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Evaluation of a Granulated Paper Waste Product as a Suitable Bedding Material for Horses

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EVALUATION OF A GRANULATED PAPER WASTE PRODUCT AS A SUITABLE BEDDING MATERIAL FOR HORSES

By

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EVALUATION OF A GRANULATED PAPER WASTE PRODUCT AS A SUITABLE

BEDDING MATERIAL FOR HORSES

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Three 14 day trials were used to compare granulated paper(GP) to pine pellets(PP) and wood shavings(WS) as bedding for horses. During days 1 through 5 stalls were cleaned daily of feces only, and stalls were cleaned of both feces and saturated areas from days 6 to 14. For stall cleanliness, PP was cleaner than both GP and WS. Initial pH was greater for both GP and WS compared to PP. The water holding capacity for WS was greater than PP and GP. On day 5 there was ammonia detected for WS during trial 1. GP and WS emitted more ammonia than PP when air flow was restricted. Bacteria found in the nasal cavity were not different among bedding types. GP had more fungal growth. Minerals were found within the maximum tolerance of horses. Results indicate the use of GP as a bedding material for horses has potential.

Key Words: Equine, Bedding Material, Granulated Paper

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Amanda Youngblood

TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Traditionally, animal bedding has consisted of several products, such as pine, cedar, hardwoods, cereal crop residues, straws, clay, and sand, and can be used in many forms, such as chips, sawdust, shavings, and pellets. Recycled newspaper has been introduced to the livestock industry as a potential bedding material. Advantages and disadvantages to these bedding types are based on availability, economics, physical properties, handling, and disposal of the product. These advantages and disadvantages of bedding types can also be affected by the region in which the bedding is being used and the time of year. For example, pine products and straw may not be readily available in the north-eastern United States but recycled newspaper is.

The use of recycled paper by the livestock industry serves as a viable economic alternative to the recycling industry when market prices are decreased (Ward et al., 2000). Moreover, the use of recycled paper in the livestock industry can benefit producers because most traditional bedding materials may be used for more valuable applications, such as fuel production, thus increasing the cost of traditional beddings.

There are concerns regarding the use of shredded paper as a bedding material such as caking when wet, dust, pollution during transportation, difficult storage issues, toxicity from heavy metals, and respiratory problems. Pelleting of recycled newspaper greatly increases the density, which helps to reduce the amount of airborne dust (Ward et al., 2000). Moreover the conditioning of the pellet such as grinding and packing amplifies the surface area thereby increasing the water binding capacity of the bedding.

Recycled newspaper is currently being used in the livestock industry, particularly for dairy cattle and poultry (Richard, 1991; Temple, 1989). In the poultry industry, researchers have compared paper products and mixed paper products to pine shavings as suitable bedding materials for chickens and obtained similar or better results compared to pine shavings (Malone and Gedamu, 1995, Lien et al., 1992, Malone et al., 1982). Malone et al. (1982) reported issues of breast blisters when using processed cardboard as bedding for broilers. Malone and Gedamu (1995) found that the adhesives used in pelleted newspaper caused caking of the bedding material after the bedding became moist. Other uses for recycled newspaper which are more recent include a cat litter box medium (PaPurr, Kadant GranTek, Inc, Green Bay WI).

There have only been a few published reports comparing recycled newspaper products to straw and wood shavings involving horses (Ward et al., 2001; 2000). Ward et al. (2000) compared the water-holding capacity of paper products to other materials such as straw, wood shavings, and peat moss and found the water-holding capacity was comparable to common beddings such as wood shavings. Furthermore temperature and humidity play a large role in the quality and efficiency of bedding materials (Ward et al., 2001). Temperature and humidity can affect the microbial populations in bedding, thus impacting the environment of the horse (Vandenput et al., 1997). Tanner et al. (1997) evaluated the respiratory effects of phone book paper compared to sawdust and found that shredded paper did not adversely affect the respiratory health of the horse.

The objective of this study was to evaluate the use of granulated paper waste as a suitable bedding material for horse stalls compared to pine wood shavings and pine pellet bedding. Health aspects such as affects on the respiratory tract were observed as well as characteristics of granulated paper waste, wood shavings, and pine pellets such as water holding capacity, heavy metal content, NH3 emission, pH, microbial loads (CFU), and cleanliness of horse and stall.

CHAPTER II

LITERATURE REVIEW

Paper Bedding

For many years the use of shredded paper as animal bedding has received much attention. Paper products have been found to be extremely absorbent compared to traditional bedding products such as pine shavings, straw, sand, peat moss, and other common products. With the declining availability of landfill space, using recycled paper such as newsprint, telephone books, and printed material as bedding material has become a popular venue for disposing of paper products. In addition to decreasing the amount of paper product put in landfills, it provides other advantages to farmers. Straw of oats and wheat require valuable land and farmers must cut the land and dry the straw at specific times. When using paper products as a source for bedding material, time of cutting is not an issue and other crops can be used for feed instead of bedding saving time and money (Apotheker, 1990). Apotheker (1990) also stated that paper bedding was more preferred because it prevented weeds because it did not have seeds to spread, it was more sterile, less expensive, more absorbent, decomposed faster, and used less storage space on the farm. Paper products are used for mulch, animal bedding, cat litter, and more. Previous research was conducted using shredded newspaper compared to wheat straw and bare soil as crop mulch (Munn, 1992). The amount of yield of soybeans and corn were greater when newspaper was used as mulch than wheat straw mulch, and wheat straw mulch had better a yield than bare soil because soil moisture and temperature were maintained with paper and wheat mulch. Shredded paper has been used successfully as a bedding material for dairy cattle, but has been less satisfactory for broilers because the shredded paper became entangled in the chicken's feet (Whitehead et al., 1983). Apotheker (1990) reported estimates of paper use stating paper bedding was used by 673 dairy herds with 45, 310 cows in the state of Pennsylvania at the beginning of 1990. There have been, however; concerns about using products such as chopped and shredded forms of paper (Misselbrook et al., 2005). Dust from grinding of the paper, environmental pollution during transportation, storage complication, toxicity of bedding from heavy metals, caking, and respiratory problems are some of the concerns which have been identified (Art and Lekeux, 2005; Ward et al., 2001; Tanner et al., 1997; Malone and Gedamu, 1995; Apotheker, 1990). Because many horses spend up to 23 hours a day in stalls, it is paramount that horses spend that time on bedding that will minimize the probability of respiratory problems such as chronic obstructive pulmonary disease (COPD). An equally important aspect of any bedding material is the need to decrease microbial growth, ammonia volitization, and dust (Fleming et al, 2008b; Ward et al., 2000; Vandenput et al., 1998a).

Pelletizing Process

Pelletizing paper waste is a process by which paper fibers are reformed into distinct particles, chips, or pellets (Malone and Gedamu, 1995). This process is performed by a pellet mill using a combination of steam, pressure, and heat. The product, in this case paper, must be ground before entering the pellet mill. A variety of different particle sizes is more efficient than a uniform particle size, because smaller particles can fill empty spaces between larger particles. Once the product is ground, the product then passes through the pellet mill where it is conditioned with steam. Then using pressure the conditioned product is forced through the pellet die by rollers. The pellet die is how the shape of the pellet is formed. Then blades cut the pellet to specific lengths as the product exits through the die. The pellets then pass through a cooler. The cooler is important to help maintain the shape and durability of the pellet. Pelletizing recycled newspaper may address some of the concerns that exist with the use of shredded paper. Pelleted newspaper has an increased density which can result in a reduction of airborne dust particles (Ward et al., 2001). Skoch et al. (1981) conducted a study to determine the effects that different conditioning practices have on pelleting variable, and found that steam improved pellet quality by increasing the durability of the pellet and decreasing the percentage of particle fines produced by handling and transportation. The pelleting process also amplifies the surface area of the product; therefore, increasing the water-binding capacity resulting with less ammonia emissions due to greater ammonia absorption (Fleming et al., 2008a). Moreover, Malone and Gedamu (1995) stated that further processing recycled paper into distinct particle shapes and sizes increase moisture-releasing characteristics in turn reducing the incidence of caking. Schmidt and Kleinebudde (1999) observed the granulation process, which occurs just before the pelleting process, and found that granulating products does affect the pellet quality and releasing characteristics. Granulating increases the structure density and decreases the releasing rate and *vise versa* (Schmidt and Kleinebudde, 1999). However, not many studies have been conducted on bedding that has been granulated.

Dust and Particle Size

The type of bedding material used has a dramatic effect on the air quality in horse stalls with respect to the amount of airborne particles that are produced over time. Dust can exacerbate and prolong respiratory diseases of horses by constant inhalation of dust (Fleming et al., 2008b). The quantity, composition, and size of airborne dust particles in the stall determine the amount of dust that a horse inhales and the site of deposition in the respiratory tract (Tanner et al., 1997). The structure of dust can appear fiber-like or bent, in flakes, spherical or polymorphic forms, which can play a large role in the health risk of horses because fiber-like particles can be more damaging than spherical particles (Fleming et al., 2008b). Depth of penetration of particles in the respiratory tract of horses is dependent on the size of the particle or dust. Particles between the sizes of 100 and 10 µm can reach as far as the larynx but not further in the respiratory tract; particle sizes <10 µm can reach beyond the larynx, but not in the non-cilia region of the lungs; where as particle sizes <5 µm can reach the alveoli (Fleming et al., 2008b). In earlier studies, particle sizes commonly found in stalls were fractioned into categories of 100 µm, 10 µm, and 5 µm (Fleming et al., 2008). The amount of dust present in the stall or air can increase or decrease over time and is dependent on the type of material used for bedding. Ward et al. (2001) reported that wood shavings contained greater quantities of particle fines, and the amount of dust from wood shavings increased over time; however, the amount of dust from pelleted newspaper peaked halfway through the experiment but then decreased over the course of the experiment. Stalls with straw as a bedding material had decreasing amounts of dust throughout the experiment which can be explained by the naturally long form of straw and less tendency to be broken down into smaller particles like pelleted newspaper and wood shavings. The particle size of the bedding material before it is used effects the amount of dust found from different bedding types. Wood shaving particles passed through sieves with a 1-mm screen, while most pelleted newspaper was retained by sieves with a 3-mm screen, more was retained by sieves with a 2-mm screen, and all of the straw was retained by sieves with a 3-mm screen (Ward et al., 2001; 2000). Tanner et al. (1997) reported similar amounts of dust were produced from phone book paper and sawdust, and Fleming et al. (2008b) reported that hemp and linen shives produced more dust particles with smaller particle sizes than straw pellets or paper cuttings. Large amounts of dust in the respiratory tract can cause COPD in horses and can compromise the horse's overall performance by negatively affecting the respiratory tract (Vandenput et al., 1998a; Vandenput et al., 1998b; Tanner et al., 1997). Respiratory function, however, is rapidly improved when dust concentrations are reduced (Vandenput et al., 1998a).

Microbial Populations

Microbes can be found mixed with dust produced from different bedding materials (Fleming et al., 2008b). Microbial populations living in bedding differ based upon type of bedding material, amount of time the animal is housed on the bedding, and seasonal/climatic conditions (Ward et al., 2001; Hogan et al., 1989). Microbes found in bedding and inhaled with dust particles can provoke respiratory diseases such as COPD. McPherson et al. (1979) stated that antigens for *Micropolyspora faeni* and *Aspergillus fumigates* are commonly found in horses diagnosed with COPD. Specific characteristics of the bedding may influence the ability to harbor microbial populations (Ward et al., 2000; Hogan et al., 1989). More microbes can be found in the breathing zone during autumn than in summer, which may be due to more time the barn is enclosed; however, more microbes can be found in the nasal passage during summer than in the autumn (Ward et al., 2000;2001). Ward et al. (2001) reported that more enteric organisms were found in wood shavings than in pelleted newspaper or straw. This may be due to the smaller particle size of wood shavings and the greater volume of particles found in the breathing zone. The fine particles along with fecal matter provide an environment rich in nutrients that allow microbial populations to thrive (Ward et al., 2001). When the particles are inhaled, microbes and bacteria can enter the respiratory system. Hogan et al. (1989) reported organic bedding materials such as sawdust, straw, and wood shavings had greater gram-negative bacteria, coliform, *Klebsiella* species, and streptococcal counts than inorganic materials such as limestone, sand, and paper bedding. This may be due to the greater moisture content of organic matter such as wood shavings and straw than of inorganic matter such as paper products, sand, and lime. The bacterial counts, when plated, did not differ between sand and lime (Hogan et al., 1989). However, in another study conducted by Hogan et al. (1990) with dairy cattle, streptococcal and staphylococcal bacteria counts, when plated, were greater for cows housed on chopped newspaper than those housed on wood shavings. Pusterla et al. (2006) used a tracheal wash, which represent bacteria found in the distal trachea, and found approximately 8% of pastured and stabled horses cultured pathogenic bacteria such as *Klebsiella pneumonia*, *β-hemolytic Streptococcus spp*., *Pasteurella spp*., and *Pseudomonas aeruginosa*. In the same study, bacteria such as *Enteobacter agglomerans*, *Bacillus spp.*, *Acinetobacter calcoaceticus*, *α-hemolytic Streptococcus spp.*, *Staphylococcus epidermidis* and *Pseudomonas stuterzi* were cultured from 24% healthy stabled horses and 64% healthy pastured horses. Cultures with *C. immitis* in combination with abnormal clinical signs are strong implications of a disease. Amount as well as the specie of bacteria present in the breathing zone, nasal cavity, and on the animal's extremities can be affected by the bedding type as well as season/climatic conditions (Ward et al., 2001). Newspaper as well as other beddings, such as wood shavings, can become a source for mold growth and subsequent endotoxin production when packed deep in unventilated conditions (Art and Lekeux, 2005; Vandenput et al., 1997). Stall design, such as having ample amount of air flow, and regular disinfection of the stall can decrease the amount of infectious agents and microbes in the living area and breathing zone. Moreover, a pH above 8 can cause the microbial population to decrease because microbes cannot thrive as well, but molds and fungus may still be viable (Ward et al., 2001).

Ammonia

The characteristic of a bedding type also affects the amount of ammonia absorption and emission. Researchers have reported that bedding type can influence ammonia emissions, but little is known regarding which characteristics influence the amount of emission. Ammonia is a noxious gas found in stables and can damage the respiratory tract (Fleming et al., 2008a). Aerosolized ammonia is a result of microbial metabolism of fecal protein and urea found in urine to produce ammonia (Tanner et al. 1997). The type of material used for bedding has a large impact on ammonia volatilization, and the characteristics of the bedding make some bedding better at ammonia absorption than others. Misselbrook and Powell (2005) reported when equal volumes of bovine urine were applied to sand, pine shavings, chopped newspaper, corn stalks, or manure solids, less ammonia was released from sand and pine shavings than from chopped newspaper, corn stalks, or manure solids. Ward et al. (2001) reported that when using pelleted newspaper there was less aerosolized ammonia because the moisture was absorbed inside of the pellet, providing less surface area for microbial colonization. This would result in less ammonia being produced from microbial fermentation. Moreover, Ward et al. (2001) reported that wood shavings emitted the greatest amount of ammonia due to a greater surface area and more available nutrients for microbial growth than either straw or pelleted newspaper. Tanner et al. (1997) reported greater ammonia concentrations in stalls bedded with sawdust than those bedded with phone book newspaper. Bedding type, however, is not the only influence of ammonia aerosolization. The way a stall is maintained or activities performed in the barn can affect ammonia concentrations in the air and breathing zone as well. Fleming et al. (2009) used three methods of maintaining horse stalls which included removing neither feces nor saturated areas from stalls, just feces was removed from stalls, and both feces and saturated areas were removed from stalls. They reported that cleaning enclosed stalls resulted with more ammonia emission than when stalls were not cleaned because aggravating the bedding while cleaning caused more ammonia to be released from the bedding. The time of year and the pH of the bedding may affect the amount of ammonia emission as well. Ward et al. (2001) conducted a study and reported that there was more aerosolized ammonia present in horse stalls during autumn than in the summer, which was due to less air flow during autumn because the doors and windows of the barn were kept closed more often than during the summer. Greater temperatures due to warmer climates can favor the growth of microbes, thus enhancing the production of ammonia as well. Detrimental ammonia concentrations have been determined for other species; however, the detrimental ammonia concentration has not been determined for horses (Tanner et al., 1997). Drummond et al. (1976) reported lambs exposed to 75 ppm of aerosolized ammonia had decreased body weight gain, decreased efficiency of feed conversion, and severe health problems such as coughing and nasal discharge with blood. Quarles and Kling (1974) reported that environmental air concentrations of 25 to 50 ppm of ammonia were detrimental to broiler chickens. Chickens housed in chambers of 50 ppm aerosolized ammonia gained less weight than chickens housed in chambers of 25 ppm ammonia. Furthermore, Drummond et al. (1978) reported young pigs housed in chambers with 50 to 75 ppm had 51% more bacteria in their lungs than young pigs housed in control chambers with no aerosolized ammonia. Research has been conducted with horses to evaluate ammonia concentrations of the bedding material used in their stalls, but a specific detrimental concentration has not been determined from these studies (Fleming et al., 2008a; Misselbrook and Powell, 2005; Ward et al., 2001; Vandenput et al., 1998a; Tanner et al., 1997).

Toxic Heavy Metals and Other Nutrients

There are 35 elements considered metals, but only 23 of them are considered heavy metals. Some which have drawn interest of researchers are cadmium, mercury, aluminum, chromium, and lead. Heavy metals are natural components of the earth and cannot be degraded or destroyed. A greater concentration of a heavy metal may result in toxicity. Research has been conducted to observe heavy metal content of paper products used as bedding for livestock, and the effects these metals may have on animals ingesting these heavy metals by eating the bedding or drinking water which bedding has contaminated the water supply in buckets, troughs, etc. Petrochemical carriers for ink production and metal content pigments contribute to the potential toxicity of paper products, and the effects of long-term exposure to these metals remain a concern even after waste has been applied to land (Ward et. al, 2000). Ward et al. (2000) compared recycled newspaper to pelleted newspaper, straw, and wood shavings, and discovered that recycled newspaper, straw, and wood shavings contained detectable concentrations of Cd, Cr, and Pb. Cadmium and Pb were reported at concentrations of <1 ppm with recycled newspaper containing the greatest concentration of Pb. Chromium concentrations were found to be greater than Cd and Pb, and the concentrations of Cr were reported as 4, 2, and 1 ppm for recycled newspaper, wood shavings, and straw, respectively. O'Connell and Meaney (1997) evaluated newspaper and discovered concentrations for Cd were undetectable, Cr was 1.4 ppm, and Pb was 0.5 ppm. Different results among studies may be attributed to the different types of laboratory equipment used and different sensitivities of the laboratory equipment. Mercury was reported to be <0.0002 ppm for straw, wood shavings, and pelleted newspaper; however, recycled newspaper contained 0.068 ppm of Hg (Ward et al., 2000). The greater concentration of Hg in recycled paper compared to pelleted newspaper may be because during the pelletizing process heat vaporized Hg. Tanner et al. (1997) reported the concentration of heavy metals of phonebook paper was less than the maximum tolerable amount recommended for horses except for aluminum. Aluminum was reported to be between 7,330 and 10,700 ppm with the recommended dose being less than 200 ppm (Tanner et al., 1997). Aluminum and magnesium in sawdust was also slightly more than the maximum recommended concentration. The maximum tolerance of dietary Cd, Cr, Pb, and Hg for domestic animals is 0.5, 1000, 30, and 2 ppm respectively (NRC, 1989).

Water Holding Capacity

The water holding capacity of bedding indicates the ability of a certain type of bedding to retain water against gravity. Not only is it important to evaluate the amount of water a bedding type can retain, but also how long it takes to reach maximum holding capacity (Ward et al, 2000). Some bedding material may reach maximum holding capacity quickly; however, other bedding materials must be broken into smaller particle sizes or the water must penetrate the bedding thereby taking longer to reach maximum holding capacity. Ward et al. (2000) reported that during the first hour after bedding was submerged in de-ionized water wood shavings and chopped newspaper absorbed water with a maximum water holding capacity of 400% on a volume basis. Wheat straw was reported to have a water holding capacity of 200%. The reduced water holding capacity of straw may be due to the inability of water to penetrate the waxy based coating which encapsulates straw. The rapid uptake of water by pelleted newspaper was less compared to other bedding material due to the inability of water to be absorbed initially. Once water penetrated pelleted newspaper, the fibers of the paper expanded, decreased the integrity of the pellet, and allowed for the maximum water holding capacity to be realized after 24 hours. Even though it took longer to achieve maximum water holding capacity, newsprint is twice as absorptive as wood shavings, almond shells, shiny paper, and rice hulls (Morse, 1994; Heimlich and Howard, 1990). Ward et al. (2000) also looked at submerged wood shavings, recycled newspaper, and straw in de-ionized water compared to non-submerged. In order to evaluate the proper method of determining the ability of bedding to absorb and retain urine Ward et al. (2000) compared bedding which was allowed to float on top of urine to bedding which was submerged in urine. The water holding capacity of straw was less when it was non-submerged because straw had a decreased water holding capacity when non-submerged. The water holding capacity of the other bedding materials, such as chopped newspaper, pelleted newspaper, and wood shavings, were similar regardless of being submerged or not. Determining the water holding capacity of bedding material can also assist with estimating other properties of the bedding such as ammonia emission, cleanliness of the stall, and ability of bedding to adhere to the horse.

Cleanliness

Type of bedding material may also affect the cleanliness of a horse's hair coat and the appearance of the stall. The longer a stall has a cleaner appearance influences the preference of horse owners for the type of bedding they use in their stalls. Bedding is used in stalls of horse's for both comfort and health, and it accomplishes these by absorbing and diluting moisture from waste materials thereby keeping the stall dry (McClain et al., 1997). McClain et al. (1997) conducted an *in vitro* study and reported wheat straw transferred less solid matter to wool flannel, which is commonly used in the horse industry for horse blankets and wraps, than wood shavings or pelleted newspaper, but wheat straw transferred more fecal matter to wool flannel. Tanner et al. (1997) visually evaluated the appearance of bedding in the stall and the appearance of the horse in terms of how clean or dirty the stall and horse were using sawdust or phone book paper. The cleanliness of the stall was scored from 0 to 3 (0=very clean with no obvious signs of manure or urine, 1= slightly dirty with one to two apparent manure piles or urine spots, 2= moderately dirty with more than two apparent manure piles or urine spots, 3= extremely dirty with apparent manure and urine throughout the entire stall). Outward appearance of the stall was affected by bedding type as phonebook paper gave a cleaner appearance of the stall. The phone book paper bedding did affect the outward appearance of the horse as the horses bedded on phone book paper had more dust on their hair coat compared to horses bedded on sawdust. Ward et al. (2000) also reported that animal cleanliness diminished with an increase of particle fines from pelleted newspaper and wood shavings compared to straw, which does not contain very many particle fines because of its elongated structure. Tanner et al. (1997) found that the hooves of mares bedded on phone book paper were drier and packed with bedding compared to mares bedded on sawdust; however, there has not been much research which evaluated the effects of paper bedding on the hoof. Other research conducted with chickens found that when paper bedding became wet it would stick between the chicken's toes (Whitehead et al., 1983).

CHAPTER III

MATERIAL AND METHODS

Twelve mares (BW= 540 ± 46.8 kg) of Quarter Horse and Thoroughbred breeds between the ages of 5 and 15 were used for three 14 day trials comparing granulated paper-clay mix (GP) to pine pellets (PP) and wood shavings (WS) as a bedding for horses (Figure 1). After each trial, mares were re-randomized for each subsequent trial. Each horse was housed in 9 m^2 box stalls for 21 hour/day. During days 1 through 5 stalls were cleaned daily of feces only. At the end of day 5 all bedding was removed from stalls and then re-bedded with clean bedding so feces and saturated bedding (wet spots) could be removed daily from day 6 through day 14. If needed, clean bedding was added to maintain initial depth of bedding. Wood shavings were provided by a local commercial supplier, pine pellets (Equine Pine Brand) were obtained from Oktibbeha County, MS Co-operative, and the granulated paper-clay mix was supplied by Kadant GranTek Incorporated. Animal procedures used during the study were reviewed and approved by the Institutional Animal Care and Use Committee at Mississippi State University.

Figure 1 Granulated paper , pine pellets , and wood shavings used to bed stalls

Notes: Each stall was randomly assigned one of the three bedding types used for this experiment: Granulated Paper (A), Pine Pellets (B), or Wood Shavings (C).

Management of Animals and Stalls

Horses were housed in a semi-enclosed, 14 stall, center isle barn (Figure 2). The barn was oriented with the north-to-south axis corresponding to the direction through the barn. The barn design included two vertical sliding doors on each end of the barn, windows that could be closed on stalls 8 through 14, open panel windows on stalls 1 through 7, and three dry lots that connected to stalls 1 through 7; however, horses were not allowed access to these lots during the study. During the winter, barn doors and windows able to be closed were shut. Only stalls 1 through 6 and 8 through 13 were used for this experiment in all three trials. All horses were in view of each other. Environmental readings such as temperature and humidity were taken both inside the barn and outside the barn, and measured with a weatherstation (H21-001, Onset Computer Corp., Bourne, MA). The outside weatherstation was placed eight meters away from the entrance to the barn and the inside weatherstation was placed in the center of the barn in the isle.

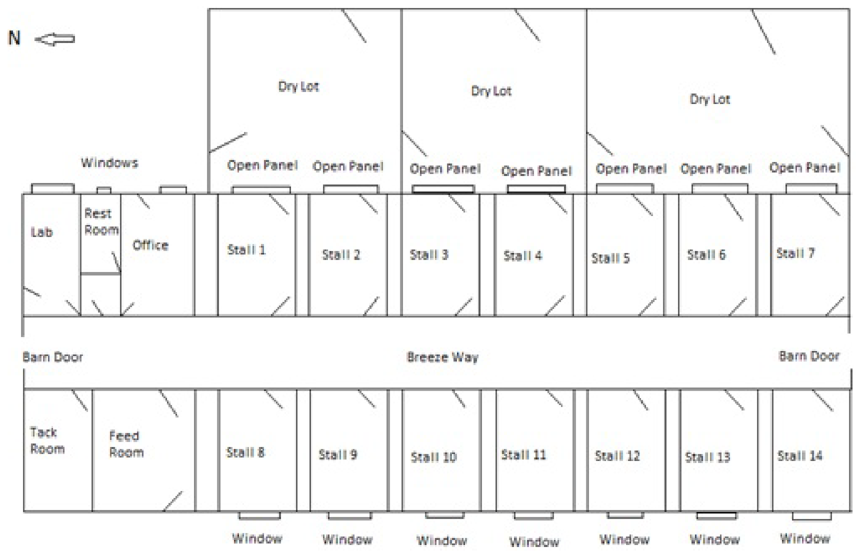

Figure 2 Mississippi State University barn layout

Each day, horses were maintained on a horse pellet ration offered at 0700 and 1600 h with ad libitum fresh water and Bermudagrass hay according to NRC (2009) recommendations. All horses were relocated to pasture from 1300 to 1600 hour for exercise and to facilitate stall maintenance, and had *ad libitum* access to Bermudagrass hay and fresh water. During the breaks between trials, horses were turned out onto pasture for 24 hour/day and given *ad libitum* access to hay and water. The volume of water drunk by horses in their stalls was recorded at 0700 and 1300 hours when water buckets were re-filled. Horses were given at least a six week break between trials.

Stalls were disinfected before and after each trial with a chlorine solution. All stall floors were uniform. Stalls were layered and packed with 5 cm of fresh red clay from wall to wall. Stall floors were then covered with rubber stall floor mats (122 cm X 183 cm X 2 cm, Davis Gates mats) prior to the commencement of the study. Initial quantities and depth of each material to a stall size of 9 m^2 were as follows: WS included 79 kg at a depth of 6 cm, PP included 145 kg at a depth of 2 to 3 cm, and GP included 177 kg at a depth of 2 to 3 cm. The density of PP and GP differed compared to WS resulting in different weights and amounts to cover the stall floors. Amounts and weights of bedding material put in each stall were recorded. Clean bedding material was sampled on day 0 of each 14 day trial period.

Daily at 1300 hours stalls were cleaned of feces on days 1 through 5 and feces and wet spots (areas saturated with urine) on days 6 through 14. The remaining bedding was mixed to make it more homogenous and piled in the center of the stall and left for 1 hour to equilibrate. The pH of the used bedding material and clean bedding material in each stall was obtained using a portable pH meter (ThermoOrion Cat. No. 090045). One liter samples were taken randomly from each pile, both around the pile and in the center of the pile, and were stored in plastic freezer bags and stored at -20^{o}C until further testing. The bedding sample was used to determine moisture content, N,P,K fertilizer value, and heavy metal analysis. Used bedding material was redistributed and clean bedding material was added if needed to maintain the initial depth and to maintain horse cleanliness and stall cleanliness. All additions and weights of clean bedding being added were recorded when clean bedding was needed. Horse cleanliness was visually assessed every day at 0700 hours observing the horse coat wetness and degree of organic matter attached to the coat using a score system of 1 to 5 (1: no residue on the horse's coat, 2:

some if any fecal stains or bedding on the horse’s coat, 3: bedding in the coat but little fecal stains on the horses coat, 4: moderate stains on the coat with bedding in the mane and tail, 5: horse is covered in bedding and fecal matter stains). Stall cleanliness was also visually assessed everyday at 0700 and 1300 hours observing the condition of the stall dust, organic matter, and appearance using a score system from 1 to 5 (1: very clean with no obvious signs of manure or urine, 2: slightly dirty with one to two apparent manure piles, 3: moderately dirty with more than two apparent manure piles, 4: moderately dirty with more than two apparent manure piles and urine spots throughout the stall, 5: extremely dirty with apparent manure and urine throughout the entire stall). Each day at 0700 hours the amount of nasal discharge was visually scored on a scoring system from 1 to 4 (1: none, 2: serous (clear), 3: mucoid (thick), 4: purulent (thick/yellow or green)). A cough test was also performed at 0700 hours to see if a cough was inducible by putting slight pressure just under the jaw on the trachea. A score system from 1 to 4 (1: none, 2: infrequent/non-inducible, 3: inducible, 4: frequent/easily induced) was used for the cough test. The score system for nasal discharge and cough was adapted from Moore et al., 1995. Scoring was done by the same individual throughout the trial for all three trials to maintain consistency throughout this experiment, however the individual was not blinded as it was easy to distinguish between the bedding types. At the end of each trial, the remaining bedding was removed from the stalls and weighed for each individual stall. The stalls were then swept, floors and walls sterilized with chlorine solution, and dried before the next trial. Throughout the trial comments of individuals cleaning the stalls were obtained for each stall on the three bedding types used and recorded to see the effects the bedding had on the individual cleaning the stall.

Laboratory Analyses

Initial and used bedding samples were stored in a -20^{o} C freezer till laboratory analyses could be performed. Subsamples were weighed out into separate plastic containers with lids and placed into a -80^{o} C freezer for 24 hours. Dry matter content was determined by lyophilization using the Freezone® 6 liter Benchtop Freeze Dry System (model 7752000). This method was used instead of oven-drying to prevent N from volatizing. Dry samples from days 1, 5, and 14 for all three trials were ground through a 2mm screen with a Thomas-Willey Laboratory Mill® (model 4). A portion of the ground clean and used bedding samples were acidified and the Kjeldahl distillation step (AOAC, 2001) was performed to measure amount of N in bedding. The remaining portion of the dried sample was sent to the Mississippi State University Soil Testing Lab to measure P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B. The Lancaster Mississippi Method as developed by JD Lancaster was used to determine mineral and heavy metal concentration of the bedding (Cox, 2001).

On days 1, 5, and 14 NH3 N in the breathing zone of horses was measured using passive diffusion tubes (Dräger Ammonia 20/a-D diffusion tubes; Luebeck, Germany) mounted to the wall in each stall. The tubes were placed between 17 and 35 cm above the ground, which is between where the horse eats from the ground and rest its head, in a place where the tube could not be disturbed by the horse. Samples were taken for 5 hours between 0700 and 1200 hours. The 5 hour duration allowed a range of measurement of 4 to 300 ppm NH3. The temperature range of 0 to 40^{o}C was appropriate for the climate conditions during this experiment. The diffusion tubes indicated the amount of aerosolized ammonia with air flow in the stall. Horses were free to move as usual within their stalls while sampling was taking place.

On days 1, 3, 5, 10, and 14 of each trial period an ammonia flux was performed to record the amount of aerosolized ammonia emitted from the bedding when air flow was restricted simulating an enclosed environment without air flow. Equilibrium ammonia concentration (ppm) was measured with a dynamic flux chamber (DFC) coupled with a photoacoustic infrared gas analyzer (Chillgard RT, Cranberry Township, PA). The DFC was constructed similar to Woodbury et al. (2006). A 46 cm diameter hood was used to close off both a dry area and saturated area (wet from urination) of each stall. The ammonia concentration measurement was taken over a 5 minute period. On days 5 and 14 an equilibrium ammonia concentration measurement was performed over a 10 minute period on top of used piled bedding after the stall had been cleaned and used bedding was mixed thoroughly to homogenize and allowed to equilibrate for 1 hour. The DFC was allowed to refresh and return to normal settings before moving to another stall. The ammonia readings were performed between 0700 and 1200 hours. Horses were moved to a dry lot to prevent them from interfering with the DFC and for the safety of the user.

On days 1 (before horses were placed on the bedding), 5, and 14 of each trial period at 0700 hours microbial colony counts from the nasal cavity of the horse were assessed. The swab was done in a 2-cm^2 area inside the nostril of each horse. After the nostril was swabbed, the swab was immediately submerged and swirled for 30 seconds in a 14 mL sterile test tube containing 1 mL of saline solution. The swab was then discarded and a screw cap secured on the test tube. Test tubes were placed in an ice box to stay chilled. Samples were sent to the Mississippi State Veterinary Microbiology Lab immediately where total aerobes on the nasal swabs were assessed using 4 serial dilutions on blood agar plates only. One order of magnitude (1 to 1000x) was used to obtained readable colony counts between 30 and 300 CFU per plate. The same individual counted plates for all three trials to maintain consistency.

On day 14 of each trial at 0900 hours a transtracheal wash (TTW) was performed on each horse as done by Pusterla and Watson (2006). Horses were sedated (detomidine; 10 to 40 micrograms/kg BW IV) prior to sample collection. An area of the middle third of the neck over the trachea was clipped and aseptically prepared. Then 3 to 5 mL of lidocaine was injected in the area clipped and prepared to provide local anesthesia. A small stab incision was made and a 10 gauge introduction catheter was placed in the trachea between the tracheal rings followed by a 70 cm, 12 gauge flushing catheter (transtracheal wash kit by MILA International). Twenty to thirty mL of sterile saline were then infused through the flush catheter and aspirated back. Recovered fluid was then submitted to the Mississippi State Veterinary microbiology lab where a cytology and microbial (aerobic and fungal) analysis was performed. A single stitch was placed to close the skin wound and the neck was wrapped. Horses were then monitored till the sedation effects were not apparent and treated, if necessary, for complications, which did not occur during this study. Once horses were alert, the horses were released to 24 hours/day pasture for at least 6 weeks before the next trial. Results were scored based on findings. Cytology was scored from 1 to 5 (1: None, 2: Mild, 3: Moderate, 4: Purulent, 5: Marked). Aerobic and fungal cultures were scored from 1 to 5 (1: None, 2: Faint, 3: Light, 4: Moderate, 5: Heavy).

Methods used to measure water holding capacity of clean, un-used GP, PP, and WS reflected modified methods used by Voyles and Honeyman (2006). Permeable nylon sacks were weighed then GP, PP, and WS were placed in individual sacks at weights of 100, 80, and 40 g respectively. Both the nylon sack and bedding were weighed using 6 replicates of each bedding material. The nylon sack was then placed and completely submerged in distilled water. After 1 hour, the sacks were removed from the water and hung to drain excess water, checking every 10 minutes to determine when the excess water had been drained. Once excess water was drained, the material was weighed. The absorbency of the bedding was calculated with the formula: 100 X (wet bedding – dry bedding) / dry bedding. This procedure was repeated for 3, 6, 9, 12, 24, 48, and 72 h to determine the saturation curve for the bedding material. Once the saturation was determined, water holding capacity for the individual bedding materials was determine at the point of complete saturation.

Statistical Analyses

Data was subjected to analysis of variance as a 3 X 3 latin rectangle (three treatments and three replicates with 12 horses) as a completely randomized design using the General Linear Model procedures of SAS. Least square means were calculated and when significant ($P < 0.05$) means were separated using Fisher's protected least significant difference. Twelve horses were used with four horses in each treatment during each replicate study. Horses were re-randomized after each replicate, but were not allowed to repeat treatments previously exposed to. Individual horse was considered as the experimental unit.

CHAPTER IV

RESULTS AND DISCUSSION

Temperatures for trial 1 averaged 25.5°C with a maximum temperature of 34.2°C and 66.6% relative humidity. During trial 2 the temperature and relative humidity averaged 12.9°C and 68.9%, respectively, and the maximum temperature was 24.3°C. For trial 3 the average temperature was 2.8°C with a maximum temperature of 16.9°C, and the relative humidity was 69.3%. Temperature and humidity were different for all three trials providing environmental contrasts (Table 1).

Table 1 Temperature, humidity, and wind speed readings for trials 1, 2, and 3

Trial	Items	T_{db}[A] °C	T_{wb}[B] °C	RH[C] %	WS[D] m/s
1	Min	14.0	12.6	66.6	0.0
	Mean	25.5	20.9		0.20
	Max	34.2	26.9		3.57
	Std Dev	4.4	3.4		0.44
2	Min	1.5	0.5	68.9	0.00
	Mean	12.9	9.9		0.09
	Max	24.3	19.1		2.27
	Std Dev	5.0	4.2		0.27
3	Min	-7.5	-8.2	69.3	0.00
	Mean	2.8	0.8		0.14
	Max	16.9	11.7		2.08
	Std Dev	5.3	4.6		0.28

[A]Dry bulb temperature - air temperature
[B]Wet bulb temperature - measure of humidity
[C]Coincident relative humidity - determined for the average wet and dry bulb temperature
[D]Wind speed

On day 5, bedding removed from stalls for GP, PP, and WS were different ($P < 0.0001$) as GP and PP had the most bedding recovered compared to WS (Table 2) similar to results reported by Ward et al. (2001) in which pelleted newspaper had a heavier final weight compared WS and straw. However, recovery of bedding for GP was 106% of the initial bedding compared to PP and WS which were 142% and 139% of the initial bedding weight. Urine absorbed into the bedding that was not removed may account for most of the excess weight obtained. On day 14 there was a trial X treatment ($P = 0.0333$) for the amount of bedding recovered. The final weight for WS was heavier in trial 3 than it was in trials 1 and 2 (Table 2). Granulated paper and PP increased in weight through trial 1 to trial 3. Wood shavings; however, had the least amount of bedding recovered compared to GP and PP during trials 1 and 2 because less was initially added to stalls and loss of bedding during removal of feces. Feces was difficult to separate from WS, thus more bedding was lost during management of the stalls. Granulated paper and PP fell away from the feces better, making separation and cleaning of the stall easier. Ward et al. (2001) reported that pelleted newspaper was easier to clean compared to wood shavings and straw because the bedding fell away from the feces more easily during maintenance of the stall. Maintenance did differ between individuals cleaning stalls as more bedding was removed with waste requiring more additional bedding to be added. Clean GP would often be removed while trying to remove fecal matter that remained as smaller particles requiring additional bedding to be added to maintain initial depth.

Table 2 Initial, final, and additional bedding weights

Item	Trial	Bedding Material GP	PP	WS	SEM	P value
Day 5						
Initial, kg		177.27	145.45	79.09	0	<0.0001
Final, kg		187.94	206.67	110.04	8.798	<0.0001
Day 14						
Initial, kg		177.27	145.45	79.09	0	<0.0001
Final, kg	1	166.14[b]	171.59[bc]	106.36[a]		
(trial x trt)	2	171.82[bc]	220.23[e]	87.5[a]		
	3	209.09[cde]	213.02[de]	175.89[bcd]	14.195	0.0333
Additions, kg						
(trial x trt)	1	44.32[b]	0[a]	34.77[ab]		
	2	105.68[c]	0[a]	29.66[ab]		
	3	34.09[ab]	4.55[a]	20.46[ab]	12.119	0.0192

[abcde]Means within bedding material differ among trials (P< 0.05)
Trial X Treatment for final bedding on day 14 and additional bedding

Addition of clean GP and WS were needed in all three trials during the second half of each trial from days 6 to 14 in order to maintain the initial depth and animal cleanliness; however, in a study conducted by Ward et al. (2001) they reported additional pelleted newspaper was not needed but additional WS and straw were needed. Additional PP were added only in one stall during trial 3 because the barn water pipe broke and some of the bedding got wet and froze. There was a trial by treatment interaction (P = 0.0192) for bedding additions because GP varied between trials. More GP was needed during trial 2 than in trials 1 and 3 (Table 2). An additional 60% of the initial bedding of GP was added during trial 2 compared to 25% and 19% additional bedding in trials 1 and 3, respectively. An additional 44%, 38%, and 27% of the initial bedding of WS was added during trials 1, 2, and 3, respectively. Additional GP was needed primarily because of the loss of bedding during regular cleaning maintenance, and additional WS were added to maintain horse cleanliness because of an apparent increase in moisture content. Additional WS were needed by Ward et al. (2001) and Tanner et al. (1997) because of increase moisture content of the bedding as well. Moreover, in the present study there was one horse that urinated more than other horses requiring more bedding to be removed, and that horse was bedded on WS during trial 1 and GP during trial 2, which were the trials where the most additional bedding for both bedding types was needed. The water holding capacity was different ($P < 0.0001$) for all three bedding types. The water holding capacity of GP was less than both PP and WS (Table 3). Different results were reported in other studies using different forms of paper such as shredded and chopped news paper. Researchers have reported paper having a greater water holding capacity than other commonly used beddings such as WS and straw (Ward et al., 2001;2000; Himlich and Howard, 1990). The decreased water holding capacity of GP could be linked to the clay mixed in with the paper to form granulated paper.

Table 3 Water holding capacity of clean, unused bedding material

		Bedding Material				
Item		GP	PP	WS	SEM	P Value
H_2O Holding Capacity	$gH_2O/100gDM$	92.07[a]	203.52[b]	239.17[c]	5.233	< 0.0001

[abc]Means within row differ ($P < 0.05$)

Average cost for bedding based on current market prices varied for the common stall sizes of 4 m^2 and 3 m^2. Average current market prices for bedding material is \$0.20/kg of GP, \$0.16/kg of PP, and \$0.55/kg of WS. Using the present study's parameters such as the amount of clean bedding used at the beginning of the trial, additional bedding needed, stall size, and time of use (14 days), yearly cost for WS would average \$1531.80 for a 4 m^2 stall. The cost of PP would average \$625.87 a year for a 4 m2 stall, and the average cost per year of GP would be \$1239 for a 4 m^2 stall (Table 4). The cost per kg of GP was very similar to PP; however, due to the amount needed to bed a stall and the additional bedding needed, the average yearly cost of GP was greater. Ward et al. (2001) reported that pelleted paper, depending on pellet size, had slightly greater cost than straw and WS but were not different. After observing the apparent moisture content of the bedding in each stall after the end of each trial, an estimation was made on how long the bedding may last before the bedding would have to be completely cleaned out and replaced with new, clean bedding. The average yearly cost was then calculated using the parameters of the study such as the amount of bedding placed in the stall at the beginning of each trial, stall size, and the additional bedding needed. The estimated lengths of time that GP, PP, and WS could be used before being removed are 4, 8, and 2 weeks respectively. The average yearly cost for a 4 m^2 stall would be \$619.50 for GP, \$156.47 for PP, and \$1531.38 for WS (Table 4). The cost of bedding can have a large effect on which bedding a consumer may use to bed their stalls.

Table 4 Average and estimated yearly cost of bedding materials for a 4 m^2 stall

	Bedding Type		
	GP	PP	WS
Initial Weight (kg)	177.27	145.45	79.09
Additions (kg)	61	5	28
Cost ($/kg)	0.2	0.16	0.55
Wks bedding can last	4	8	2
Total cost (2 wks)	47.654	24.072	58.8995
Average Yearly Cost*	1239.004	625.872	1531.387
Estimated Yearly cost**	619.502	156.468	1531.387

* Average cost yearly for bedding using the stall size, length of time (2 weeks), initial clean bedding weight, and additional bedding needed
** Estimated average cost for bedding based on an estimation of how long bedding can be used before being replaced while also using stall size, initial clean bedding weight, and additional bedding needed from the current study

Stall cleanliness in the morning was different (P < 0.0001) among beddings. Pine pellets and WS had a cleaner appearance followed by GP (Table 5). These results were different from what was found in a study by Tanner et al. (1997) who reported that phone book paper had a cleaner appearance compared to sawdust. In the present study, fecal matter in stalls bedded with GP would become dry and break into smaller particles that were hard to remove causing the stall to look unclean. There was a trial X treatment (P = 0.0295) for stall cleanliness in the afternoon. Granulated paper and WS had a dirtier appearance than PP during the afternoon. However, the trial X treatment may be due to variation in horse’s stall habits. Pine pellets were cleaner during trials 2 and 3, and WS were cleaner during trial 3 than in trials 1 and 2 (Table 5). Granulated paper remained consistent. Horse cleanliness is a priority in the horse industry as well as stall cleanliness. Horse cleanliness was not different among bedding types (Table 5). McClain et al. (1997) reported that WS were different and transferred more fecal matter to horse coats, and

Tanner et al. (1997) reported that phone book paper caused horses to have a dustier appearance compared to horses bedded on sawdust.

Table 5 Stall cleanliness (morning and afternoon) and horse cleanliness scores

		Bedding Type				
Item	Trial	GP	PP	WS	SEM	P Value
Stall						
A.M.		3.3^{b}	2.79^{a}	3.09^{ab}	0.106	< 0.0001
P.M.	1	3.77^{c}	3.48^{bc}	3.7^{c}		
	2	3.68^{c}	2.85^{a}	3.83^{c}		
	3	3.77^{c}	3.02^{ab}	2.89^{a}	0.19	0.0295
Horse		1.99	1.78	2.12	0.131	0.217

^{abc}Means with P.M. stall cleanliness differ among trials ($P < 0.05$)
Trial X Treatment for P.M. stall cleanliness

Nasal swabs did not differ among bedding types for the initial, day 5, and day 14 collection days (Table 6). Cough and discharge did not differ ($P = 1.0$ and 0.05 respectively) as well. A cough was not heard or provoked among bedding types for any trial. Some discharge was reported, which may be from the change in weather as it was not consistent from day to day. The transtracheal wash (TTW); however, preformed at the end of each trial was different for fungal culture ($P = 0.02$) among bedding types. Granulated paper received more light readings for the fungal culture compared to PP and WS (Table 7). Cytology and aerobic culture were not different among bedding types, similar to a study conducted by Tanner et al. (1997). Granulated paper received moderate readings for the cytology as compared to PP and WS which received more mild reading; however, during trial 1 results showed that there were predominantly neutrophils with rare macrophages, eosinophils, and lymphocytes present in cytologies performed on two horses bedded on GP. There was also one horse stalled on PP in trial 3 with resulting stating there were non-degenerated neutrophils. The horse from trial 3 was also one of the horses from trial 1 bedded on GP paper with similar results. For the aerobic culture, GP received light readings as compared to PP and WS which received faint readings (Table 7). During trial 1 *Streptococcus equi ssp zooepidemicus* was isolated from the aerobic test from one horse bedded on GP and one horse bedded on PP. Also during trial 1, *Klebsiella pneumonia ssp pneumaniae* was isolated from the aerobic test from one horse bedded on GP and two horses bedded on PP. *Klebsiella pneumonia* is not commonly seen in horses; however, *Streptoccus zooepidemicus* is commonly seen. Even though GP was different than PP and WS the readings were not of any health concern. Tanner et al. (1997) reported that horses bedded on phone book paper had pigmentation found in TTW, but that it was not of any health concern as well to horses.

Table 6 Nasal swab microbial counts (CFU) from horses bedded on material

		Bedding Type				
Item	Trial	GP	PP	WS	SEM	P Value
Nasal Swab						
day 1, CFU		45,975	58,783	96,825	20,785.4	0.083
day 5, CFU		111,416	86,108	113,800	26,615.6	0.572
day 14, CFU		187,350	114,808	122,425	30,774.9	0.085

Table 7 Transtracheal wash scores

Item	Trial	Bedding Type GP	PP	WS	SEM	P Value
TTW						
Cytology		2.83	2.92	2.25	0.266	0.1753
Aerobic Culture		2.25	1.75	1.75	0.182	0.0999
Fungal Culture		2.17[b]	1.67[a]	1.67[a]	0.142	0.0267

[ab]Means within row differ (P < 0.05)

Diffusion tubes did not detect any aerosolized ammonia in the stalls before the trials were started, which was expected. On day 5 there was a trial X treatment (P = 0.036). Some ammonia was detected during trial 1 for WS, but during trials 2 and 3 there was not ammonia detected (Table 8). There was also no ammonia detected for GP and PP for any of the trials, which is similar to results reported by Tanner et al. (1997). This interaction may be due to the greater temperatures during trial 1 as compared to trials 2 and 3. Moreover, the horse that urinated more than the others was bedded on WS during trial 1, and the N from urea present in urine volatilizes into ammonia. There was not a difference (P = 0.23) between treatments for day 14. Ammonia was only detected for all three bedding types during trial 1, which again can be explained by the greater temperatures during trial 1 (Table 8). The ammonia detected by the diffusion tubes was not of any concern because it was detected in such small amounts. Ward et al. (2001); however, reported that greater concentrations of NH_3 N was present in stalls bedded with pelleted newspaper and was present more in the autumn than in the summer. Environmental conditions and type of bedding could be the reason for the difference in results. The ammonia flux; however, detected greater concentrations of ammonia when air flow was restricted when all bedding was piled and mixed together. There was a trial X treatment for the ammonia flux on both d 5 ($P < 0.0001$) and d 14 ($P < 0.0001$) for piled bedding. Day 5 and d 14 were only different during trial 1, which may be due again to greater temperatures during the trial. Granulated paper had the greatest concentration of ammonia detected followed closely by WS (Table 9), and PP were not different than the rest of the bedding in trials 2 and 3. On day 14 GP had the greatest ammonia concentration, and PP and WS were the same as the bedding in trials 2 and 3 (Table 9).

Table 8 Aerosolized NH_3N detected using the diffusion tubes

		Bedding Type				
Item	Trial	GP	PP	WS	SEM	P Value
Diffusion Tubes						
Day 1, ppm		0	0	0	0	-
Day 5, ppm	1	0^a	0^a	2.38^b		
	2	0^a	0^a	0^a		
	3	0^a	0^a	0^a	0.4571	0.0360
Day 14, ppm		0.917	0.229	0.313	0.229	0.2256

[ab]Means within bedding material differ among trials ($P < 0.05$)
Trial X Treatment for Day 5

Table 9 Aerosolized NH_3N detected using an ammonia flux

		Item	
Trial	TRT	Day 5, ppm	Day 14, ppm
1	GP	253.50[c]	124.75[b]
	PP	8.25[a]	15.00[a]
	WS	147.00[b]	19.75[a]
2	GP	11.50[a]	2.50[a]
	PP	0.50[a]	6.50[a]
	WS	7.75[a]	6.75[a]
3	GP	0.50[a]	0.75[a]
	PP	1.25[a]	0.25[a]
	WS	1.00[a]	3.25[a]
	SEM	23.079	23.079
	P Value	<0.0001	<0.0001

[abc]Means within column differ ($P < 0.05$)
Trial X Treatment for both Day 5 and Day 14

The initial pH was greater ($P < 0.0001$) for both GP and WS compared to PP (Table 10), and increased with use, which agrees with results of different paper products reported by Ward et al. (2001;2000) and McClain et al. (1997). On day 5 and day 14 the pH of PP was the least compared to WS and GP which were similar (Table 10). There were differences found among bedding types for the mineral analysis and trial X treatment for some minerals. All concentrations of minerals and metals for GP, PP, and WS were within the maximum tolerable amounts according to NRC (1989). There was a difference for N ($P <0.0001$) and Ca ($P <0.0001$). Pine pellets and WS had smaller concentrations of N and Ca present compared to GP (Table 11). Bedding types were also different ($P = 0.0004$) for P. Granulated paper had the least amount of P present compared to PP and WS (Table 11). There were trial by treatment interactions for K ($P = 0.004$), Mg ($P = 0.0165$), S ($P < 0.0001$), Fe ($P < 0.0001$), Mn ($P < 0.0001$), Zn ($P = 0.0012$), Cu ($P = 0.0003$), and B ($P = 0.0002$). Granulated paper and PP remained consistent for K through all trials, but WS had a greater concentration of K during trial 1 as compared to trials 2 and 3 (Table 11). Pine pellets varied among trials for Mg. Concentrations of Mg were greater during trial 2 than in trials 1 and 3, and GP and PP remained consistent. Wood shavings varied among trials for S, as there were greater concentrations of S during trial 1, and GP and PP remained consistent (Table 11). When Fe was analyzed, greater concentrations were found during trial2 for GP and WS as compared to trials 1 and 3. Pine pellets; however, had greater concentrations in trials 2 and 3 as compared to trial 1. Pine pellets increased greatly in concentration of Mn in trial 2, but then decreased in trial 3 (Table 11). Wood shavings had a greater concentration of Mn during trial 1 and GP remained consistent among all three trials. When Zn was analyzed, GP had greater concentrations present in trials 1 and 3, but PP and WS remained consistent (Table 11). Copper was found in greater concentrations in GP during trial 3 compared to trials 1 and 2, and in WS greater concentrations were found during trial 1 (Table 11). Wood shavings varied with concentrations of B, as it had greater concentrations during trials 1 and 2 as compared to trial 3 (Table 11). Mineral results were comparable and similar to results reported by Ward et al. (2000); however, granulated paper differed from the pelleted newspaper product used for N, Mg, Fe, and Mn. Nitrogen and Fe were found in less concentrations than that reported in the study by Ward et al. (2000), while Mg and Mn were found in slightly greater concentrations. Both studies were within the maximum tolerable amount for horses.

Table 10 pH of initial (clean, unused) and used bedding

Item	Trial	Bedding Material GP	PP	WS	SEM	P Value
Intial day 5		7.37^{c}	4.91^{a}	5.68^{b}	0.064	< 0.0001
Day 5		8.13^{b}	6.16^{a}	7.96^{ab}	0.135	< 0.0001
Initial day 14		7.57^{c}	4.85^{a}	5.50^{b}	0.051	< 0.0001
Day 14		8.21^{b}	6.79^{a}	8.04^{b}	0.132	< 0.0001

^{abc}Means within row differ (P < 0.05)

Table 11 Mineral and nutrient analysis of bedding material

Item	Trial	Bedding Type GP	PP	WS	SEM	P Value
Minerals						
*N, ppm		4980.02^{b}	3332.83^{a}	3584.86^{a}	157.54	<0.0001
*Ca, %		0.2044^{b}	0.0583^{a}	0.0611^{a}	0.003	<0.0001
*P, %		0.0264^{a}	0.0406^{b}	0.0456^{b}	0.0041	0.0004
**K, %	1	0.1367^{ab}	0.2725^{d}	0.3975^{e}		
	2	0.1058^{ab}	0.2392^{d}	0.165^{bc}		
	3	0.0925^{ab}	0.2683^{d}	0.22^{cd}	0.0243	<0.0001
**Mg, %	1	0.2017^{de}	0.0517^{a}	0.0667^{bc}		
	2	0.2142^{e}	0.0692^{c}	0.0517^{a}		
	3	0.1975^{d}	0.0542^{ab}	0.65^{abc}	0.0053	0.0165
**S, %	1	0.065^{b}	0.04^{a}	0.0758^{c}		
	2	0.0625^{b}	0.0367^{a}	0.0325^{a}		
	3	0.06^{b}	0.035^{a}	0.0342^{a}	0.003	<0.0001
**Fe, ppm	1	223.33^{d}	28^{a}	70.5^{c}		
	2	271.67^{e}	60.08^{bc}	38.67^{ab}		
	3	220^{d}	63.42^{bc}	83.08^{c}	9.809	<0.0001

Table 11 (Continued)

Item	Trial	Bedding Type GP	PP	WS	SEM	P Value
Minerals						
**Mn, ppm	1	17[a]	97.83[b]	130.42[e]		
	2	15.83[a]	128.75[e]	111.92[c]		
	3	16.67[a]	120.17[d]	114.25[cd]	2.636	<0.0001
**Zn, ppm	1	56.42[c]	12.25[a]	20[ab]		
	2	30.75[b]	11.58[a]	8.75[a]		
	3	72.5[c]	9.67[a]	10[a]	6.251	0.0012
**Cu, ppm	1	12.33[c]	2.08[a]	3.67[b]		
	2	12.42[c]	2.42[a]	2.08[a]		
	3	13.25[d]	1.83[a]	2.17[a]	0.2801	0.0003
**B, ppm	1	7.58[de]	5.67[bc]	6[c]		
	2	7.25[d]	5.92[c]	5.08[a]		
	3	7.67[e]	6[c]	5.5[b]	0.1398	0.0002

*[ab]Means within row differ ($P < 0.05$)
**[abcde]Means for K, Mg, S, Fe, Mn, Zn, Cu, and B differ among trials ($P < 0.05$)
Trial X Treatment for K, Mg, S, Fe, Mn, Zn, Cu, and B

Implications

Quantities as well as cost of materials used may vary among sources of animal bedding products and how it was processed. Water holding capacity, cleanliness, pH, and mineral concentrations may vary depending on type of bedding and environmental conditions, such as change in weather. Health aspects of the horse such as microbial populations, cough, and nasal discharge may also vary among environmental condition and individual horse. Personal preference varies as well. Someone may choose GP because it is more environmentally friendly as it is the product of recycled paper.

However, someone else may choose PP over GP because it is cheaper, or they may choose WS because it is not as heavy as PP and GP. Preference is a large factor in what bedding type is used most by consumers. Comments by individuals cleaning stalls were made that GP was dusty when all bedding was removed at the end of the trials, but individuals found PP dusty from the very beginning and found it very hard to breathe when cleaning. Furthermore, individuals cleaning stalls reported it was hard to breathe when cleaning WS as well, but because of the aerosolized ammonia in the stall. This was only a problem for WS during the first trial because of increased temperatures. Moreover, observations were made during the experiment on the effect of the bedding on the horse's hooves as bruising and visible lameness was observed on horses bedded on GP. Dry hooves and packing of paper bedding was also reported by Tanner et al. (1997). Thus, different management strategies should be used for different bedding types to reduce dust or reduce lameness. A short study was conducted after this study after the company (Kadant Gran Tech) that furnished the bedding improved upon their bedding. The study observed strictly the effect of the improved granulated paper on the horse's hooves. There were not any signs of lameness or bruising at the end of the short study, and hooves did not become as impacted as before in the current study, for more information refer to appendix A. Granulated paper and PP were also found in the bottom of water buckets. It is not known if horses were eating the GP and PP, or if it was just getting stuck the horse's nose and falling off in the water bucket. Use of granulated paper has potential as a suitable bedding material for horses.

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APPENDIX A

EVALUATION OF THE HORSE’S HOOF AND SIGNS OF LAMENESS USING IMPROVED GRANULATED PAPER AS A BEDDING

Materials and Methods

Four quarter horses, two 2 yr olds and two 10 yr olds, were used for a 12 day trial period to look at the effect that granulated paper bedding had on the horse's hooves and tendons. Horses were individually stalled in 9m^2 box stalls. Two horses had their hooves picked out and cleaned once a day (stalls 2 and 4) and the other two horses did not receive any regular cleaning maintenance of their hooves (stalls 1 and 3). Horses were randomly assigned stalls and treatments.

Stall floors were already layered with red clay dirt and had rubber mats from wall to wall. Stalls were bedded with granulated paper provided by Kandant GranTek Inc at a depth of 2 to 3 cm.

Each day, horses were maintained on a horse pellet ration and Bermudagrass hay offered at 0700 and 1600 hours with *ad libitum* fresh water according to NRC (2009) recommendations. Horses were allowed access to dry lots from 1300 to 1600 hours for exercise and to facilitate stall maintenance. The horses had *ad libitum* access to Bermudagrass hay and fresh water. Each day at 1600 hours each horse was examined and a lameness score test using a score system of 1 to 5 (Score 1 (sound): The animal walks and stands with a level back and has a normal gait (tested here at the trot in horses) - the hind-feet in line with the corresponding fore-feet, no shortening of the stride or nodding of the head. There is minimal abduction / adduction. Weight is generally distributed evenly on all limbs when standing still. Score 2 (mildly lame): The animal stands with a level back but may adopt an arched back while walking. The gait may show some abduction / adduction or other slight abnormalities, which may be exaggerated by manipulation of the limb. Score 3 (moderately lame): The animal may adopt an arched back while standing or walking. Its gait is affected and is best described as short striding with at least one limb. Score 4 (lame): Same as for score 3, plus the gait is more severely affected the animal deliberately taking one step at a time. Weight may be taken off one or more limbs/feet (favored) when standing. The animal has more difficulty turning. Score 5 (severely lame): The animal shows an inability or extreme reluctance to bear weight on one or more limbs and may be reluctant to rise (if lying) or move (if standing). The animal may stop frequently.) was performed by walking and trotting the horses the length of the barn and back.

On day 12 at 1300 hours a veterinarian/farrier evaluated the hooves of all 4 horses for lameness or soreness. Thermographs were taken every day at 0900 hours using a FLIR SC 660 camera. Pictures were taken of the knees, fetlocks, pasterns, hocks, front tendons, back tendons, hoof wall, and the bottom of the hoof (sole, frog, heel).

Results

Lameness Score Test

Horses were scored for an initial score before being placed on the bedding. All horses except for the horse going into stall 4 received normal scores. Stall 4 received a score of 2 at the trot but we think that was due to her being run up on gravel 30 minutes before because she did not show any signs of lameness throughout the rest of the trial (Figure 3). Horses in stalls 1, 2, and 4 received a score of 1 for both the walk and trot throughout the trial consistently. Horse is stall 3, which was one of the horses that did not have its feet cleaned out routinely, received a score of 2 for both the walk (Figure 4) and trot (Figure 3) on days 5,6, 8, 9, and 10. Horse in stall 3 received a score of 2 for just the trot on days 11 and 12 showing some improvement in soreness.

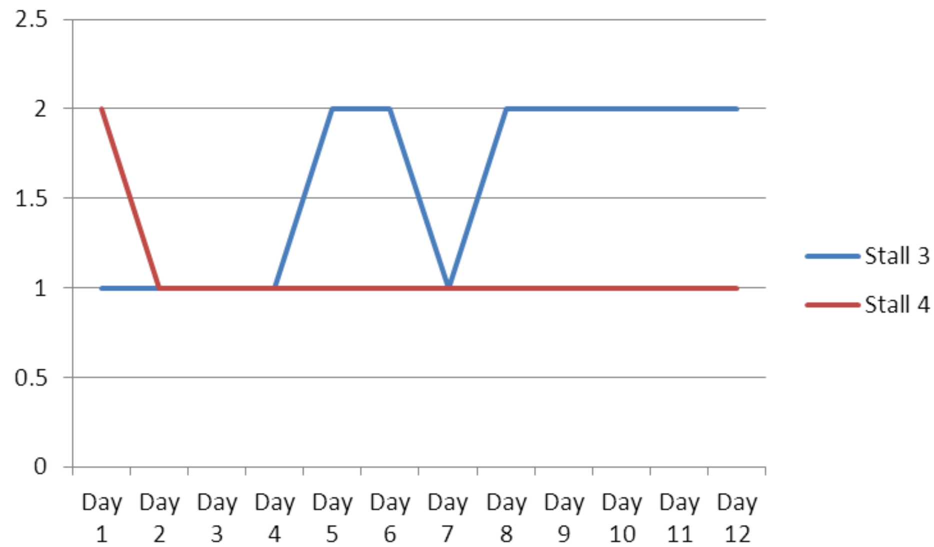

Figure 3 Lameness scores for the trot assigned to horses in stalls 3 and 4

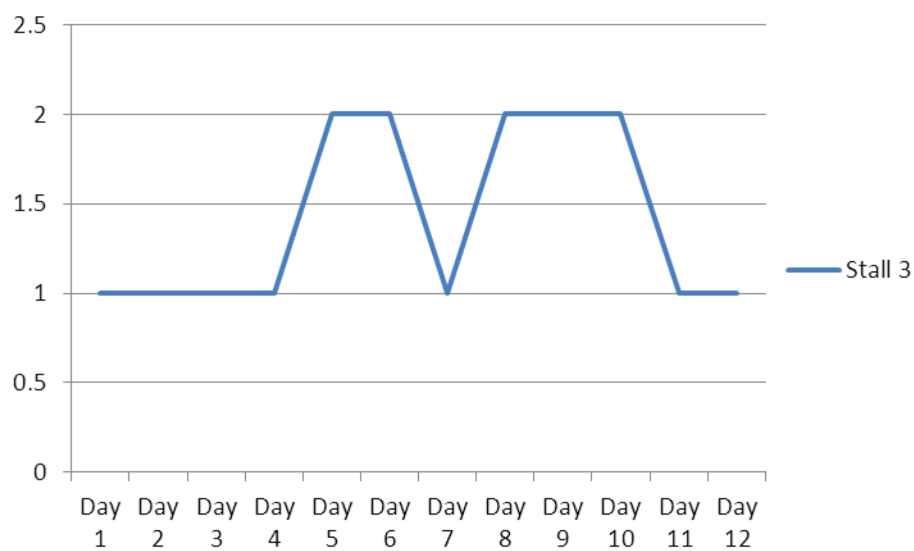

Figure 4 Lameness scores assigned to the horse in stall 3 for the walk

Soreness seemed to improve for the horse in stall 3 when large amounts of used bedding was replaced with new, clean bedding on days 7 and 10. The horse in stall 3 also defecated and urinated more than the other three stalls throughout the trial causing stall 3 to be dirtier than the rest and causing more impaction in the hoof. Moreover, stall 3 was assigned the treatment where the hooves did not receive regular cleaning and maintenance. Packing of bedding in the hooves was evident starting on day 5, which was when the bedding seemed to become visually dirty. On day 12 when the veterinarian/farrier came to hoof test the horses for soreness, he found there was no bruising of the sole or soreness in any of the horses. This was an improvement from the bedding experiment conducted before this one where the horses on GP on all three trials had soreness and evident bruising of the sole.

Thermographs

Images were taken of horses before being placed on the bedding to see how the horse is normally, then every day after that for 12 days pictures were taken of the horse at the same time (Figure 5). All horses will not show the same temperature readings, it all depends on the individual horse. For the purpose of this report I am focusing on the areas where lameness and soreness were evident in the last bedding trial preformed on GP which includes: front view of the right and left hoof, the right and left fetlock, the right and left sole of the hoof, the right and left frog of the hoof, and the right and left heel. The max temperature was analyzed for each area.

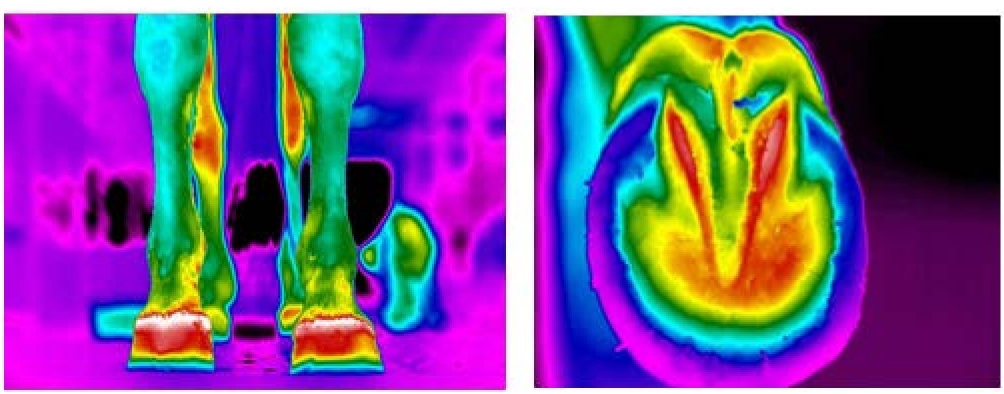

Figure 5 Thermograph images of the horse in stall 3 on day 6

On day 1, thermographs of the right hoof for horses in stalls 1, 2, 3, and 4 showed temperatures of 12.4, 10.8, 7.4, and 14.4^{o}C respectively (figure 6). Thermographs of the left hoof showed temperatures of 13.7, 10.4, 7.5, and 13.3^{o}C for horses in stalls 1, 2, 3, and 4 respectively (figure 6). On day 6, the thermographs of the right and left hoof showed a dramatic increase in temperature for horses in stalls 1,3 (figure 5), and 4 with temperatures of 22.5, 24.4, and 24^{o}C respectively for the right hoof and 26.8, 21.4, and 21.2^{o}C respectively for the left hoof (figure 6). However, the only horse with clinical signs of lameness was the horse in stall 3. The increase in temperature of horses in stalls 1 and 4 proves there is a potential for soreness or lameness but there is not clinical evidence of lameness. On day 12, horses in stalls 1 and 2 returned to normal initial temperatures. The horse in stall 3 showed an increased in temperature to 29.1^{o}C for the right hoof and 28.4^{o}C for the left hoof (figure 6). The horse in stall 4 showed hardly any change from day 6 with a temperature of 24^{o}C in the right hoof and 29.2^{o}C in the left hoof (figure 6).

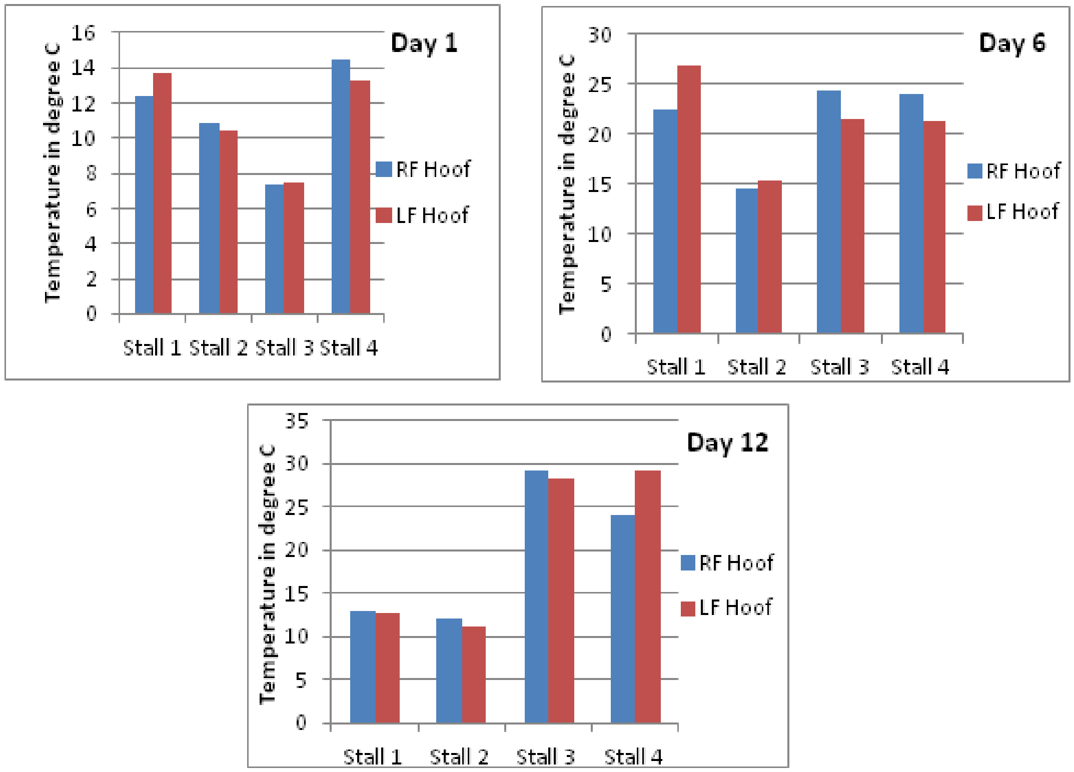

Figure 6 Maximum temperatures for the right and left hoof for day 1, 6, and 12

On day 1, thermographs of the right fetlock for horses in stalls 1, 2, 3, and 4 were 14.1, 13.1, 8, and 13.9°C respectively. Thermographs of the left fetlock showed a temperature of 15.6, 13.2, 10.5, and 13.5°C for stalls 1, 2, 3, and 4 respectively. On day 6, there was a large increase in temperature for horses in stalls 1 and 3 with temperatures of 23.7°C and 22.5°C respectively for the right fetlock and 24.8°C and 21.7°C respectively for the left fetlock. Horses in stalls 1 and 3 were in the treatment where the hooves were not cleaned out. There was only a slight increase in heat shown by the thermograph for the horse in stall 4 with a temperature of 19.3°C for the right fetlock and 18.8°C for the left fetlock. Only the horse in stall 3 showed clinical signs of soreness or lameness when moving. On day 12, horses in stalls 1 and 2 returned to normal temperatures shown on day 1. The horse in stall 3 showed a large increase in temperature in the left fetlock at

33.3°C, and the horse in stall 4 remained about the same as day 6 with temperatures of 18.3°C for the right fetlock and 21.8°C for the left fetlock. Results are shown in figure 7.

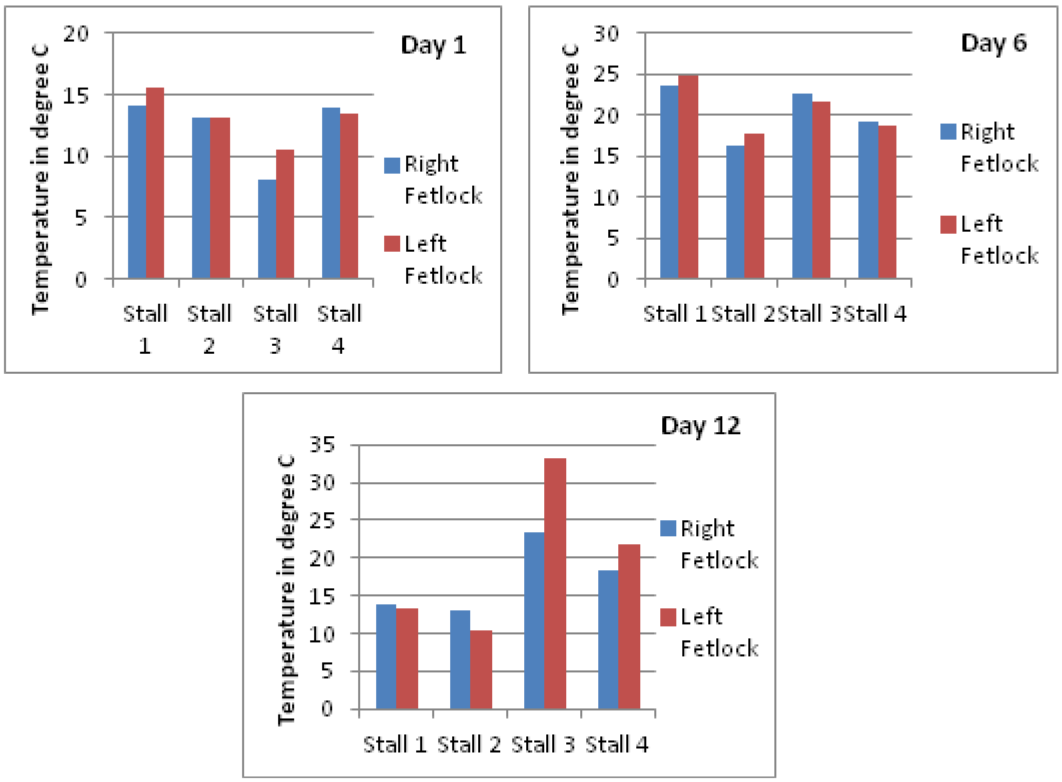

Figure 7 Maximum temperatures for the right and left fetlock of all horses on days 1, 6, and 12

On day 1, thermographs of the right and left sole of the hoof for horses in stalls 1, 2, 3, and 4 showed temperatures of 16.1, 11, 9.5, and 21.5°C respectively for the right sole and 15.7, 11.6, 10.2, and 20.1°C respectively for the left sole. On day 6, the horse in stall 3 showed the greatest increase in temperature with a temperature of 30.6°C for the right sole and 30.3°C for the left sole. Horses in stalls 1 and 4 also showed an increase in heat with temperatures 24.2 and 31.2°C respectively for the right sole and 28.2 and 30.6°C respectively for the left sole. On day 12, the horse in stall 1 returned to normal temperatures as on day 1. Horse in stall 3 showed a slight increase in heat from day 6 with a temperature of 33.3°C for the right sole and 34.2°C for the left sole. Horse in stall 4 did not change much from day 6 with temperatures of 30.2°C for the right sole and 33.8°C for the left. Horse in stall 2 did not show any signs of abnormal heat in the sole throughout the study, and only stall 3 show clinical signs of lameness throughout the trial. Results are shown in figure 8.

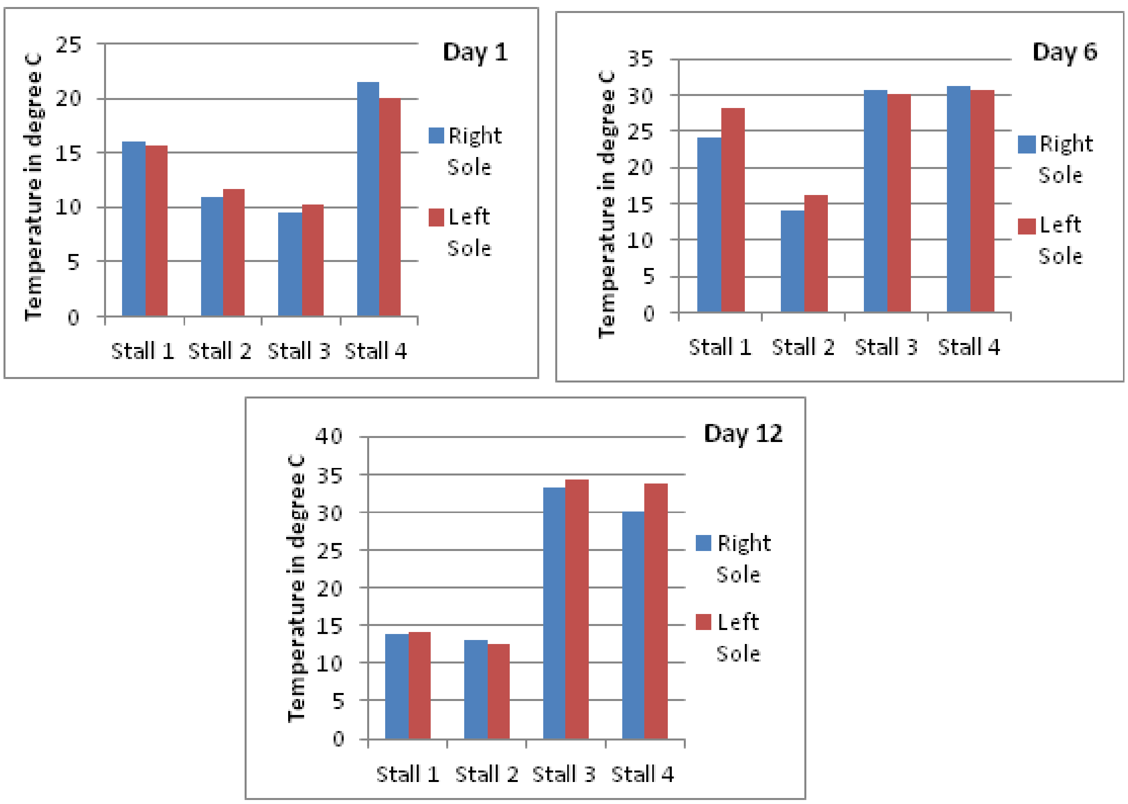

Figure 8 Maximum temperatures for the right and left fetlock of all horses on days 1, 6, and 12

On day 1, thermographs of the right frog for horses in stalls 1, 2, 3, and 4 showed a temperature of 14.3, 9.6, 9.4, and 20.5°C respectively. Thermographs of the left hoof showed a temperature of 15.4, 11.6, 9.9, and 17.2°C for horses in stalls 1, 2, 3, and 4 respectively. On day 6, horses in stalls 1 and 3 had the greatest increase in heat with temperatures of 21.7 and 25.5^{o}C respectively for the right frog and 25.2 and 23.8^{o}C respectively for the left frog. Horse in stall 4 showed a slight increase in heat with temperatures of 25.4^{o}C for the right frog and 23.8^{o}C for the left frog. Horse in stall 2 remained similar to day 1. On day 12, horses in stalls 1 and 2 showed normal readings like on day 1 thermographs. Horse in stall 4 showed a decrease in heat on the right frog at 23.4^{o}C and a slight increase on the left at a temperature of 28.4^{o}C, and the horse's thermographs in stall 3 showed a slight increase in heat of both frogs with temperatures of 28.9^{o}C for the right frog and 29.3^{o}C for the left frog. Results are shown in figure 9.

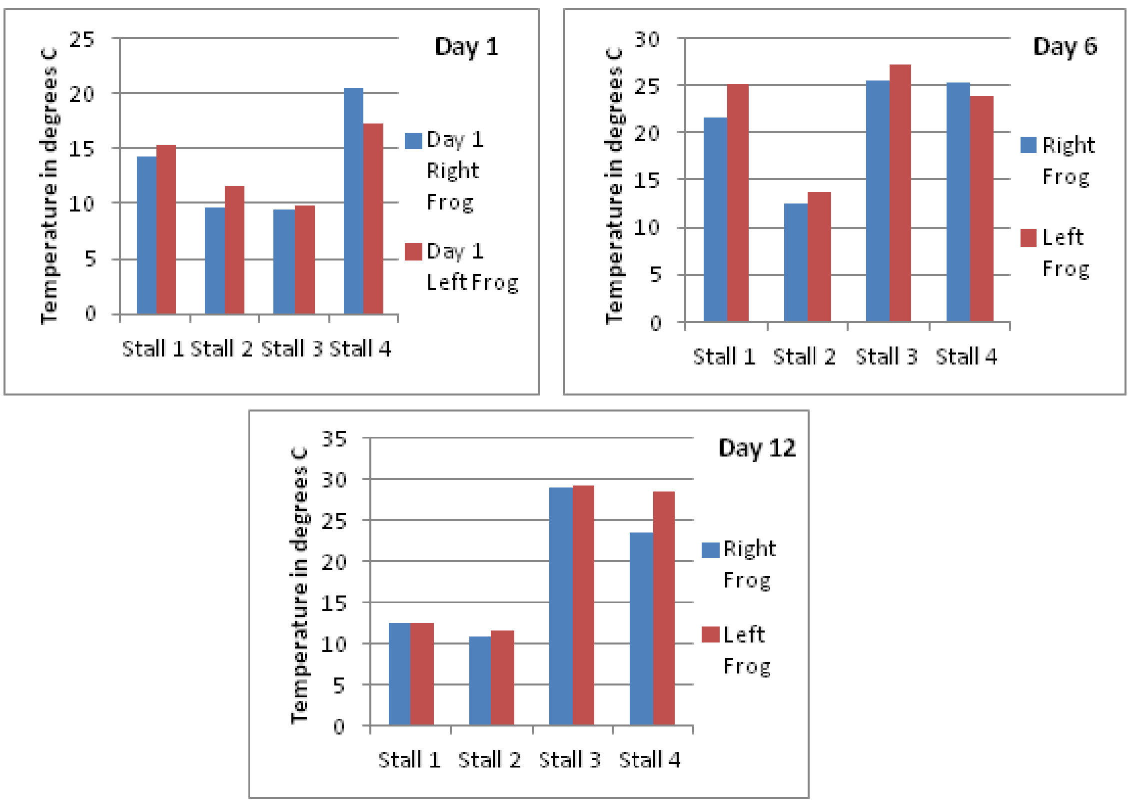

Figure 9 Maximum temperatures for the right and left frog for all horses on days 1, 6, and 12

On day 1, thermographs of the right heel for horses in stalls 1, 2, 3, and 4 showed a temperature of 15, 10.5, 8.6, and 21.3°C respectively. Thermographs of the left heel showed a temperature of 18.3, 12.8, 9.4, and 20.1°C for horses in stalls 1, 2, 3, and 4 respectively. On day 6, horses in stalls 1 and 3 had the greatest increase with temperatures of 21.5 and 28.1°C respectively for the right heel and 25.9 and 27.3°C respectively for the left heel. Horses in stall 2 and 4 showed only a slight increase by 3 degrees. Horses in stalls 1 and 3 were assigned the treatment where the hooves were not cleaned out daily. On day 12, horses in stalls 1 and 2 returned to normal readings like on day 1. Horses in stalls 3 and 4 showed a decrease of about 1 degree on the right heel but an increase on the left with temperatures of 29.9 and 29.5°C respectively. Results are shown in figure 10.

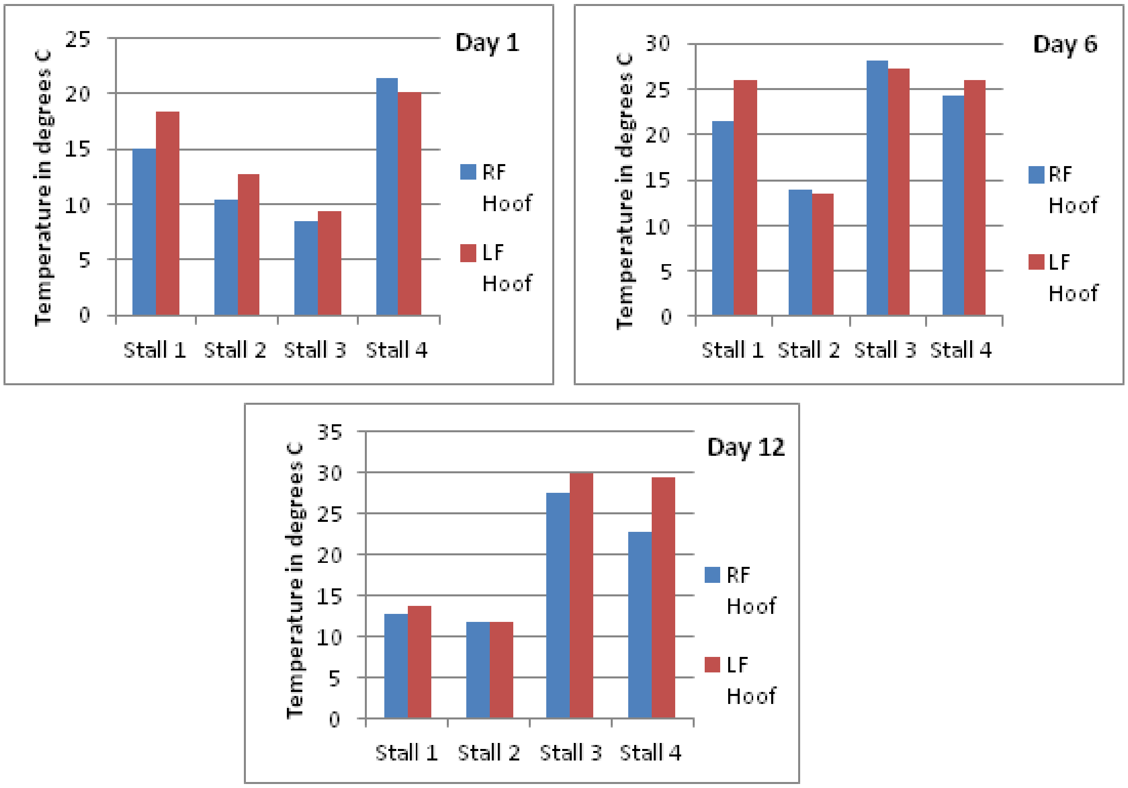

Figure 10 Maximum temperatures for the right and left heel of all horses for days 1, 6, and 12

Discussion

Throughout the trial the thermograph picked up increases in heat throughout the hoof and fetlock that were abnormal from the initial thermographs that were taken which were the horse's normal. This shows that there is a potential for soreness or lameness that is not being seen yet clinically. The horse in stall 3 did show clinical signs during the trial of soreness and lameness which also appeared in the thermographs, and consistently had greater changes in heat throughout the hoof and fetlock. The horse in stall 3 was also assigned the treatment where the feet were not cleaned out every day. There were circumstances where horses in both stall 1 and 3 (both on the same treatment) showed an increase in heat where as horses in stall 2 and 4 (feet cleaned out regularly) did not. This was seen in the heel, frog, and fetlock. Horse in stall 2 had times where there were never any heat changes, which shows that it depends on individual horses the outcome of what is seen on the thermographs. A hoof test was done at the end of the trial to test for soreness in the sole and wall of the hoof as well as looking for bruising that may have occurred from the bedding and none was found not even on stall 3. I would highly recommend that horse's hooves be cleaned out when being bedded on GP for long periods of time and to keep stall bedding clean. When the majority of dirty bedding was removed on days 7 and 10 of the trial and new clean bedding was added to the bedding that was left in the stall there was a great improvement seen in the horse's feet through the lameness exam.