculate nutrient digestibility. The use of markers can over or underestimate apparent digestibility from total collection. Acid insoluble ash (AIA) has been shown to accurately estimated total collection results by some (Sutton et al., 1977, Miraglia et al., 1999), particularly when using hay-based diets (Bergero et al., 2009), while (Karlsson et al., 2001) suggested that AIA over-estimated digestibility.

Effects of Dental Care on Equine Production

Digestibility. Horses typically have their teeth floated once per year. Horse teeth grow throughout their lifetime and must be filed, also termed floated, in order to remove sharp points that occur when the teeth do not meet up correctly which can be a function of a high grain diet. The modern diet of horses is softer than that of their ancestors and so not as much wear occurs during eating. Irregular wear patterns can cause poor feed digestion, weight loss, performance problems, and pain in the horse when wearing a halter or bridle (Jeffrey, 2009). An improvement in feed digestion has been documented in horses consuming northern forages, however this finding has not been tested in horses consuming southern forages (Allen et al., 2013). Southern forages are higher in fibers and lower in crude protein than northern forages and this tends to have a negative effect on digestibility, causing digestibility to be lower than that of northern forages (Allen et al., 2013). Improvements in the digestion of crude protein and ADF and NDF fiber have not been observed at 4 weeks post teeth-floating in horses consuming northern forages (Ralston et al., 2001).

In contrast to the above results, dental floating had no effect on short-term changes in body weight, body condition score, feed digestibility, or fecal particle size in pregnant mares (Carmalt et al., 2004). The diet was found to impact weight gain, but dental floating had no effect. A limitation of this study was fecal particle size was directly associated with the four different feed groups. Carmalt et al. (2004) used dried feces for their fecal measurements, and the use of agitation with rubber balls may have artificially reduced the overall mean fecal particle size. The study did not examine the effect of dental floating on long-term dental health. When only observing the correlation between dental lesions and feed digestibility, molar occlusal angles between 6 and 19 degrees did not influence feed digestibility, water balance, or fecal particle size in pregnant mares (Carmalt et al., 2005). Optimal molar table angles are twelve to eighteen degrees, depending on the breed, head shape and size of the horse (Jeffrey, 2009). The upper and lower quadrants should have matching curve of occlusion and curvature of Spee and Wilson, where the curve of Spee is a one dimensional caudal to rostral curve in the arcade of the cheek teeth. The curve of occlusion is a three-dimensional curve that has identical biomechanical planes. This allows for occlusal surfaces to align and reduce food particle sizes traveling across the arcades of the cheek teeth (Jeffrey, 2009). The limiting factor behind this study was the varying feed groups used for a wide range of horses, as there was no consistency in diets for these study groups. Also, no dentistry was actually performed in this study.

No changes were found in both routine or performance correction digestion of dry matter, crude protein, neutral detergent fiber, or acid detergent fiber relative to pre dental work or controls in eight mature horses (Ralston et al., 2001). Therefore, it is possible that correction to small points and hooks does not significantly improve digestion. Performance floating does not greatly affect digestion 2-4 weeks post procedure. Changes in the molar occlusal angle may affect digestion of protein and fiber. The limiting factor of the study was removal of only sharp points using hand-held rasps. The rounding of edges and bit seats does not involve balancing molar tables, which is where mechanical digestion takes place. Four weeks after the routine correction group was tested, they corrected the performance horses and limiting control factors. The primary limitations of this study were that the authors were inconsistent with when they did dental work for treatment groups. There was a lack of significant alterations in digestion. There was limited detection of small differences in digestion between treatment trials of mature horses (Ralston et al., 2001).

In an experiment using nine warmblood horses, the digestibility of dry matter, energy, crude fiber, and non-fiber extract increased significantly after floating. Horses received a grass hay diet from the first cutting of a meadow used throughout the study. Concentrates consisted of barley, oats and ground maize. Additionally, 100 g of a mineral supplement was fed per day and horse. The results showed that there was an improved digestibility in horses eating larger amounts of grain, but there was no effect on fecal particle size (Zwirglmaier et al., 2013). The limiting factor of this study was the enamel points were only smoothed and the molar table was not balanced, and there was a small sample size.

O'Neill et al. (2010) compared dental abnormalities in stabled horses and free-grazing horses. It was expected that those horses that freely graze for sixteen plus hours a day will have less dental abnormalities than those kept in a stable. Sixty Thoroughbred horses all between the ages of five and fifteen years of old were used. Results showed that the stable-kept horses showed higher total number of abnormalities compared to the free-grazing horses (O'Neill et al., 2010). The stable-kept horses also had prevailing transverse ridges, overgrowth ramps, and signs of periodontal disease. Sharp buccal and lingual edges occurred in both treatment groups. The diet of the free-grazing horses resulted in fewer abnormalities, but it is common to find sharp points in dentition coming from any diet.

Based on the group of colic horses in O'Leary's study (2016), increasing age and diastemata or gap between the teeth were the most common dental abnormalities found. Of the cases where weight loss was occurring, 20% of the horses also experienced dental abnormalities. These dental abnormalities primarily included sharp enamel points that produced buccal mucosal ulcerations that restrike the lateral excursion of the mandible. This limited the power-stroke that performed mastication and allowed for digestibility of fiber. It has been found that around 60% of overgrown teeth were associated with diastemata (O'Leary, 2016). Reduced lateral excursion or dental abnormalities will reduce masticatory function and therefore cause a loss in weight.

Data collected on twenty different horses with permanent dentition showed no significant changes between the jaw quadrants and the Triadan position using 3D-images to measure occlusal angles of cheek teeth (Listmann et al., 2016). The angles ranged from 15.1 degrees to 20.2 degrees. As you moved from rostral to caudal inside the horse's mouth, the angle increased across the arcades (Listmann et al., 2016). The limiting factor of this study was the excursion from left and right sides of the horse's jaw.

A finite element analyses was performed on a 3-D model for the maxillary and mandible molar tables for horses split into age groups: 0-5 years old, 6-15 years, and greater than 15 years (Periodontal biomechanics, 2012). They determined calculations for non-striking, closing stroke, and power stroke patterns. The results showed a uniform distribution of low strain energy density for the closing stroke and high stress strain for the power stroke. The force that is present during the power stroke can cause tissue necrosis and inflammation which is a significant limiting factor. When this happens, local trauma occurs and microorganisms begin to settle in these environments. The finite elements showed high forces that occurred in the periodontal ligament of older horse's teeth (Periodontal biomechanics, 2012). This gives way to periodontal disease occurring in geriatric horses.

Table 1. Common Forages Fed to Horses

Hay	% Crude Protein	% ADF	%NDF
Bermudagrass hay	11.161	35.383	66.547
MML hay	17.749	35.084	48.521
Legume hay	21.301	30.68	38.873
MMG hay	12.489	38.239	59.841

Note: These values obtained from Equi-Analytical, (2020).

Conclusion

The goal of any horse owner is to provide quality health for their horse's life. Nutrient digestion in horses may be dependent on proper dental correction. This study was conducted testing the hypothesis that performing dental work on horse's teeth will improve nutrient digestion. Based on these findings, the researchers in this study hypothesize that dental work will improve nutrient digestibility in horses consuming southern forages.

CHAPTER IV

MATERIALS AND METHODS

Prior to the start of this study, the Sam Houston State University Institutional Animal Care and Use Committee approved all procedures involving animals.

Study 1

One hundred and two horses were used for the age prediction study. Data were collected during routine dental exams from horses that were registered with a breed organization and owners supplied photographs of registration papers following the exam. Horses ranged in age from 5 to 24 years and included 52 mares, 27 geldings, and 23 stallions. Breeds consisted of 97 American Quarter Horses and 5 American Paint Horses.

Images of both the upper and lower incisor teeth were captured during a routine dental exam using the same mobile phone camera for all horses and taken by certified equine dentist (A. Starrett). Imaged were then uploaded to the Computerized Horse Aging Program (CHAP). Horse's mouths were held open by inserting a hand between the bars of the horse's gums. The procedure took less than 1-2 minutes to collect adequate pictures of the occlusal surfaces of the upper and lower incisors.

The app provided a two-year age range based upon the cup and ring system analysis. The app does not evaluate the Galvayne's groove method or Vegas hook. The cups are detected by computer vision, then analyzed through artificial intelligence, and compared to a database of horses with known age. When the structure of the cup becomes smaller than half the surface of the tooth and the dental star appears, the cups are considered worn. The cups start wearing on the middle incisor teeth first and then laterally till all cups are worn on incisors. A similar pattern then repeats the process with rings. The ring is a characteristic found at the very bottom of the cup. It disappears on the central incisors and then proceeds laterally till all rings disappear.

Study 2

Animals and Experimental Design. Sixteen non-pregnant Quarter Horse mares (age 4-17 years) were used in the study. None of the horses had received dental work in the 12 mo. Horses had been maintained as a group in a pasture consisting of *ad libitum* Bermuda grass (*Cynodon dactylon)* hay (10.9 CP%, 1.13 ADF%, and 3.56 NDF%) A balancer concentrate (22.8 CP%, 1.09 ADF%, and 1.55 NDF%) of 1.36 kg was offered in individual buckets in a group pen, daily prior to and during the study in order to meet or exceed the National Research Council recommendations for maintenance energy, protein and macrominerals (National Research Council, 2007). Horses were monitored to make sure they all had access to feed. All horses had a body condition score between 5 and 6, as assessed using the Henneke body condition score system (Henneke, 1983). Horses were not moved from their existing housing living situation and remained on dry lot for the duration of the study.

All horses received an aging examination using the Computerized Horse Aging Program (CHAP) as previously described in this manuscript. Prior to the start of the study, all mares received a dental examination, and buccal points located on the molar tables were assessed to block mares to treatments (Jeffrey, 2009). Mares were blocked by severity of buccal points, with 4 mares having severe points, 11 mares having moderate points and 1 mare having minimal points; therefore, a severe block and a moderate/light block were developed. Within blocks, horses were randomly assigned to one of two groups, untreated controls (CON; n=8) and those who received dental work (DENT;

n=8). Each treatment contained 2 horses with severe points, while the CON treatment contained 6 moderate points horses and the DENT treatment contained 5 moderate and 1 light point horses. Mean age of CON horses was 10.3 ± 1.5 years, while the mean age of DENT horses was 10.8 ± 1.5 years. The study began mid-August 2020 (Day 0) and ended 56 days later in October 2020 (Day 56).

Dental Corrections. Dental work was performed by International Association of Equine Dentist (IAED) certified equine practitioner (A. Starrett). All horses received an aging examination using the Computerized Horse Aging Program (CHAP) including those not receiving dental work. DENT treatment horses underwent the balancing of molar tables (12-18 degrees), incisor angle adjustments (8-12 degrees), bit seats, and lateral excursion of the mandible. Two of the DENT treatment horses underwent the removal of wolf teeth. The dental corrections were carried out with use of Jerry's Equine Dental Tools (Jerry's Equine Dentistry, Atascadero, CA) and Capps Manufacturing instruments (Capps Manufacturing, Inc., Cortland, NE). In addition, Jerry's motorized hand pieces were used for incisor adjustments with a small (1 ¼") and monster (3 ¼") cut-off wheel and mandrels (Jerry's Equine Dentistry, Atascadero, CA). All sharp buccal and lingual points, wedges, ventral curves, and malocclusion were corrected.

Images of both the upper and lower incisor teeth were captured during a routine dental exam and uploaded to Computerized Horse Aging Program (CHAP). Horse's mouths were held open by inserting a hand between the bars of the horse's gums. The procedure took less than 1-2 minutes to collect adequate pictures of the occlusal surfaces of the upper and lower incisors.

Fecal Collection for Estimation of Digestibility. Apparent digestibility of nutrients was estimated using the AIA marker method according to the methods of Müller (2012), it does not involve total collection but instead a single sample collected once per day over several days. In the present study, samples of feces were collected once per day for four days, both before and eight weeks after dental work. On each of these days, aliquots of feces were collected by rectal palpation between 0800 and 1200. Horses were rectally palpated in a random order, feces were weighed to ensure adequate collection, and then stored at -20 °C until analyses.

Blood Collection. Blood samples were obtained via jugular venipuncture on Day 0 and Day 56, after an overnight fast (FAST) and then 90 minutes after consuming their pelleted ration (FED). Blood was then transferred into heparinized, evacuated tubes (Vacutainer, BD). A total of 80mL of blood volume was collected for each horse (20 mL per collection point, 4 collection points). Following blood collection, samples were centrifuged for 10 min at 1500 × g and 4°C, after which plasma was harvested and frozen at -20 °C until analysis.

Body Condition Scores and Body Weight. Body Condition Scores (BCS) were subjectively appraised by the same researcher prior to starting the digestion study. Scores were assigned using the 9-point scale described by Henneke et al. (1983), ranging from BCS 1 (very poor) to BCS 9 (extremely fat). Body weight was determined through use of a cloth weight tape on Day 0 (Purina Mills, Gray Summit, MO). The same weight tape was used for all horses.

Feed Samples. Hay samples were collected by hand from several round bales offered in the same pasture as the horses. Samples were collected following dental treatment at day 0. The Coastal Bermuda grass (*Cynodon dactylon)* hay used for this study all came from the same cutting. Concentrate samples were taken as grab samples from the bags of concentrate at day 0. Hay and concentrate were analyzed by Cumberland Valley Analytical Services.

Sample Analysis. Fecal samples were thawed and then dried in a drying oven at 100 degrees Celsius (Thermo Electron LED GmbH, Langenselbold, Germany). Dried fecal samples were then ground in a centrifugal mill with a 1 mm screen (IKA Works, Inc., Wilmington, NC). Fecal and feed samples were analyzed for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and dry matter (DM) at the TRIES Lab (Huntsville, TX). Analysis of AIA in feed and fecal samples was performed by Cumberland Valley Analytical Services (Waynesboro, PA).

Acid Detergent Fiber. The ADF content was determined following the procedures described by Ankom Technology. A solvent resistant marker was used to label the filter bags used for the analysis. Weight of each empty filter bag was recorded, and the balance zeroed. A prepared sample of 0.45-0.50g was recorded and weighed in the filter bags. A heat sealer was used to completely seal each filter bag closed within 4mm of the top to encapsulate the sample. Three bags were placed on each of the eight Bag Suspender Trays and stacked with each level rotated 120 degrees in relation to the tray below it. An empty ninth tray was added to the top. The Bag Suspender was inserted into the Vessel and a weight placed on top of the empty ninth tray to keep trays submerged. 1900-2000 mL of ambient temperature AD solution was added to the fiber analyzer vessel. Samples were agitated for 60 minutes with heat. After the solution was exhausted, 1900-2000 mL of 70–90°C water was rinsed over the trays. Water was agitated for five minutes three times repeatedly until pH paper was no longer acidic. Filter bags were then removed, and excess water was gently pressed from bags. Bags were placed in 250mL beaker, and enough acetone was added to cover bags and soak for 3-5 minutes. Filter bags were removed from acetone and placed on a metal sheet for air-drying. Samples were completely dried in an oven at 102 ± 2°C for 2-4 hours. Residue was then stored in a desiccator for 30 minutes until the crucible was room temperature and to avoid sample becoming moist. The sample was weighed, and the remaining acid detergent residue (ADR) was removed from crucibles and stored for pure residue from NIRS spectrum. All samples were analyzed in duplicates.

The acid detergent fiber of the sample was calculated according to Equation 1:

Eq. 1: ADF (%) = $((\mathrm{P1} + \mathrm{P2}) - \mathrm{P1})/P3 \times 100$,

P1 is the crucible weight, P2 is the weight of the residue. P3 is the weight of the sample.

Neutral Detergent Fiber. The NDF content was determined following the procedures described by Ankom Technology. A solvent resistant marker was used to label the filter bags used for the analysis. Filter bags were weighed and recorded empty and the balance zeroed. A 0.45-0.50g of prepared sample was placed in empty filter bags and weighed. A heat sealer was used to completely seal each filter bag closed within 4mm of the top to encapsulate the sample. Three bags were placed on each of the eight Bag Suspender Trays and stacked with each level rotated 120 degrees in relation to the tray below it. An empty ninth tray was added to the top. The Bag Suspender was inserted into the Vessel and a weight placed on top of the empty ninth tray to keep trays submerged. A 1900-2000 mL of ambient temperature ND solution was added to the fiber analyzer vessel. A 20g of sodium sulfite and 4.0 mL of alpha-amylase was added to the solution in the vessel. Heat was added to the vessel and samples were agitated for 75 minutes. After solution was exhausted, 1900-2000 mL of 70-90°C water with 4.0 mL alpha-amylase was added to the first and second rinses. Vessel was agitated for 5 minutes during each rinse. A third hot water rinse was performed following. Filter bags were removed from vessels and excess water was gently pressed out before being added to a 250ml beaker for an acetone soak for 3-5 minutes. Filter bags air dried on a metal sheet before being completely dried in an oven at 102 ± 2°C for 2 to 4 hours. Residue was then stored in a desiccator for 30 minutes until the crucible was room temperature and to avoid sample becoming moist. The sample was weighed, and the remaining neutral detergent residue (NDR) was removed from crucibles and stored for pure residue from NIRS spectrum. All samples were analyzed in duplicates.

The neutral detergent fibre of the sample was calculated according to Equation 2:

Eq. 2: NDF (%) = $((\mathrm{P1} + \mathrm{P2}) - \mathrm{P1})/P3 \times 100$,

P1 is the crucible weight, P2 is the weight of the residue. P3 is the weight of the sample.

Crude Protein. Crude protein was examined using the Flash 2000 elemental analyzer (Thermo Fisher Scientific, Waltham, MA). The Elemental Analyzer operates by flash combusting each pellet sample and analyzing the resultant gas. For this analysis, a sample weighing 5-10mg was weighed into a tin cup (Thermo Fisher Scientific, Waltham, MA) and placed as a pellet on the machine. The machine automatically loaded each pellet sample using a CHNS chamber and oxygen gas. Nitrogen was quantified from the combustion by a helium flow to a second reactor filled with copper. This swept through CO_2 and H_2O traps and a gas chromatography column. A Thermal Conductivity Detector (TCD) calculated the percent nitrogen and then the result was multiplied by 6.14 in the Eager*Smart* software.

Calculation of Nutrient Digestibility. Nutrient digestibility was calculated using AIA in feeds and feces according to the methods of (Müller, 2012).

Dry matter digestibility= (1- Acid Insoluble Ash in hay/ Acid Insoluble Ash in feces)

Nutrient digestibility= Dry matter digestibility x (Nutrient in hay/ Nutrient in feces) x 100

Glucose. Plasma concentrations of glucose were determined using a colorimetric assay according to the manufacturer's instructions (Eton Biosciences, San Diego, California, USA). All reagents were thawed and brought to room temperature before use. Diluted 500µL of 800µM Glucose Standard was mixed with 500µL of dH2O to prepare a 400µM Glucose Standard. Samples were prepared by diluting 20 fold in ddH_20 and 50µL of diluted samples were added to each well. Samples and glucose standards were assayed in duplicate. A standard curve was run for each assay and consisted of 50µL, 40µL, 30µL, 20µL, 10µL, 5µL, 1 µL, and 0µL of Glucose Standard added to a total volume of 50 µL dH2O. A volume of 50µL of Glucose Assay Solution was added to each well and samples were incubated for 15 mins at 37°C. The reaction was stopped by adding 50µL of 0.5 M acetic acid per well followed by a brief gentle agitation. Air bubbles were eliminated in the wells using a needle prior to measurement. The absorbance was measured at 490nm using a microplate reader. The inter and intra assay CV's were 10.5 and 5.8%, respectively.

Amino Acids. Plasma free amino acid concentrations were measured by use of reverse-phase HPLC of phenyl-isothiocyanate derivatives as described (Urschel et al. 2011). Prior to derivatization, plasma samples were mixed in a 1:1 ratio with 0.4mM L-norleucine and deproteinated by centrifugation at 14,000 X *g* in a 10-kd cutoff centrifuge filter for 30 minutes at 4°C.

Statistical Analysis

Study 1. Estimated ages were compared to actual ages using Bland Altman agreement analysis and the MIXED procedure of SAS (SAS v 9.4), to determine whether the difference between predicted and actual age, e.g. the residual, was different from 0.

Study 2. All statistical analyses were performed using repeated measures ANOVA and the MIXED procedure of SAS (v.9.4, SAS Inst., Inc., Cary, NC). Digestibility of ADF, NDF, and CP were analyzed for the effects of day (0 vs. 56), treatment (DENT vs. CON) and the day by treatment interaction. For these analyses, the repeated term was day, and initial digestibility was included as a covariate ($P < 0.05$). Plasma nutrient concentrations were analyzed for the effects of day, treatment, and time (fasting vs. 90 min post feeding), and all interactions. For these analyses, the repeated term was time within day. For all repeated models, the lowest AICC index was used to select the covariance structure. Data are presented as means bounded by the 95% confidence interval.

CHAPTER V

RESULTS

Study 1

CHAP accurately predicted age using upper incisors in horses between 8 and 13, while lower incisors were accurate between 8 and 10 years (Table 1). Age prediction in horses greater than 14 years and less than 8 years was not accurate.

Table 2. Differences in estimated ages from actual ages in horses aged 5 to 24

Actual Age	N per age	Upper Incisor Residual	*P*	Lower Incisor Residual	*P*
5-7	18	-2.3±0.6	<0.001	-1.1±0.4	0.021
8-10	19	-0.6±0.6	0.317	-0.2±0.4	0.631
11-13	26	0.4±0.5	0.393	1.2±0.4	0.003
14-16	17	1.2±0.6	0.045	1.1±0.5	0.018
17-19	12	2.2±0.7	0.004	2.0±0.6	<0.001
21-24	10	3.9±0.8	<0.001	5.8±0.6	<0.001

Study 2

Body Condition Scores and Body Weight. Body Condition Scores ranged from 4.5 to 6.5 for horses prior to starting the digestion study. Scores were assigned using the 9-point scale described by Henneke et al. (1983), ranging from BCS 1 (very poor) to BCS 9 (extremely fat).Average body weight ranged from 960-1310 pounds based on a cloth weight tape at day 0.

Nutrient Digestibility. There was a tendency for an effect of the day by treatment interaction for ADF (P=0.073), whereby ADF digestibility was not different for groups at day 0 (50.3±0.8% vs. 50.3±0.8%; $P > 0.9$) but was greater ($P = 0.014$) in DENT treated horses (59.5±0.8%) than CON (56.5+0.8) at day 56 (Table 2).

NDF digestibility was not affected by the day by treatment interaction or treatment (P>0.1). However, NDF digestibility was greater at day 56 (86.3±1.3%) than day 0 (67±1.3%) across treatments (P<0.001).

There was a tendency for dentistry to improve the day by treatment interaction for CP (P = 0.093), whereby CP digestibility was not different for groups at day 0 (50.2±0.8% vs. 49.9±0.8%; P > 0.9) but tended to be greater (P = 0.088) in dental treated horses (59.5±0.8%) than controls (56.5+0.8) at day 56.

Table 3. Mean (±SEM) percent digestibility of acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP) in horses eating a diet of *ad libitum Cynodon dactylon, or* Bermudagrass and a balancer concentrate of 1.36 kg, prior (Day 0) to dental treatment (DENT) and 56 days later and in untreated controls (CON)

Treatment	Day			*P* value		
	0	56	SEM	Treatment	Time	Treatment x Time
		--NDF--				
CON	67.8	84.2	1.8	0.4	<0.001	0.1
DENT	66.9	88.4	1.8			
		--ADF--				
CON	50.3	56.5	0.8	0.07	<0.001	0.07
DENT	50.3	59.6	0.8			
		--CP--				
CON	7.1	6.8	0.3	0.1	0.4066	0.09
DENT	7.1	7.8	0.3			

Nutrient Absorption. Glucose concentrations were not affected by the day by treatment interaction or day (P > 0.1), but were influenced by time, whereby glucose was greater (P < 0.001) after eating (6.64 mmol/L [6.41-6.87]) than after a 12 h fast (4.15 mmol/L [4.01, 4.30]).

Plasma amino acid concentrations were not affected by the day by treatment interaction or day, but showed an effect by time, where they all increased 90 min after eating (P < 0.05).

Table 4. Mean (lower 95% CI, upper 95% CI) plasma glucose concentrations (µmol/L) in horses after an overnight fast (T0) and 90 min (T90) after consuming 3 pounds of a pellet meal, prior (Day 0) to dental treatment (DENT) and 56 days later and in untreated controls (CON)

Treatment	T0	T90
	---Day 0---	
CON	4226 (3933, 4541)	6747 (6279, 7249)
DENT	4125 (3857, 4411)	6489 (6067, 6941)
	---Day 56---	
CON	4056 (3774, 4359)	6598 (6139, 7091)
DENT	4210 (3936, 4501)	6713 (6276, 7180)
	---Day by Treatment Mean---	
Mean	4153 (4011, 4300)	6636 (6409, 6871)***

Note: ***Within row, means differ $P < 0.001$.

Table 5. Plasma amino acid concentrations prior to feeding (T0) and 2 hours post consumption (T90) of 1.8 kg of pelleted ration prior to dentistry (Day 0) and post dentistry (Day 56)

	T0		T90	
		Arginine		
Day of Study	CON	DENT	CON	DENT
Day 0	52.4 ± 12.2	71.9 ± 12.2	112.8 ± 12.2	132.8 ± 12.2
Day 56	48.6 ± 12.2	55.5 ± 12.2	93.1 ± 12.2	103.6 ± 12.2
Time mean	57.1 ± 6.1		110.6 ± 6.1***	
		Alanine		
Day of Study	CON	DENT	CON	DENT
Day 0	250.7 ± 25.2	216.8 ± 25.2	350.6 ± 25.2	313.2 ± 25.2
Day 56	183.8 ± 25.2	180.9 ± 25.2	262.9 ± 25.2	250.5 ± 25.2
Time mean	208.0 ± 12.6		294.3 ± 12.6**	
		Aspartic acid		
Day of Study	CON	DENT	CON	DENT
Day 0	14.7 ± 4.2	17.4 ± 4.2	27.0 ± 4.2	40.9 ± 4.2
Day 56	10.6 ± 4.2	8.9 ± 4.2	16.7 ± 4.2	18.4 ± 4.2
Time mean	12.9 ± 2.1		25.8 ± 2.1**	
		Glutamine		
Day of Study	CON	DENT	CON	DENT
Day 0	258.9 ± 24.5	227.7 ± 24.5	414.4 ± 24.5	388.7 ± 24.5
Day 56	225.7 ± 24.5	204.7 ± 24.5	367.5 ± 24.5	353.1 ± 24.5
Time mean	229.3 ± 12.3		380.9 ± 12.3**	
		Glutamate		
Day of Study	CON	DENT	CON	DENT

(continued)

Day 0	104.1 ± 6.4	98.2 ± 6.4	110.2 ± 6.4	98.8 ± 6.4
Day 56	74.3 ± 6.4	64.0 ± 6.4	88.7 ± 6.4	76.9 ± 6.4
Time mean	85.1 ± 3.2		93.6 ± 3.2***	
		Glycine		
Day of Study	CON	DENT	CON	DENT
Day 0	527.2 ± 48.0	494.2 ± 48.0	665.5 ± 48.0	588.8 ± 48.0
Day 56	458.6 ± 48.0	473.5 ± 48.0	541.1 ± 48.0	527.1 ± 48.0
Time mean	488.4 ± 24.0		580.6 ± 24.0*	
		Histidine		
Day of Study	CON	DENT	CON	DENT
Day 0	62.9 ± 5.8	60.1 ± 5.8	92.1 ± 5.8	87.4 ± 5.8
Day 56	51.3 ± 5.8	50.7 ± 5.8	74.0 ± 5.8	69.0 ± 5.8
Time mean	56.3 ± 2.9		80.6 ± 2.9**	
		Isoleucine		
Day of Study	CON	DENT	CON	DENT
Day 0	42.9 ± 3.8	41.7 ± 3.8	63.4 ± 3.8	53.5 ± 3.8
Day 56	39.3 ± 3.8	42.1 ± 3.8	52.2 ± 3.8	48.5 ± 3.8
Time mean	41.5 ± 1.9		54.4 ± 1.9**	
		Leucine		
Day of Study	CON	DENT	CON	DENT
Day 0	79.8 ± 7.9	75.1 ± 7.9	117.2 ± 7.9	98.4 ± 7.9
Day 56	70.8 ± 7.9	68.5 ± 7.9	91.6 ± 7.9	79.3 ± 7.9
Time mean	73.5 ± 4.0		96.6 ± 4.0*	
		Lysine		
Day of Study	CON	DENT	CON	DENT
Day 0	167.8 ± 12.3	180.4 ± 13.1	211.3 ± 12.3	226.1 ± 13.1
Day 56	152.5 ± 12.3	147.9 ± 13.1	187.6 ± 12.3	181.1 ± 13.1
Time mean	162.1 ± 6.3		201.5 ± 6.3**	
		Methionine		
Day of Study	CON	DENT	CON	DENT
Day 0	21.9 ± 2.3	23.4 ± 2.3	30.9 ± 2.3	30.0 ± 2.3
Day 56	22.6 ± 2.3	25.9 ± 2.3	30.5 ± 2.3	29.3 ± 2.3
Time mean	23.5 ± 1.2		30.2 ± 1.2*	
		Phenylalanine		
Day of Study	CON	DENT	CON	DENT
Day 0	44.9 ± 4.2	45.6 ± 4.2	63.7 ± 4.2	60.8 ± 4.2
Day 56	41.8 ± 4.2	44.8 ± 4.2	55.5 ± 4.2	52.8 ± 4.2
Time mean	44.3 ± 2.1		58.2 ± 2.1*	
		Proline		
Day of Study	CON	DENT	CON	DENT
Day 0	64.2 ± 9.1	63.5 ± 9.1	128.2 ± 9.1	128.3 ± 9.1
Day 56	49.7 ± 9.1	51.5 ± 9.1	102.9 ± 9.1	92.0 ± 9.1

(continued)

Time mean	57.2 ± 4.5		112.9 ± 4.5**	
		Serine		
Day of Study	CON	DENT	CON	DENT
Day 0	243.1 ± 24.7	238.9 ± 24.7	362.9 ± 24.7	335.2 ± 24.7
Day 56	196.0 ± 24.7	189.8 ± 24.7	273.9 ± 24.7	253.0 ± 24.7
Time mean	216.9 ± 12.4		306.3 ± 12.4**	
		Taurine		
Day of Study	CON	DENT	CON	DENT
Day 0	47.8 ± 5.1	45.8 ± 5.1	60.3 ± 5.1	56.9 ± 5.1
Day 56	37.1 ± 5.1	42.3 ± 5.1	43.0 ± 5.1	45.4 ± 5.1
Time mean	43.3 ± 2.5		51.4 ± 2.5**	
		Threonine		
Day of Study	CON	DENT	CON	DENT
Day 0	70.6 ± 8.7	88.5 ± 8.7	109.0 ± 8.7	106.0 ± 8.7
Day 56	58.5 ± 8.7	58.8 ± 8.7	87.1 ± 8.7	81.1 ± 8.7
Time mean	69.1 ± 4.4		95.8 ± 4.4**	
		Tryptophan		
Day of Study	CON	DENT	CON	DENT
Day 0	50.5 ± 5.2	48.8 ± 5.2	83.7 ± 5.2	71.5 ± 5.2
Day 56	46.9 ± 5.2	48.7 ± 5.2	60.4 ± 5.2	59.0 ± 5.2
Time mean	48.7 ± 2.6		68.6 ± 2.6*	
		Tyrosine		
Day of Study	CON	DENT	CON	DENT
Day 0	52.6 ± 6.6	47.6 ± 6.6	85.7 ± 6.6	70.1 ± 6.6
Day 56	55.8 ± 6.6	49.6 ± 6.6	76.6 ± 6.6	62.6 ± 6.6
Time mean	51.4 ± 3.3		73.7 ± 3.3*	
		Valine		
Day of Study	CON	DENT	CON	DENT
Day 0	123.8 ± 9.2	123.1 ± 9.2	175.4 ± 9.2	161.4 ± 9.2
Day 56	118.4 ± 9.2	118.0 ± 9.2	152.9 ± 9.2	140.5 ± 9.2
Time mean	120.8 ± 4.6		157.5 ± 4.6*	

Note: ***Within row means differ P<0.001

CHAPTER VI

DISCUSSION

The primary objective of this study was to determine the effect of dental correction on nutrient digestion and absorption when measured 56 d after undergoing dentistry as compared to untreated controls. Specific objectives were to examine ADF, NDF, crude protein digestion and plasma glucose and amino acid concentrations before and after eating. A secondary goal was to evaluate the accuracy of a computerized horse aging app.

The main finding of this study was that dental correction tended to improve fiber and crude protein digestion from forages, as estimated by the AIA marker method. The current study's results are in agreement with those of a previous study that found improvements in apparent digestibility of fiber after dental correction, although the previous study did not note changes in crude protein digestibility (Zwirglmaier et al., 2011). The effect of dental work to improve fiber digestion may be due to increased capacity for mechanical destruction of feeds by the teeth. When the opposing teeth are fully in occlusion and making contact between the upper and lower arcades, greater destruction of food particles can occur (Jeffrey, 2009). Secondly, a greater capacity to chew could lengthen chewing time and research examining the chewing patterns of dairy cattle showed that chewing time is associated with saliva production that in turn promotes fiber digestion (Beauchemin and Buchanan-Smith, 1990). Future research should investigate the role of malocclusions on length of time spent chewing each bite of feed and whether saliva production is enhanced in horses following dental correction. Interestingly, Zwirlgmaier et al. (2004) demonstrated that voluntary hay intake was not altered by dental correction, and so greater digestibility is unlikely to be due to greater nutrient intake.

In previous work by Zwirlgmaier et al. (2004), digestibility was measured after 4 wk, whereas the current study measured digestibility at 8 wk. This length of time could have played a role in the differing results observed, suggesting that a longer time may be necessary to observe changes in some digestion parameters. The previous study also utilized a variety of diets ranging from 0.6 - 4.1 kg of concentrate daily, and when separating horses by concentrate intake level, those with concentrate intakes greater than 3 kg also had greater nutrient digestibility. In the current study, horses were offered 1.36 kg of concentrate daily, putting them at a lower range of concentrate intake, a level that corresponded to lower overall nutrient digestibility.

Type of dental correction was also different between the two studies, with Zwirlgmaier et al. only smoothing enamel points and not floating the chewing surface. In contrast, the current study employed a more complete dental correction that additionally floated malocclusions on the chewing surface. Food particles are broken down from larger particles to smaller particles on the occlusal surface of the molar teeth. Therefore, the level of dental correction may impact nutrient digestibility. Floating the chewing surface allows the mandible and maxilla jaw to fully extend from left to right without catching on sharp points or malocclusions, which maximizes the amount of time that feed particles are crushed between opposing teeth. Malocclusions would then negatively affect digestion of nutrients because opposing teeth do not make contact with each other. When horses have moderate to severe lesions on the molar tables, feeds cannot be fully broken down in the chewing cycle because teeth do not come in contact during the closing stroke

(Peffers, 2016). Proteins in plants exist in a complex matrix that requires physical destruction for greater digestibility (Hymøller et al., 2012). Our digestibility results suggest that correcting the chewing surface may have enabled horses on the current study to have greater capacity to mechanically digest forage to extract crude proteins. Intriguingly, the observed tendency for increased forage crude protein digestibility disagreed with postprandial amino acid concentrations that did not change after dental correction. However, blood samples were collected after consuming a concentrate meal, and concentrates contain more digestible protein sources than does hay. Alternatively, the tendency for crude protein digestion from hay may not have been biologically significant enough to extend to concentrate digestion. Preliminarily, it appears that smoothing of enamel points improves fiber digestion from forages while fully correcting the chewing surface may be required for improving crude protein digestion.

In the current study, trends were observed for increased nutrient digestion, however, these did not reach true significance. Factors that could have influenced these results are the small number of horses used, the marker method of estimating apparent digestibility, individual variation in nutrient digestion due to intestinal tract health, the lack of horses with severe lesions, forage quality, and a short evaluation period of 56 d. The number of horses included was determined from a power analysis of data from previous studies, however, the current study included different diets than those previously used, which could have contributed to the inability to achieve significance. For instance, Coastal Bermudagrass hay is typically higher in fiber and lower in protein than cool season grasses, resulting in overall lower nutrient digestibility (Ball et al., 2001). The current study also employed an estimate of apparent digestibility, which Bergero et al.

(2009) adequately estimate apparent digestibility of hay-based diets in horses under field conditions. However, other studies found this method to overestimate digestibility (Palmgren-Karlson). The current method was employed because total collection was not feasible.

A second factor influencing the results was that our horses aged from 4 to 17 years of age. As horses age, more variation in tooth growth, wear patterns, and digestive tract damage occur, which will produce more inherent variability in nutrient digestion (Gibbs and Potter, 2002). Controlling for age was not possible in the current study but is recommended for future research. It is also possible that the mares started the study without enough severe lesions to have impacted basal nutrient digestibility. For instance, Carmalt et al. (2005) found that molar occlusal angles did not significantly associate with digestibility at 16-19 degrees, but outside of these ranges did have an impact. In the current study molar occlusal angles were corrected to 10-18 degrees for mares receiving dental work. Prior to correction all horses had steep occlusal angles of 18 degrees or more, and these angles were also observed in the control horses. Lastly, the current study lasted 56 days, but further research should follow horses monthly through the course of a full year to investigate peak nutrient digestibility. For this study, horses' teeth were not re-assessed at day 56 so we are unsure if dental correction is maintained for at least 56 days or has already begun to decline to pre-correction levels.

An additional finding of this study was the overall increase in digestibility from day 0 to 56 for NDF. Although all hay was from the same batch, bale to bale variations do occur and could be a factor (Orloff et al., 1999). For this study, grab samples were collected from a random sampling of bales, and samples were homogenized prior to analysis. Future research should also include individual bales fed during digestion trials in order to control for this potential variation.

A further examination on postprandial nutrient concentrations in plasma was performed. It was hypothesized that increased nutrient digestion would correspond to increased nutrients appearing in plasma after eating a concentrate meal, although this was determined to not be the case. Glucose is a digestion product of starch and is also in the diet in the form of simple sugars, while amino acids are digestion products of proteins. Commercial concentrates contain ground grains and extracted protein sources, which may already have maximal digestibility. Future research should be performed investigating the effect of dentistry on nutrient absorption following a hay meal.

A second objective of this study was to determine the accuracy of a computerized horse aging app (CHAP). CHAP is an innovative mobile application using the old technique of aging horses by viewing the occlusal surface of the incisors to train a computer module to predict the age of a horse based off a photo of that horse's teeth (Jeffrey, 2009). Based on current knowledge, there is no other mobile device that competes on the market. The current study found that CHAP could accurately predict age using upper incisors in horses between 8 and 13, while lower incisors were accurate between 8 and 10 years. Age prediction in horses greater than 14 years and less than 8 years was not accurate and this could have been because there were fewer horses of these ages in our study. Older horses have consumed a variety of feeds throughout their lifetimes which can influence age prediction. More horses need to be used in this mobile prediction in order to investigate its accuracy for younger and older horses. The CHAP app finds its predominant usefulness in horses of unknown age, and for these horses, if the app predicts the age between 8 and 13 then it is accurate within 1 year of age. Users should take upper and lower teeth into consideration when using the app. The mobile app can be used for speed and objectivity for those who already know the ages within the range. It is a good aid to people doing their own aging of horses and can provide them with comfort that the data is supporting their estimated age within those margins as well.

Implications

This study's results are consistent with previous work showing that dentistry can improve fiber digestion. More complete dental work, including balancing the chewing surface has the potential to improve forage crude protein digestion. Future studies should control for age and follow horses for a longer period of time. Plasma concentrations of glucose and amino acids following a concentrate meal are not influenced by dental work.

CHAPTER VII

CONCLUSION

Digestion starts in the mouth with well-formed teeth, healthy saliva flow, and a balanced dental occlusion. A study was conducted testing the hypothesis that performing dental work on horse's teeth will improve digestion of fiber and crude protein from forages. When measured at 56 days post dentistry crude protein and fiber digestion tended to be improved in horses consuming a primarily forage-based diet of coastal bermudagrass hay. However, glucose and amino acid absorption was not changed following consumption of a concentrate meal. Horses consuming high amounts of grains might not show physiologically relevant increases in nutrient digestion following dentistry. A second objective of the study investigated the accuracy of a computerized horse aging app. The CHAP app is useful for horses of unknown age presumed to be between 8 and 13 and users should take upper and lower teeth into consideration.

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VITA

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Missouri Southern State University, Joplin, MO, Diploma May 2018, GPA 3.594/4.0
Sam Houston State University, Huntsville, TX, Current Grad Student, GPA 3.71/4.0

Professional Experiences

Denim Dentistry, Texas, January 2019-present

•**Owner-** I am a certified equine dental practitioner performing dental exams in conjunction with veterinary practices or horse owners. I evaluate soft tissue, wolf teeth removal, extractions, caps, incisor and canine maintenance, balance molar tables, examine bite and limited excursion, provide charting, and biting care. I also provide nutrition and diet education for clients as well. I have instructed equine dental educational classes for local horse-riding clubs, SHSU, and the Equine Dental School of Texas.

Computerized Horse Aging Program (CHAP), Texas, January 2019-present

•**Representative-** I promote the CHAP app at equine dental conferences and am a sales person for horse owners. The Computerized Horse Aging Program is a great tool for my practice as I spend a lot of time aging horses for my clients who buy, sell, and trade in the horse industry. The mobile device captures the occlusal surface of the incisor teeth and this image is uploaded to the app, which analyzes the surface using the cup and ring method. Within thirty seconds the app will provide an age based on the cone shaped cup located in the top third of a developed tooth.

Qualifications & Skills

•SHSU Equine Science Teaching Assistant
•Certified and Practicing Equine Dentist
•Computerized Horse Aging Program Sales Representative (CHAP)

Awards & Honors

•FFA STAR Area Agribusiness Ownership
•FFA Food Processing Award
•State FFA Degree
•American FFA Degree
•Rotaract Club President (2016-2018)
•Rotary Paul Harris Fellow
•Rotary International Convention Attendee (2017)
•Alpha Chi Honors Society
•Homer McCallum Scholar
•Trustee Scholar
•Dr. E Samuel Gibson Scholar
•Lion Ambassador Scholar
•Putnam County Alumni Scholar
•Rotary District 6040 GSE Team Member-Australia

Publications

- Starrett, A., Urso, P. M., Smith, R., & Suagee-Bedore, J. K. (2021). 82 Effect of dental floating on ADF and NDF digestion after 8 weeks. *Journal of Equine Veterinary Science*, *100*, 103545.
- Starrett, A., & Suagee-Bedore, J. K. (2021). 91 Accuracy of a computerized horse aging program. *Journal of Equine Veterinary Science*, *100*, 103554.

Professional Associations

- International Association of Equine Dentist- Member
- Rotary International Dual Member (Unionville, MO Rotary Club and MSSU Rotaract Club)
- Rotaract International (SHSU Club Member)

Grants & Fellowships

- Sam Houston State University Ag Development Grant $500
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Licenses & Certificates

- International Association of Equine Dentist Certificate
- Equine Dentistry School of Texas Certificate
- Rotary Leadership Institute Certificate
- University of Missouri-Kansas City Dental School AEP Certificate
- Unbound Foundations Course Certificate

Volunteer Work

- International Association of Equine Dentist Membership Committee
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