REDUCING CRIBBING FREQUENCY IN HORSES THROUGH

DIETARY SUPPLEMENTATION OF TRYPTOPHAN

AND CALCIUM CARBONATE

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REDUCING CRIBBING FREQUENCY IN HORSES THROUGH DIETARY SUPPLEMENTATION OF TRYPTOPHAN AND CALCIUM CARBONATE

Blaine O'Reilly

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REDUCING CRIBBING FREQUENCY IN HORSES THROUGH

DIETARY SUPPLEMENTATION OF TRYPTOPHAN

AND CALCIUM CARBONATE

Blaine O'Reilly

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Date of Graduation

VITA

Blaine O'Reilly, son of Dr. George and Barbara O'Reilly, was born on December 27, 1973 in Washington DC. At the age of six months, he, his parents, and sister moved to Huntsville, Alabama where he lived until 1992. After graduating from Randolph High School in 1992, he attended Auburn University until the spring of 1997 studying Animal and Dairy Sciences. Blaine then spent three years working in Huntsville until he returned to Auburn University in January of 2000. He graduated with a Bachelor of Science Degree in Animal and Dairy Sciences in the spring of 2000. He next began graduate school at Auburn University, working towards a Master of Science in Animal Sciences. In the fall of 2002, Blaine began veterinary school at Auburn University's College of Veterinary Medicine as a member of the class of 2006.

THESIS ABSTRACT

REDUCING CRIBBING FREQUENCY IN HORSES THROUGH

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Blaine O'Reilly

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Cribbing is an equine stereotypy that results in damage to both the horse and the facilities. Serotonin is a neurotransmitter that has calming effects on many animals' behavior (Fernstrum, 1994). It has been shown that tryptophan is the precursor for serotonin and supplementing this amino acid will result in increased serotonin production (Fernstrum, 1994). The resultant mood change may calm the horse and lead to a decrease in cribbing activity. Some authors hypothesize that an acidic gastric pH will result in increased cribbing activity in horses (Nicol et al., 2002). Perhaps the supplementation of an antacid such as calcium carbonate will increase the pH of these horses and decrease their cribbing activity. The objectives of this study were two fold: first, to determine if either tryptophan or calcium carbonate supplementation would decrease the number of

cribbing bouts per day, the number of crib-bites per bout, or the duration of each bout, and second, to determine if either supplement resulted in an increase in fecal pH, a gross estimate of gastric pH. Nine horses were utilized in a split-plot design in which the whole plot was designed as a replicated 3 x 3 Latin square with three replicates. The horse*period*treatment term was used as the error term to test for treatment differences. Each horse spent three weeks on each treatment: 3 g feed grade tryptophan mixed in 30 cc corn syrup, 42.5 g calcium carbonate mixed in 30 cc corn syrup, or 30 cc corn syrup control. Treatments were administered at 0700, 1100, 1500, 1900, and 2300 hrs. Each horse was observed on day 7, 14, and 21 of each treatment for a 17 h period. A washout period of 1 week was implemented between each treatment.

There was no effect of treatment on fecal pH ($P > 0.18$), with values ranging from 6.4 ± 0.1 to 6.8 ± 0.1 over the three week test period. There was also no effect of treatment on the total number of cribbing bouts per day ($P > 0.3$), with values ranging from 48.7 ± 6.1 to 72.6 ± 6.1 over the three week test period. Similarly, there was no significant change of the average duration of each bout ($P > 0.9$), with these values ranging from 260.8 ± 24.7 to 334.6 ± 24.7 for the test period. Finally, there was no effect of treatment on the average number of crib-bites per bout ($P > 0.8$), with a range of 24.5 ± 2.7 to 32.7 ± 2.2 over the three week period. These results show that neither tryptophan nor calcium carbonate, at the given doses, will affect cribbing frequency or duration in horses.

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TABLE OF CONTENTS

LIST OF TABLES

I. INTRODUCTION

Cribbing is an equine stereotypy that is bothersome to many horse owners because of the damage it may cause to both the horse and the farm itself. While cribbing, the horse places his upper incisors on the surface of an object, flexes his neck, rocks backwards, and emits an audible grunting noise. Often horses that are still useful are sold, slaughtered, or euthanized when they display this behavior. Many horsemen believe that cribbing is learned by horses, although there is no documented proof that horses learn via observation (Baker and Crawford, 1986). A cure for this behavior would benefit both the horse owner and the horse, possibly preventing its premature death.

There are many beliefs as to why horses begin and continue to crib. Most believe that horses crib in response to boredom or frustration (Kiley-Worthington, 1983; Marcella, 1988; Houpt and McDonnell, 1993; Simpson, 1998; Nicol, 1999). There is also evidence that there is a heritable component to cribbing (Vecchiotti and Galanti, 1986; Marsden, 1995). A decrease in gastric pH also has been shown to increase the frequency of cribbing in horses (Gillham et al., 1994). Finally, it has been hypothesized that horses crib to increase their serotonin or endorphin levels (Dodman et al., 1987; Bagshaw et al., 1994; Miller, 1996).

There are several commercial supplements available that claim to decrease the frequency of cribbing in horses or stop it completely. The main ingredients in these

supplements are often a combination of tryptophan and calcium carbonate. There are mixed results from horse owners as to whether or not these products work. Tryptophan is a precursor for serotonin, and administering pure tryptophan can result in serotonin synthesis (Fernstrum, 1994). The synthesis of serotonin occurs in the brain, and the resultant increase in neurotransmitter will have effects on the animal's behavior (Fernstrum, 1994). Large levels of tryptophan in food have resulted in a drowsy or euphoric state in humans (Fernstrom, 1994), and decreasing levels resulted in a deterioration in the mood of women (Delgado et al., 1990). Bagshaw et al. (1994) showed that tryptophan supplementation reduced the occurrence of head twisting in a horse. Therefore, it is likely that supplementation of tryptophan in a horse's diet may alter its mood such that it remains calmer and consequently decrease cribbing frequency and/or duration per bout.

Calcium carbonate ($CaCO_3$) is a base that will raise pH levels in solution and decrease acidity (Maton and Burton, 1999). It has been shown that horses that have an acidic environment in their stomachs will be more likely to begin cribbing (Nicol et al., 2002). It also has been proven that horses that spend more time grazing at pasture or eating hay will produce more saliva and consequently buffer their stomach contents and crib less (Meyer et al., 1985). There have been mixed opinions in the literature as to whether or not calcium carbonate will increase or decrease the acid content of the stomach. Some authors have found that high doses of calcium carbonate may increase bicarbonate load to the duodenum and consequently increase gastric acid secretion in the stomach (Fordtran and Collyns, 1968; Levant et al., 1973, Behar et al., 1977; Brodie et al., 1977). This acid rebound has many researchers questioning the use of $CaCO_3$ as an

antacid. However, it has been proven that despite the occurrence of acid rebound, the overall pH of gastric fluids still increases when $CaCO_3$ is used due to its buffering abilities (Caldwell et al., 1976). Therefore, at the proper dosage, the addition of a buffer to the diet such as $CaCO_3$ may raise the pH in the stomach of horses, thus resulting in fewer or shorter cribbing episodes.

The objectives of this study were to: determine if tryptophan or calcium carbonate can decrease the frequency of cribbing; and determine if either tryptophan or calcium carbonate changes fecal pH in the horse (a gross indication of GI tract pH)

II. LITERATURE REVIEW

Cribbing

Cribbing is a stereotypic behavior in horses that may result in damage to both the horse and its surroundings. A stereotypy is defined as, “a repeated, relatively invariant sequence of movements with no obvious function” (Broom and Kennedy, 1993). For events to be considered a stereotypy they should be performed at a higher than normal rate (Dantzer, 1986). These habits have been reported in many animal species, including laying hens in battery cages, veal calves in crates, and sows in tethers or crates (Dantzer, 1986). Stereotypies in horses can be divided into two categories: first, locomotor stereotypies, including weaving, head bobbing, pawing, and wall kicking, and second, oral stereotypies, which include cribbing, wood chewing, and flank biting. Each of these can vary in frequency and amplitude, with some horses spending so much time on their stereotypy that they become unthrifty and unkempt. McGreevey et al. (2001) found that cribbing horses spent less time eating and visiting water sources than non-cribbers. Generalized behavior of these animals also can fluctuate. Some appear flighty and nervous while others seem calm and composed (Houpt and McDonnell, 1993).

Cribbing can be defined as a series of movements performed by the horse that are repeated but may not all be present with each occurrence and may vary between individual horses. With many horses, licking of the object comes first (McGreevy et al.,

1995b), followed by grasping of the object with the upper incisors. Next, the horse flexes its neck and rocks backwards, this manipulates the larynx and results in a "grunting" sound (Marcella, 1988). The muscles involved in this act are the sternomandibularis, omohyoideus, sternothyroideus, and sternohyoideus muscles (Forssell, 1926). Wood chewing is a redirected behavior in horses that is not quite as involved as cribbing, but some horse owners confuse the two acts (McGreevey et al., 1995a). Here, the horse simply bites and chews on wooden objects in the barn or pasture, actually removing pieces of wood with each bite. Some say this is due to a horse's craving to spend more time grazing at pasture (Marcella, 1988). Both cribbing and wood chewing cause damage to the barnyard, but cribbing is the only one that may damage the horse's teeth.

Many authors erroneously state that air is swallowed during cribbing (Marcella, 1988; Houpt and McDonnell, 1993). McGreevy et al., (1995b) used endoscopy to determine the function of the pharynx and esophagus, as well as any movement of air, during cribbing. They determined that the epiglottis and soft palate remained in their resting positions, and therefore the nasopharynx was not occluded, thus preventing deglutition. However, a small pocket of air was observed in the proximal esophagus at the time of each crib-bite, but this air was not moved down the esophagus via peristalsis. Instead, this air returned through the esophageal sphincter to the nasopharynx. These researchers attribute the characteristic "grunting" sound heard during cribbing to the movement of the esophageal sphincter, but note that this air is not swallowed due to an increase in pressure in the pharynx. Instead, it is a build up of negative pressure in the proximal esophagus due to the dilation of the thyropharyngeal and cricopharyngeal muscles of the esophageal sphincter coupled with the contraction of the sternocephalicus

and sternohyoideus muscles of the neck that permits the movement of air to the proximal esophagus only.

There are many opinions as to the etiology of equine stereotypies, including cribbing. Some authors claim that cribbing is a learned behavior (Kiley-Worthington, 1983), but there has been no solid scientific data to back these claims that horses learn via observation (Baker and Crawford, 1986). It seems likely that young horses, due to the fact that they are teething and constantly exploring, may be more likely to adapt this behavior, but the data is not there to support this (Houpt and McDonnell, 1993). Further evidence to support this is shown because most stereotypies, including cribbing, begin around 20 weeks of age, a time when young foals are learning rapidly and developing routines (Nicol, 1999; Waters et al., 2002). Feeding patterns and types of feed also have been implicated in an increased rate of equine stereotypies. On average, domestic horses on pasture graze for 16 hours per day (Kiley-Worthington, 1983). When fed a concentrated diet, this supplies more energy than a diet consisting solely of grass, which is more typical of a diet for a horse in the wild. This allows horses to consume enough feed in only a few hours to meet energy needs, so they potentially have more time during the day to develop or continue their stereotypic behavior. Additionally, certain types of feed may increase cribbing in horses. Gillham et al. (1994) found that feeding horses grain and sweetened grain significantly increased cribbing rates as compared to pelleted alfalfa hay or no feed at all. The authors attribute this increase to the fact that more palatable rations increase morphine potency and pain threshold (Kanarek et al., 1991; Roane and Martin, 1990). The β-endorphin levels consequently increase and activate dopamine pathways which increase stereotypic behaviors (Goodman et al., 1983).

There is growing evidence of a heritable component to cribbing. Vecchiotti and Galanti (1986) examined 1035 Thoroughbred horses to determine if there was a higher incidence of cribbing (and other stereotypies) in families of horses versus the entire population. They determined that the incidence of cribbing was 2.4% in the entire population, but it was 30% in families of horses that previously exhibited the cribbing stereotypy. Foals imitating mares cribbing was excluded as a reason for the foals cribbing in the experiment. The authors do concede that this merely suggests an inherited component to cribbing, but they did not determine the exact inheritance pattern in this study. In another study by Marsden (1995), certain families were noted to have a much higher incidence of stereotypic behavior than others, with some families rising as high as 67%. This study examined multiple stereotypies, including cribbing, pacing, circling, and headswinging. The authors determined that the stereotypy exhibited by the offspring will not necessarily mimic the one displayed by the sire or dam, but stereotypic behavior exhibited by the sire and/or dam does increase the incidence of stereotypic behavior in the offspring. In families where a granddam, grandsire, or sibling is affected and neither the dam nor sire exhibit stereotypic behavior, the occurrence in foals is 25%. If either dam or sire are affected, the occurrence increases to 60%, and if both are affected, the occurrence becomes 89%.

Finally, environment has been shown to play a major role in the occurrence of many equine stereotypies, including cribbing. According to McGreevey et al. (1995a), horses that spend more time confined in stalls are more likely to exhibit stereotypic behaviors. Also, they note that horses in stalls bedded with material other than straw have an increased occurrence of stereotypies. Further, horses housed in stalls which reduce

visual, tactile, auditory, and/or olfactory input will have an increased occurrence of cribbing (Kiley-Worthington, 1983). She continues to state that horses that are further restricted, i.e. tied in a stall, have even a higher likelihood of a stereotypy developing. Marcella (1988) noted that stereotypies often develop when a horse's needs are not met. He lists these needs as related to those of horses in the wild, such as being a member of a small group with a set hierarchy and having the ability to graze slowly throughout the day while moving freely. Confinement to a stall or small paddock, coupled with being fed as few as two times a day with little access to pasture may lead to a frustrated animal and consequently the development of stereotypies. Being confined in a stall also restricts the animal from fleeing from stressful situations, which is their natural reaction to stress (Luescher et al., 1991). In addition, relocating horses on the same farm or onto other farms may upset the established hierarchies and have similar results (Marcella, 1998). Erratic training habits also may contribute to the development of stereotypies in horses (Luescher et al., 1991). Waters et al. (2002) reported that one environmental factor that resulted in the onset of cribbing in young horses was the type of feed received postweaning. Foals fed concentrate feed were 4 times as likely to begin cribbing as those that did not. These researchers also noted that of the foals that started cribbing, 74% demonstrated wood-chewing prior to the onset of cribbing. They further state that foals weaned by abrupt separation from their dams in a box stall may begin to suckle at various indiscriminate objects in the stall, leading to the onset of cribbing in these foals. Miller (1996) states that there may be ways to avoid the establishment of cribbing in foals housed in box stalls. One way is to install mirrors in the stall to alleviate the sense of isolation, and the other is to construct the upper portion of the stall with bars or grills

instead of solid wood. This will allow the foal to socialize and visualize other horses, thus reducing stress and seclusion.

It is hypothesized that management and environment can facilitate cribbing, therefore, it is apparent that simple changes in these practices may result in a decreased occurrence of this stereotypy. Simply increasing the amount of exercise, contact with other horses, and time allowed for grazing often decreases the act of cribbing in horses (Simpson, 1998). Probably the most widespread, cheapest, and easiest control of cribbing is a cribbing collar or strap. This device, when applied properly, prevents the contraction of the strap muscles, thus preventing the larynx from being pulled in a caudal direction (Fjeldborg, 1993).

There are also surgical and pharmacological solutions to cribbing, although Nicol (1999) notes that these should not be explored until the underlying causes are eliminated. Initially, Forsell (1926) suggested complete myectomy of the muscles involved in cribbing. This results in a success rate of around 80%, but considerably disfigures the animal's ventral neck (Hamm, 1977). Additionally, according to Forssell (1926), it is not advised to perform this procedure on horses less than three years of age due to the risk of tracheal malformation. Hamm (1977) suggested a different technique in which the ventral accessory nerve was transected to denervate the muscles involved in cribbing. Although Hamm reported success, Firth (1980) reported no success after performing the surgery on eight horses. Greet (1982) combined accessory nerve transection with partial myectomy of the omohyoideus, sternohyoideus, and sternothyroideus muscles. This seems favorable because there is limited cosmetic damage and horses under the age of three can undergo this operation. However, Hakansson et al. (1992) reported the original Forssell's

procedure resulted in better long term prevention (89%) as compared to the combined myectomy/neurectomy technique (50%). Further, there was a higher incidence of relapse with the combined technique (31%) as compared to Forssell's original procedure (11%).

There have also been attempts made to use narcotic antagonists to treat cribbing in horses. Dodman et al. (1987) injected several narcotic antagonists either intravenously or intramuscularly to test their effects on cribbing. The results varied, with naloxone stopping cribbing for about 20 minutes but nalmefene and diprenorphine ceasing any cribbing for four or more hours. They also determined that a continuous supply of nalmefene (5 to 10 mg per hour) would have positive results for up to one week. Obviously, such a routine is not practical for the common horseman due to time and money, but these results are intriguing in that with further research scientists may develop a treatment that is both economical and time friendly.

Tryptophan

Tryptophan is one of the essential amino acids that are the subunits of protein molecules. Its side chain consists of an indole group that characterizes it as a non-polar, aromatic amino acid (Voet and Voet, 1995). Tryptophan, as with the other aromatic amino acids, is synthesized via the shikimate pathway with chorismic acid as the intermediate. Chorismic acid is the common intermediate for the synthesis of many cellular compounds containing benzene rings. The precursors for chorismic acid are phosphoenolpyruvate and erythrose-4-phosphate. When these two compounds react with each other they form 3-deoxy-D-arabino-heptulosonate-7-phosphate or DAHP. The enzyme that is required for this reaction to take place is DAHP synthase. Next, the DAHP

is converted to 3-dehydroquinate, and then through a series of other steps shikimate is formed which is finally converted to chorismic acid (Garrett and Grisham, 1999). Once the chorismate intermediate is reached, the pathway for tryptophan biosynthesis branches in another direction from that of phenylalanine and tyrosine, the other aromatic amino acids.

The process of forming tryptophan from chorismic acid involves six steps. The first step requires the enzyme anthranilate synthase, which is an α2β2-type protein. The β-subunit essentially provides the –NH2 group for the resulting compound anthranilate. The α-subunit from anthranilate synthase is actually the catalyst for the second step in the synthesis of tryptophan. This reaction is termed the phospho-ribosyl-anthranilate transferase reaction and the compound 5-phosphoribosyl-1-pyrophosphate (PRPP) is the product. The PRPP next rearranges in step three such that the ribose moiety is isomerized to the ribulosyl form and enol-1- (o-carboxyphenylamino)-1-deoxyribulose-5- phosphate is the result. Next, the decarboxylation and subsequent ring closure yield the indole ring and form indole-3- glycerol phosphate. The enzyme catalyzing this fourth reaction is indole-3-glycerol phosphate synthase. Finally, in the fifth and sixth reactions, serine takes the place of glyceraldehyde-3-phosphate to form tryptophan. Tryptophan synthase is the enzyme involved in this reaction. This enzyme is also an α2β2-type protein. The α-subunit forms the indole and 3-glycerol phosphate by cleaving indoleglycerol-3-phosphate. The β-subunit then adds the indole to yield the final product (Garrett and Grisham, 1999).

The other aromatic amino acids, tyrosine and phenylalanine, along with tryptophan, are the precursors for the neurotransmitters serotonin, dopamine, and

norepinephrine (Fernstrom, 1994). Neurotransmitters are chemical signals that affect various target cells throughout the body (Cooper et al., 1991). Because of their role in brain function, any increase in the formation and release of these neurotransmitters will have an effect on behavior (Fernstrum, 1994). Tryptophan is the precursor for serotonin, and the synthesis of serotonin can be accomplished by the administration of pure tryptophan (Fernstrum et al., 1991). For tryptophan to be converted to serotonin it must first cross the blood-brain barrier between the blood stream and the brain fluid. It is composed of endothelial cells that line the capillaries of the brain. These cells are connected to one another by tight-junctions. The junctions are formed by the endothelial cells being tightly fused with one another as opposed to having pores between them as with most capillaries in the body (Guyton and Hall, 2000). The blood-brain barrier exists in most areas of the brain with the exception of certain areas of the hypothalamus, pineal gland, and area postrema. These areas require rapid diffusion of certain substances because they contain sensory receptors that respond to certain bodily changes which may require immediate regulation (Guyton and Hall, 2000). There are certain substances that are permeable across this barrier, such as water, carbon dioxide, oxygen, and most lipid-soluble substances (Guyton and Hall, 2000). Amino acids, however, are not readily permeable across the barrier and therefore require other routes of entry.

The endothelial cells of the blood-brain barrier are saturated with transport mechanisms that control the passage of substances into and out of the brain (Rapoport, 1976). The transport of the non-polar, aromatic amino acids is competitive. Therefore, the uptake of tryptophan into the brain depends not only on the amounts of tryptophan circulating in the blood, but also on the amounts of tyrosine and phenylalanine present

(Fernstrom, 1994). For example, ingestion of a nonprotein (carbohydrate) meal causes blood tryptophan to rise and the blood levels of the other large, neutral amino acids to fall, an indirect consequence of insulin secretion (Fernstrom, 1994). This results in a competitive advantage for the uptake of tryptophan into the brain. Contrarily, the ingestion of a meal containing moderate amounts of protein raises blood tryptophan *and* the blood levels of the other large, neutral amino acids about equally, thus causing no change in competition at the large, neutral amino acid transporter and, therefore, no change in the level of brain tryptophan (Fernstrom, 1994). The reason for this competition is that tryptophan uptake into the brain is mediated by a neutral amino acid carrier. This carrier also mediates the passage of tyrosine and phenylalanine and therefore their concentrations may alter the rate of tryptophan uptake (Oldendorf, 1971). Yuwiler et al. (1977) conducted an experiment to prove this fact. They used tyrosine at two concentrations: low levels to represent the normal blood levels of tyrosine in the body, and high levels to approximate the sum of all neutral amino acids in the blood (excluding threonine). In the solution containing free tryptophan and the high concentration of tyrosine, uptake of tryptophan is 54% of the solution containing tryptophan alone. In the solution containing tryptophan bound to albumin, uptake was only 36% of that found in pure tryptophan solutions. This follows because tryptophan bound to albumin decreases the concentrations of effective tryptophan in the bloodstream.

A second factor that affects tryptophan uptake in the brain is the pH of the blood. As McMenamy and Oncley (1958) reported, the binding of tryptophan to albumin is pH dependent. Again, Yuwiler et al. (1977) found that 67% of the tryptophan was bound to albumin at pH 6.8 and 86% was bound at pH 7.6. In this experiment, the pH of the buffer

mixture did not appear to affect the uptake of tryptophan. Once more, the uptake of tryptophan bound to albumin was less than that of free tryptophan.

A third factor that affects tryptophan uptake in the brain is the overall concentration of tryptophan itself. Brain uptake index, or BUI, decreased with increasing tryptophan levels, but relative tryptophan uptake into the brain increased 8 fold (from 24 to 515 μM) when free tryptophan levels were elevated 21 fold (Yuwiler et al., 1977). The BUI, as defined by Yuwiler et al. (1977), is calculated as the ratio of isotopes in the brain sample divided by the ratio in the starting material. As usual, the presence of albumin decreased the uptake into the brain.

Finally, the presence of fatty acids may alter tryptophan uptake in the brain. By competing with tryptophan for binding with albumin, fatty acids such as palmitate may decrease the overall uptake of tryptophan by the brain (Yuwiler et al., 1977). The researchers pretreated albumin with palmitate and found that the proportion of free to bound tryptophan increased and that the apparent Ka of the tryptophan-albumin complex decreased. The uptake into the brain, however, did not change as much as the levels of free tryptophan in the blood. In this experiment, the free tryptophan levels increased four fold while the tryptophan uptake into the brain only increased 1.4 fold.

Once the tryptophan has traveled through the circulation, reached the brain, and passed through the blood-brain barrier, it may affect behavior. It has been demonstrated that rats, when exposed to stress, have increased concentrations of 5-hydroxyindoleacetic acid (5-HIAA) in their blood. Because 5-HIAA is the primary metabolite of serotonin (5-HT), its increase, coupled with the fact that serotonin levels do not change, suggests that serotonin is being synthesized and subsequently metabolized under these conditions

(Kennett and Joseph, 1981). Several stresses also resulted in elevated brain tryptophan levels (Curzon et al., 1972). Fernstrum and Wurtman (1971) showed that an elevated brain tryptophan concentration is connected to an increased rate of serotonin synthesis. Joseph and Kennett (1983) hypothesize that this tryptophan increase is required to prevent exhaustion of serotonin availability. This explains why serum serotonin levels do not increase or decrease under stressful conditions even though the concentration of its primary metabolite, 5-HIAA, does increase (Curzon et al., 1972; Kennett and Joseph, 1981; Bagshaw et al., 1994).

It has been argued that the change in the serum tryptophan: large neutral amino acid (LNAA) ratio after a meal is in fact large enough to have an influence on brain function and therefore mood (Fernstrom, 1994). When subjects consumed a breakfast containing no protein, the serum tryptophan: LNAA ratio increased. Also, despite additional meals, this ratio remained high if the subsequent meals were also void of protein. When the breakfasts containing moderate amounts of protein were fed, the ratio did not rise after the meal. After consuming the subsequent meals that also contained moderate amounts of protein, the ratio remained at low levels as compared with levels from the diet that contained no protein. Finally, when breakfast was fed that contained large amounts of protein, the serum tryptophan: LNAA ratio began to fall and continued to fall throughout the day despite the other meals. Again this was because of the high protein content of the additional meals. Therefore, if a person ate one breakfast one day that was low in protein and then ate a breakfast high in protein the next day, the serum tryptophan: LNAA ratio could vary from about 0.15 the first day to 0.08 the next. The average difference in serum ratios would be around 0.07 (Fernstrom, 1994).

Studies by Delgado et al. (1990) and Weltzin et al. (1994) have shown that an increase in the serum tryptophan: LNAA ratio of this amount (0.07) is large enough to alter brain function. Delgado et al. (1990) administered pure amino acid mixtures to human subjects either containing or lacking tryptophan. The idea was that ingestion of an amino acid mixture that lacked tryptophan would reduce brain tryptophan uptake, decrease serotonin synthesis, and therefore depress the mood of the subject. The day that the experimental mixture that contained no tryptophan was administered, subjects reported a significant deterioration in mood. However, on the day that the control mixture was given, there were no reports in any changes in mood. Furthermore, when the mixture containing no tryptophan was given, a significant drop in serum tryptophan was observed. Weltzin et al. (1994) quantified the serum tryptophan: LNAA ratio changes in a separate experiment using the same amino acid mixtures. This experiment also showed that serum tryptophan decreased after the mixture containing no tryptophan was administered. It also showed that serum levels of the other LNAAs increased.

These two studies confirm the fact that the serum tryptophan: LNAA ratio can be altered in man with meals of varying protein content, and that changes in mood can result from a ratio decrease of 0.07 to 0.09 (Fernstrom, 1994). It might be thought from these conclusions that the change in brain function could be linked to serotonin neurons, but unfortunately not enough research has been conducted to confirm this speculation as it relates to food.

One study conducted by Spring et al. (1983) showed increased sleepiness in women, but not men, after consuming a high carbohydrate breakfast. This carbohydrate load should increase tryptophan uptake by the brain and therefore increase the synthesis

and subsequent release of serotonin in the brain. This result is consistent with the findings of Hartman (1983), but that study only documented sleepiness and did not look at mood specifically. Lieberman et al. (1986) examined obese subjects with and without a prior craving for carbohydrate meals. The subjects that were not "carbohydrate cravers" demonstrated an increase in sleepiness and a decline in mood after ingestion of the carbohydrate snack. Those who reportedly often consumed such snacks reported no sleepiness as well as an elevation in mood. These results are somewhat inconsistent with previous studies. The subjects who did not prefer the carbohydrate snack would be expected to display an increased level of sleepiness, but they should not demonstrate a decline in mood. This worsening of mood is unusual because most literature shows that mood deteriorates when serotonin synthesis and secretion declines. Similarly, the increase in mood seen in the subjects who prefer carbohydrate snacks would agree with most research, but the lack of any sleep effects is inconsistent. The craving of these snacks is said to raise brain tryptophan uptake and therefore increase the synthesis and secretion of serotonin. The problem arises in that the snacks used in the Lieberman (1986) study contained high enough levels of protein to prevent the stimulation of serotonin secretion.

pH

The pH of a solution is defined as the negative log to the base 10 of the hydrogen ion concentration and it determines the acidity of the solution (Voet and Voet, 1995). Chief cells in the mucosal lining of the stomach secrete hydrochloric acid (HCl), which contributes to the majority of the acidity of the stomach in mammals. When excess HCl is

produced, or not enough buffer is introduced to the gastric juices, a harsh environment is created that may be detrimental to the stomach itself, and may lead to the onset of certain equine stereotypies such as cribbing (Nicol et al., 2002).

It is widely known that there are many problems associated with the digestive tracts of equids. Most attention, however, is focused on the small and large intestines and not on the stomach itself. The occurrence of gastric ulcers in horses can be quite high depending on the diet and use of the animal, and these ulcers may induce cribbing. It has been shown that feeding a high concentrate diet will increase the occurrence of cribbing in horses (Waters et al, 2002). Pagan (1997) stated that the major cause of gastric ulcers in horses was the prolonged exposure of the fundic region of the stomach (the non-glandular region) to gastric acid. This non glandular portion of the stomach does not secrete mucus to protect it from the acid produced. In this situation, saliva production is one way to buffer the stomach and help prevent irritation. Meyer et al. (1995) showed that horses fed a high grain diet produced 206 g of saliva per 100 g of dry matter consumed per day. Contrarily, if horses consume a diet consisting of hay and fresh grass, they will produce 400 to 480 g of saliva per 100 g dry matter consumed per day. The reduced saliva production when on a high grain diet is because saliva is secreted only during mastication (Alexander and Hickson, 1970). Furthermore, gastrin, a hormone that stimulates the release of gastric acid, is stimulated by grains and pelleted concentrates (Smyth et al., 1988). Gastrin is not secreted as much with diets composed primarily of hay, therefore showing another reason for increased acidity if on a high grain diet. Horses have evolved as foraging animals that spend the majority of their day grazing and

therefore chewing. The high grain diet is a man-made phenomenon which may contribute to the onset of cribbing in horses.

Nicol et al. (2002) performed a study to test the relationship of gastric inflammation and ulceration and cribbing in horses. They suggest that horses on high grain diets may be seeking an alternative way to stimulate their parotid salivary glands in order to increase saliva production and therefore increase the pH of the stomach. Although it is not known whether or not horses can detect the acidity of their stomach contents, behavioral signs such as bruxism have been shown to correlate with gastric damage in horses (Murray, 1998). Nicol et al. (2002) compared 15 cribbing foals to nine normal foals endoscopically to determine the presence of inflammation and/or ulceration of the stomach. The cribbing foals had significantly higher combined ulceration and inflammation scores as compared to the normal foals. The researchers further report that evidence of salivary release by the salivary glands during cribbing is needed to confirm their hypothesis. They think that the parotid gland is most likely involved, but again they have no evidence. Later research has shown that cribbing horses do produce less saliva than non-cribbers, supplying further support for this hypothesis (Moeller, 2000). Nicol et al. (2002) also note that this study does not eliminate the possibility that an acidic stomach content may be the result of cribbing rather than a cause of it or that the two may be independent results of diet.

Antacids consist of aluminum, calcium, magnesium, or sodium salts or a combination of any of the above. Commonly used antacids include calcium carbonate, sodium bicarbonate, and magnesium or aluminum hydroxide (Maton and Burton, 1999). When antacids are used, it is expected that they either will decrease the hydrogen ion

and/or pepsin activity, or aid the gut in resisting these compounds. They accomplish this by either reducing the total number of hydrogen ions available for reverse-diffusion through the gastric mucosa or by absorbing pepsin to alter its activity. If pepsin levels rise too far, the mucosal lining of the stomach may be eroded, thus preventing it from secreting mucus for protection (Guyton and Hall, 2000). Further, if the antacid is capable of raising the pH of the gastric contents to above 6.0, it will inactivate any pepsin present in the stomach. At a pH of 6.0, optimal levels of proteolysis are passed (Morrissey and Barreras, 1974). Most gastric juice is normally neutralized once it contacts the bicarbonate that is found in the duodenum, so any decrease in the amount of acid reaching the duodenum will enhance this procedure, thus decreasing the amount of acid throughout the small intestine as well.

Calcium carbonate is one of the most widely used antacid on the market today. It reacts slowly with the hydrochloric acid of the stomach to produce calcium chloride, carbon dioxide, and water (Maton and Burton, 1999). Some studies have shown that the addition of calcium carbonate to the stomach may actually increase the amount of gastric acid secretion (Fordtran and Collyns, 1968; Levant et al., 1973; Behar et al., 1977; Brodie et al., 1977). Calcium's role in acid production is to activate calmodulin kinases, which in turn produce acid secretion (Hade and Spiro, 1992). However, in a study by Hurlimann et al. (1995), similar doses of antacids were used with and without calcium with no such effect occurring. Further, Caldwell et al. (1976) reported no acid rebound when using calcium carbonate as an antacid in humans. One determining factor seems to be dose because if the amount of antacid supplemented exceeds the amount of gastric acid present, the excess bicarbonate will be reabsorbed in the duodenum. This will in turn

increase gastric acid secretion in the stomach (Morrissey and Barreras, 1974). In one study by Frey et al. (2001), acid rebound did occur when 595 mg/kg calcium carbonate was given to horses. This dose probably exceeded gastric acid production, thus stimulating gastric acid secretion due to excess bicarbonate levels in the duodenum.

There are several adverse reactions that can occur with the use of calcium carbonate as an antacid. Belching and flatulence has been reported in humans, and prolonged usage has resulted in hypercalcemia. Also, calcium carbonate doses as low as 4g per day can enhance chronic renal failure in humans (Maton and Burton, 1999).

III. MATERIALS AND METHODS

Animals and Observations

Horses used in this experiment were maintained at the Auburn University Horse Unit according to a protocol (PRN# 0406-R-2437) that had been approved by the Institutional Animal Care and Use Committee. The animals used in the experiment were nine mature horses ranging in age from two to fifteen years that were previously known cribbers. There were four American Quarter Horses consisting of two geldings, one mare, and one stallion; four Thoroughbreds consisting of three geldings and one mare; and one American Saddlebred gelding.

Horses were fed a grain-molasses concentrate and oat hay diet formulated to meet National Research Council (1989) equine nutritional guidelines. The concentrate was fed twice daily, at approximately 0700 and 1500 h. The horses were maintained on Bermudagrass pasture with oat hay and water available ad libitum. The three treatments were: 3g feed grade tryptophan, 42.5 g calcium carbonate, or 30cc corn syrup carrier as a control. Each treatment was administered by oral dose syringe five times daily. The dosing times were 0700, 1100, 1500, 1900, and 2300 h. Tryptophan and calcium carbonate were weighed and placed in small cups and then transferred to the syringe with 30 cc of corn syrup for tryptophan and 30 cc corn syrup Q.S. with water for calcium carbonate immediately prior to administration. These dosages were formulated to

resemble suggested dosages found in commercial calming agents for horses. Horses were rotated through each treatment so that every horse received every treatment. Horses were maintained on each treatment for three weeks with a one-week washout period between treatments. During the washout periods all horses were administered 30 cc of corn syrup control five times daily.

Prior to the experiment, an initial 24-h observation period was implemented to determine peak cribbing times. It was determined that visual observation hours would be from 0600 until 2300 on day 7, 14, and 21 of each treatment. Three horses were observed during this time by two to three observers. On the morning of each observation day, all nine horses involved in the project were confined in a smaller section of their pasture in a 51 x 19 m pen that consisted of fourteen wooden fence posts along the edge that were used as cribbing objects. The stallion remained in its 12 x 61 m paddock that contained two wooden posts for cribbing. These conditions best mimicked their normal cribbing objects at pasture. Free choice water and hay were then supplied and the horses were allowed a 45 min adjustment period before observations began. On one day, observations were ceased for a period of 30 min due to the threat of tornadoes. This did not seem detrimental to the study because most cribbing activity ceased during periods of hard rain anyway.

Through initial observations prior to the experiment, it was observed that many horses often lick objects and even place their teeth upon it without actually performing a crib-bite. Therefore, the beginning of a cribbing bout was defined as the moment when the horse initially contracted the strap muscles of its neck. Once the strap muscles were contracted, the observer started a stopwatch to record the length of time for each bout.

During the bout, the number of actual contractions, or crib-bites, was recorded. A cribbing bout ended if the horse stopped contractions of the strap muscles for more than 60 sec. The succeeding activity of the horse also was noted. On observation days feeding and dosing times remained the same to maintain consistency. During hours of darkness flashlights were used to ease observation, and these did not seem to alter the cribbing habits of the horses or startle them in any way. At the end of each observation period the horses were released back into their regular pasture. They were maintained on this pasture throughout the study. On each observation day, the pH of each observed horse's initial defecation was determined with a digital pH meter (Omega PHH26, Omega Engineering, Inc., Stanford, CT).

Design and Analysis of the Experiment

The experiment was conducted according to a split-plot design. The whole plot was designed as a replicated 3 x 3 Latin square with three replicates. The split plot was created by recording the data during three cycles. Data were analyzed using the GLM procedure of SAS (SAS Institute, Cary, NC). The horse*period*treatment term was used as the error term to test for treatment differences. In the split plot, the only effects considered to be of possible importance were the effect of cycle and the interaction of cycle with treatment. Effects in the split plot were tested using the residual term.

Table 1

Schedule of Treatments

Horse	Tx. #1	Tx. #2	Tx. #3
Zook	Control	$CaCO_3$	Tryptophan
Stormy	Tryptophan	Control	$CaCO_3$
Bourbon	$CaCO_3$	Tryptophan	Control

(a.) Group A

Horse	Tx. #1	Tx. #2	Tx. #3
Dutch	Tryptophan	Control	$CaCO_3$
Nilla	Control	$CaCO_3$	Tryptophan
Bar	$CaCO_3$	Tryptophan	Control

(b.) Group B

Horse	Tx. #1	Tx. #2	Tx. #3
Oakie	Control	$CaCO_3$	Tryptophan
Nie	$CaCO_3$	Tryptophan	Control
Flashy	Tryptophan	Control	$CaCO_3$

(c.) Group C

IV. RESULTS AND DISCUSSION

Fecal pH

Each horse spent a total of three weeks on each of the two treatments, tryptophan and calcium carbonate ($CaCO_3$), as well as the corn syrup control, and was observed once weekly for 17 h for each treatment. When analyzed by week, the data for fecal pH showed a significant change ($P < 0.02$) during each three-week period (Table 2). However there was no treatment by week interaction ($P > 0.8$), and examination of weekly means revealed no meaningful pattern to weekly pH fluxuation. When fed the control, the mean fecal pH started at 6.59 during week one, decreased to 6.35 during week two, and then increased to a high value of 6.60 during week three. These data suggest that mean fecal pH naturally fluxuates over time for horses under normal conditions. It is interesting to note that the mean fecal pH of cribbing horses in this experiment, 6.51, is closer to the mean fecal pH of non-cribbing horses in the Nicol et al. (2002) study, 6.58. Nicol et al. (2002) found the fecal pH of cribbing horses to be much lower at 6.07. This could be attributed to certain environmental factors, such as type and amount of feed eaten by the horses in the different experiments. Both experiments do, however, indicate that fecal pH will fluxuate over time. The fecal pH of the horses also fluxuated while on the $CaCO_3$ supplement. The mean fecal pH during week one was higher than week two, but again week three's values were higher than week one's

(Table 2). This shows that the addition of $CaCO_3$ at the levels given will not significantly affect fecal pH and the values will fluxuate similarly to the control values. This was a bit unexpected because it was thought that the buffering ability of $CaCO_3$ might have resulted in a higher mean fecal pH value. There are a couple of reasons as to why this might have occurred. First, although fecal pH has previously been used in literature as a gross indicator of pH throughout the gastrointestinal tract (Nicol et al., 2002), this may not be an accurate assumption. It is well known that there is a low pH in the stomach (3.0 to 6.0) to facilitate protein digestion, but this pH gradually increases as material passes through the intestines of horses. Bicarbonate secretion begins in the duodenum and increases through the jejunum until it reaches its peak output in the ileum, with the pH of material entering the cecum registering 6.5 to 7.0. The pH remains relatively constant throughout the cecum and colon, resulting in a fecal pH of similar value (Gordon and Allen, 1988). Therefore, due to a normal rise in pH throughout the GI tract, fecal pH may not be an accurate measure of gastric pH in horses and the pH of the foregut may have increased. Second, there have been reports of a phenomenon referred to as acid rebound when $CaCO_3$ is administered as an antacid in humans as opposed to other antacids such as sodium bicarbonate or magnesium sulfate (Fordtran and Collyns, 1968; Levant et al., 1973; Behar et al., 1977; Brodie et al., 1977). These researchers all concluded that it was the calcium found in $CaCO_3$, not its neutralizing capability, that resulted in the rebound. Acid rebound is defined as a temporary increase in gastric acid secretion that follows oral administration of an antacid (Hade et al., 1992). Although there have been some reports that acid rebound in association with $CaCO_3$ does not exist (Caldwell et al., 1976; Hurlimann et al., 1995), most researchers agree that it does occur. However, it seems

apparent that despite the increase in gastric acid secretion, the overall gastric pH still increases due to the superior buffering properties of $CaCO_3$ (Fordtan and Collyns, 1966; Hollander and Harlan, 1973). Further, when Deering et al. (1979) demonstrated acid rebound after administration of aluminum and magnesium hydroxide, they also concluded that the total amount of acid entering the duodenum was decreased. It is important to note that the preceding experiments were all performed on humans, and all were testing antacid effects on gastric or duodenal ulcers. Perhaps the desired pH change is different to acquire decreased cribbing frequency than to relieve ulcers. Also, no studies were found in the literature concerning horses and acid rebound, so it has yet to be determined whether or not it occurs in horses, although it is likely. Therefore, although $CaCO_3$ seems to result in an overall decrease in gastric pH, perhaps the resultant acid rebound created enough gastric acid secretion to maintain pH at a low enough level to sustain cribbing. Finally, there were similar findings when mean fecal pH was analyzed for the period the horses were on the tryptophan supplement (Table 2). Once again, mean fecal pH was lowest in week two, but increased to its highest value during week three. This would be expected because tryptophan does not have buffering capability, so any supplementation should not change pH values of the feces. Overall, treatment had no significant effect on fecal pH ($P > 0.8$).

Table 2

Least Squares Means and Standard Errors for Fecal pH of Horses on Each Treatment According to Week

	Mean pH + SE		
	Control	Tryptophan	$CaCO_3$
Week 1	6.6 ± 0.1	6.6 ± 0.1	6.7 ± 0.2
Week 2	6.4 ± 0.1	6.6 ± 0.1	6.5 ± 0.1
Week 3	6.6 ± 0.1	6.7 ± 0.1	6.8 ± 0.1

Number of Cribbing Bouts per Day

There were some interesting results when the total number of cribbing bouts per day was examined, although they were not statistically significant ($p > 0.2$) (Table 3). When the horses were fed the corn syrup control, the total number of bouts per day decreased continuously from week one (72.6 +/- 6.1) through week three (58.3 +/- 5.1). Although this decrease was not statistically significant ($p>0.2$), this was the only consistent trend shown when analyzing this variable. Obviously, the control should not affect the total number of bouts per day, therefore these results were not considered to have biological significance either. Most other experiments evaluate cribbing bouts per hour, with Nicol et al. (2002) finding an overall decrease in the number of bouts per hour (21.4 from 23.4) after treating with 125 g per day of Neighlox (KPL Technology), which contains dihydro-aluminum sodium carbonate and aluminum phosphate as the active ingredients. The cribbing frequency actually increased in the middle of their study to 33.2

bouts per hour, which corresponds to frequency changes in our study. While fed tryptophan or $CaCO_3$ supplements, the highest number of bouts was observed during week two, but values again varied over the entire three weeks, and no significant differences in the number of bouts were detected ($p > 0.2$). Week one of tryptophan treatment showed the lowest total number of bouts per day for any treatment at 48.7. Although not significant, it might indicate an initial effect of tryptophan on cribbing, with the horses developing some type of tolerance for the treatment over time. To this author's knowledge there is no other data currently available that tests long term effects of tryptophan on horses. The random pattern of total bouts per day again could be a result of environment, with rain, ambient temperature, and distractions from other horses or events at the horse unit all possibly playing a role in the cribbing habits of the test horses. Although efforts were made to maintain an environment as close to normal as possible, the smaller pen used to confine the horses was necessary for observation, but it did not allow horses the range they normally have. Often submissive horses were displaced by more dominant horses, thus cutting their cribbing bout short. Although this happens under normal conditions as well, it might have occurred more frequently in the smaller area, thus slightly skewing results. Overall, the number of cribbing bouts per day was not affected ($P > 0.2$) by treatment.

Table 3

Least Squares Means and Standard Errors for Total Cribbing Bouts Per Day on Each Treatment According to Week

	Total cribbing bouts per day ± SE		
	Control	Tryptophan	$CaCO_3$
Week 1	72.6 ± 6.1	48.7 ± 6.1	63.1 ± 6.1
Week 2	65.6 ± 5.1	61.3 ± 5.1	72.0 ± 5.1
Week 3	58.3 ± 5.1	58.9 ± 5.1	60.6 ± 5.1

Duration of Cribbing Bout

Again, the differences in the average duration of each cribbing bout per treatment were not significant ($p > 0.2$) (Table 4). While fed the control, bout duration again decreased steadily over the three-week period, ranging from 295.6 +/- 29.5 (week one) to 260.77 +/- 24.7. Again, the control should not have any effect on cribbing bout duration, and these results are not considered biologically significant. The $CaCO_3$ supplement resulted in the same fluxuation as seen in the number of cribbing bouts per day, with week one being highest, 318 +/- 29.5 s, and week two being lowest, 248.5 +/- 24.7 s. Week three was lower than week one at 283.33 +/- 24.7 s, but this was not statistically significant ($P > 0.2$) over the entire period. Although this decrease might seem promising, with a P value of 0.2 this cannot even be termed a trend towards success, although a similar experiment had similar results. Nicol et al. (2002) found a decrease in time spent

cribbing from 547 s per hour to 431 s per hour when foals were treated with 125 g per day of Neighlox (KPL Technology) over a three-day period. These results were also not stastically significant, however. Finally, there was a trend found when horses were treated with tryptophan. The average duration of each bout actually increased from 241.77 to 334.55 over the three-week test period ($p<0.1$). These results, however, did not coincide with previous experiments. Bagshaw et al. (1994) found a decrease in head twisting behavior when tryptophan was administered to horses. Further, studies in man have shown that tryptophan consumption has had a calming effect on mood (Fernstrum, 1994). Therefore, it was plausible that supplemented tryptophan would calm the horses and result in a decrease in various aspects of cribbing, including the average duration of each bout.

Table 4

Least Squares Means and Standard Errors for Average Durations of Each Cribbing Bout on Each Treatment According to Week

	Bout duration (s) ± SE		
	Control	Tryptophan	$CaCO_3$
Week 1	295.6 ± 29.5	241.8 ± 29.5	318.6 ± 29.5
Week 2	281.0 ± 24.7	242.6 ± 24.7	248.6 ± 24.7
Week 3	260.8 ± 24.7	334.6 ± 24.7	283.3 ± 24.7

Average Number of Crib-Bites per Bout

No significance was found between any treatment and the average number of crib-bites per bout ($p>0.7$) (Table 5). Again, when fed corn syrup, the horses' average crib-bites per bout decreased steadily over the three week period, ranging from 33.13 +/- 2.7 to 25.67 +/- 2.2. This finding was not significant, with factors such as weather and environment being possible causes for the decrease. When fed the $CaCO_3$ supplement, the numbers of crib-bites per bout decreased overall for the three-week period, but week three (27.1 +/- 2.2) was slightly higher than week two (26.6 +/- 2.2). These findings were not significant and natural variation was attributed for the changes. The average number of crib-bites per bout increased when horses were fed the tryptophan steadily over the three-week period, although not significantly. The increase from 24.5 +/- 2.7 crib-bites per bout to 32.7 +/- 2.2 is again considered to be due to natural variation. No other data

were found in the literature to compare to the findings of the present experiment. However, it follows that if the duration of each bout increased while fed the tryptophan supplement, the average number of crib-bites per bout would also increase. Although not significant, these increases in both bout length and the average number of crib-bites per bout while being fed tryptophan are intriguing. Miller (1996) states that most stereotypies are a result of stress and animals perform them to elaborate the production of endorphins. Endorphins are opiate-like compounds that are formed in the brain that have a calming effect on the animal when released. Around twelve naturally occurring opiate-like substances are presently recognized, and all are breakdown products of larger proteins: proopiomelanocortin, proenkephalin, and prodynorphin. Fibers originating in the raphe magnus nucleus secrete serotonin, and this in turn results in enkephalin secretion from the dorsal horns of the spinal cord (Guyton and Hall, 2000). Therefore, it seems likely that supplementation of tryptophan resulting in the synthesis of serotonin and consequently enkephalin would decrease cribbing in horses. In human medicine, serotonin is a key substance in the study and production of antidepressants. Fluoxetine, or Prozac, is a serotonin reuptake inhibitor, and it is commonly used as an antidepressant today. It is interesting to note, however, that this drug must be taken for four to six weeks before any antidepressant effects are seen (Ganong, 2001). Therefore, the three-week period implemented in this experiment may not have been long enough to create the desired effect of decreased cribbing frequency.

Table 5

Least Squares Mean and Standard Errors for Average Number of Crib-Bites per Bout on Each Treatment According to Week

	Avg crib-bites per bout ± SE		
	Control	Tryptophan	$CaCO_3$
Week 1	33.1 ± 2.7	24.5 ± 2.7	30.7 ± 2.7
Week 2	28.3 ± 2.2	28.1 ± 2.2	26.6 ± 2.2
Week 3	25.7 ± 2.2	32.7 ± 2.2	27.1 ± 2.2

General Discussion

Although efforts taken in this experiment did not cease or even reduce cribbing frequency in any of the horses examined, there is still the possibility of finding a cure for this equine stereotypy. The first step in finding a cure, or at least a treatment, should come in defining the exact cause, or causes, of cribbing. It seems likely that pH of the stomach and/or the rest of the gastrointestinal system plays a major role in the onset of cribbing in horses. Nicol et al. (2002) showed that young horses that crib are more likely to have gastric ulcers than non-cribbing horses. These horses also had a lower mean fecal pH than the non-cribbing group. In their experiment, the antacid diet given not only improved the condition of the stomach, but it also reduced the cribbing behavior in these horses. The type and the amount of antacid given differed in the Nicol et al. study as compared to the current one. Nicol et al. (2002) included 125 g of Neighlox (KPL Technology), of which the active ingredients are dihydro-aluminum sodium carbonate

and aluminum phosphate, mixed in with the normal diet of commercially available yearling cubes. The current experiment supplemented the horses with 42.5 g of $CaCO_3$ five times daily in addition to their normal diet of grain-molasses concentrate and oat hay. As stated earlier, the $CaCO_3$ antacid has been reported by some to create a hypersecretion of gastric acid, thus decreasing pH more commonly than the dihydro-aluminum sodium carbonate (Fordtran and Collyns, 1968; Levant et al., 1973; Brodie et al., 1977). Perhaps this explains the contrasting results. Further, the horses used in the Nicol et al. (2002) test were much younger than those in the current study. Therefore, the cribbing habit had been established for a much longer period of time, perhaps being engrained in the animal's behavior pattern and resulting in a resistance to change regardless of treatment in the present study. Therefore, by the time the $CaCo_3$ was supplemented, the horses seemed unresponsive.

This brings about another interesting point regarding gastric pH and cribbing. Most horses begin cribbing around twenty weeks of age (Nicol, 1999; Waters et al., 2002), which also corresponds with the time that many horses are first exposed to sweet feed or substances that are high in grain content. Gillham et al. (1994) showed that increasing the amount of grain fed to horses previously known to crib increases cribbing rates. Perhaps it is the introduction of this diet that decreases gastric pH, resulting in the onset of cribbing habits. The idea hypothesized by Nicol et al. (2002) that horses crib to increase saliva production and consequently increase buffering of the stomach content seems valid, and there has been proof that cribbing horses produce a lower baseline of saliva as compared to non-cribbers (Moeller, 2000). It may be necessary to perform future experiments testing the effects of varying amounts of $CaCo_3$ on gastric pH to see if

this could be an effective tool to use against cribbing. For the best results, a direct reading of gastric pH should be taken and analyzed, rather than fecal pH. Time and supplies prevented this from being possible in this experiment.

Along with an acidic environment in the stomach, it seems likely that curiosity itself could play a role in the onset of cribbing in horses. The inherent desire of foals to explore their environment with their mouths makes one wonder if this could lead to the act of cribbing. If so, the foals may quickly adapt this behavior as a stereotype and continue it for years. The data stated earlier that most cribbing begins in horses around the age of twenty weeks (Nicol, 1999; Waters et al., 2002) adds to this suspicion since this is a time that foals are becoming increasingly curious as well as brave and exploring more. Additionally, foals experience a period of stress and change during this time as they are weaned, introduced to new feeds, and begin training. Further, the fact that there seems to be some type of inherent aspect to cribbing could explain an early start to this stereotypy. Data shows that horses do not learn via observation (Baker and Crawford, 1986), so there may be an innate drive to begin cribbing in some foals. Perhaps it is a combination of the increasing grain content in the diet, curiosity, along with genetics that leads to cribbing in young horses. It is reasonable to believe that many factors contribute to the stereotypy of cribbing in horses.

Although tryptophan supplementation did not result in a decrease in cribbing frequency in this experiment, it has been shown to decrease the incidence of head twisting, another equine stereotypy (Bagshaw et al., 1994). In this same experiment, mares' heart rates decreased during periods of isolation stress when they were supplemented with tryptophan. It also has been demonstrated that tryptophan has a

calming effect on humans when supplemented (Liebermann et al., 1986), but no other reports concerning the effects of tryptophan on equine behavior were found. In the human studies, the resultant sedate behavior was attributed to a subsequent increase in serotonin levels due to the supplementation of tryptophan. The Bagshaw et al. (1994) trial, however, found no increase in blood serotonin levels in horses after the tryptophan supplementation. The present experiment was also testing the behavioral effects of tryptophan supplementation on behavior in horses, specifically cribbing. The current experiment required oral supplementation of 3 g feed grade tryptophan while the Bagshaw et al. (1994) study orally administered 0.1 mg kg^{-1} of body weight or 0.05 mg kg^{-1} of body weight of tryptophan (high and low doses, respectively). The head twisting was reduced in a linear fashion with dose. In retrospect, it is unfortunate that blood serotonin levels were not measured in the current experiment. Although it is clear that the tryptophan supplement did not decrease cribbing frequency, it would be interesting to know if blood serotonin levels correlated with tryptophan supplementation in the present study. Although there has been success with the supplementation of tryptophan on behavior in humans, studies to date are contradictory as to its effects on equine stereotypies. It is interesting that heart rates decreased in mares when on tryptophan in the Bagshaw et al. (1994) study. Perhaps this provided enough of a calming effect to decrease the incidence of head swinging in the horses studied. Cribbing, however, seems to be a result of many factors, and possibly a slight decrease in the feeling of stress is not enough to curb its occurrence. It may be that cribbing becomes almost second nature to the horse, as if eventually it performs the act without being consciously aware that it is doing it. If this is the case, then the horse may not be cribbing to gain some satisfaction,

and preventing or completely eliminating this stereotypy may prove even more difficult than previously thought.

There have been other pharmacological agents used in attempts to decrease cribbing frequency in horses. These efforts, although promising, also have provided mixed results. Dodman et al. (1987) established that narcotic antagonists do prevent cribbing in horses for at least short periods of time. However, these treatments do not work if given orally, and IV injection proved to be short acting, requiring frequent infusion for long term results. This type of treatment would be costly and complicated for the average horse owner. Further research may lead to long term termination of cribbing in horses following a single injection, but present technology does not make this possible. Perhaps the cure will be found when the correct combination of drugs and/or supplements is discovered. For instance, due to time and financial constraints, the combination of both $CaCO_3$ and tryptophan was not examined. This author was interested in the effects of each substance individually, not the combination of both. However, because the combination of the two is what seems to have a positive effect in commercial substances, it would have strengthened the experiment if the combination had been inspected as well. That being the case, it appears that neither tryptophan nor $CaCO_3$ individually will decrease cribbing behavior at the given doses. Perhaps higher doses may prove more effective, although there has been a report of induced acute hemolytic anemia in horses when given 0.6 g of tryptophan per kilogram of body weight via stomach tube (Paradis et al., 1991), so dosage must be calculated carefully.

Besides the use of various drugs and supplements, there have been many other proposed methods for preventing cribbing in horses. Several surgical procedures have

been created that either remove the strap muscles of the neck or render them useless via neurectomy (Forsell, 1926; Hamm, 1977; Firth, 1980; Greet, 1982; Hackansson et al., 1992; and Fjeldborg, 1993). These procedures have proven successful in preventing cribbing, but they are expensive, invasive, and some are even disfiguring to the horse. To date, the most inexpensive and successful device available for the prevention of cribbing is the cribbing collar. This device, when used properly, will prevent the contraction of the strap muscles of the neck, thus making it virtually impossible to crib (Fjeldborg, 1993). Some horses inevitably will find a way to crib regardless of whether or not they are wearing a cribbing collar. Unfortunately, these horses often are euthanized or slaughtered because of the lack of the ability of the owner to prevent damage caused by the horse. However, this author still believes that a cribbing collar is the best way to prevent cribbing in horses. It is important to note that once the collar is removed, the horse will resume cribbing as if it had never ceased at all.

Cribbing is an equine stereotypy that has created problems for both horse and horse owner for many years. There have been several attempts made using various methods to reduce the frequency of its occurrence or stop it completely. It seems apparent that there are many factors that combine to induce this behavior as well as facilitate it once started. To find a cure, the exact etiology, or combination of etiologies, must be determined. It also must be known exactly what factors enhance the behavior once it has started. Until then, there are many supplements, devices, and surgical techniques available that have resulted in mixed success in many horses. It must be kept in mind that individual horses may respond differently to each treatment, so a continued effort of trial and error must be employed to find the most successful treatment for each horse.

V. IMPLICATIONS

The hypothesis that tryptophan would result in adequate endorphin release and/or that $CaCO_3$ would increase gastric pH enough to reduce cribbing was not validated in the current study. Neither tryptophan nor $CaCO_3$ supplementation had any effects on the occurrence of cribbing in this experiment. There was also no significant difference in fecal pH by treatment in this experiment. Commercial supplements that have achieved mixed results in reducing cribbing behavior often contain a combination of tryptophan and $CaCO_3$, and perhaps this combination is the critical factor for this success. Another possibility for the commercial products success could be that there was a lack of adequate control in their experiment. In the current study, tryptophan, at the given dose, may not result in enough serotonin production to have any effect on behavior, or perhaps the hypothesis that horses crib to produce serotonin or endorphins is incorrect. $CaCO_3$ may have been ineffective at the given dose because not enough buffer was available to increase pH or because of acid rebound.

Perhaps continued research investigating the etiology of this stereotypy will lead to a successful and long term cure. Until an exact etiology is determined, current methods of control must be implemented. There have been reports of success using narcotic antagonists (Dodman et al., 1987), surgical techniques (Forsell, 1926; Firth, 1980; Fjeldborg, 1993) or mechanical devices (Fjeldborg, 1993) to control cribbing in horses,

but further research is needed to determine if there are any supplements or drugs available that will reduce cribbing habits in horses at minimal costs and management.

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APPENDICES

APPENDIX 1

Statistical Analysis (GLM Procedure) for the Dependent Variable of pH

Source	df	Type III SS	Mean square	F value	Pr>F
Square	2	1.48351768	0.74175884	7.51	0.0018
Horse (square)	6	0.45697379	0.07616230	0.77	0.5973
Period (square)	6	1.90928787	0.31821465	3.22	0.0120
Treatment	2	0.24755952	0.12377976	1.25	0.2973
Horse*period*square*treatment	10	0.60208482	0.06020848	0.61	0.7954
Cycle	2	0.84524230	0.42262115	4.28	0.0213
Treatment*cycle	4	0.15758118	0.03939529	0.40	0.8080

APPENDIX 2

Statistical Analysis (GLM Procedure) for the Dependent Variable of Total Number of Cribbing Bouts Per Day

Source	df	Type III SS	Mean square	F value	Pr>F
Square	2	3402.47707	1701.23854	7.38	0.0018
Horse (square)	6	70376.05820	11729.34303	50.86	<0.0001
Period (square)	6	1641.34454	273.55742	1.19	0.3322
Treatment	2	1293.49107	646.74554	2.80	0.0719
Horse*period*square*treatment	10	4375.44709	437.54471	1.90	0.0729
Cycle	2	692.52646	346.26323	1.50	0.2345
Treatment*cycle	4	1342.68783	335.67196	1.46	0.2328

APPENDIX 3

Statistical Analysis (GLM Procedure) for the Dependent Variable of Average Duration of Each Cribbing Bout

Source	df	Type III SS	Mean square	F value	Pr>F
Square	2	64006.5511	32003.2756	5.84	0.0058
Horse (square)	6	505172.5597	84195.4266	15.37	<0.0001
Period (square)	6	105281.6495	17546.9416	3.20	0.0111
Treatment	2	1322.9950	661.4975	0.12	0.8866
Horse*period*square*treatment	10	90316.9742	9031.6974	1.65	0.1262
Cycle	2	18484.9709	9242.4854	1.69	0.1974
Treatment*cycle	4	52883.8307	13220.9577	2.41	0.0639

APPENDIX 4

Statistical Analysis (GLM Procedure) for the Dependent Variable of Average Number of Crib-Bites Per Bout

Source	df	Type III SS	Mean square	F value	Pr>F
Square	2	499.728670	249.864335	5.61	0.0069
Horse (square)	6	6696.716576	1116.119429	25.04	<0.0001
Period (square)	6	768.822598	128.137100	2.88	0.0194
Treatment	2	12.418664	6.209332	0.14	0.8703
Horse*period*square*treatment	10	617.896842	61.789684	1.39	0.2198
Cycle	2	31.869001	15.934500	0.36	0.7015
Treatment*cycle	4	496.882827	124.220707	2.79	0.0385