IMPROVING DONKEY (*EQUUS ASINUS)* WELFARE THROUGH ENHANCED MANAGEMENT, TRAINING AND EDUCATION

By

Amy K. McLean

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ABSTRACT

IMPROVING DONKEY (*EQUUS ASINUS)* WELFARE THROUGH ENHANCED MANAGEMENT, TRAINING AND EDUCATION

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Mankind has depended on donkeys *(Equus asinus)* for thousands of years. Donkeys have helped farming communities cultivate land, harvest crops, and transport goods to the markets. To the present day, the "beast of burden" is still being relied upon as a major source of traction in many developing countries such as Mali, West Africa. These animals are able to live and work in regions of the world where food and water are often scarce. The welfare and management of donkeys in both industrialized nations and developing countries is often ignored. Donkeys in countries such as the United States are often faced with obesity and little physical exercise, while donkeys in places like Mali are faced with low body conditions and are overloaded and overworked. Problems encountered in either scenario could be addressed with pro-active management techniques. For example, training methods used to train donkeys to drive are often too harsh, leading to lesions and decreased longevity. Gentler training methods are available and can be equally, if not more, effective and will enhance donkey welfare.

A survey conducted by Diarra et al. (2007) indicated that most donkeys in Mali were subjected to poor working conditions. The productive life expectancy of a donkey in

Mali is on average about 2 years. Since a donkey can be purchased for $100-150 USD, a Malian must pay this amount every two years to replace his donkey, and this is approximately 1/3 of a smallholder farmer's annual income. The

survey conducted in Mali, indicated a need for education and training on better husbandry and management practices, including proper harnessing and cart usage to reduce injuries and increase the productive life of donkeys in Mali.

This research has taken an instructional approach to studying improved training, harnessing, and nutritional management for donkeys in both the United States and in Mali. The management tools and techniques were shared with other professionals, such as veterinarians, technicians and secondary and college students at agricultural institutions in Mali, so that they can eventually transfer this knowledge to donkey owners in the field. Informative articles and seminars have also been published in the United States on donkey behavior, metabolic conditions, and improved training and management techniques.

This dissertation is dedicated

To all the donkeys and mules that have to work for a living, to the people who try to make a difference in their lives and to all the longears that I have known!

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LIST OF ABBREVIATIONS

ADL	Acid detergent lignin
ADF	Acid detergent fiber
AIRg	Acute Insulin Response to Glucose
BCS	Body condition scores
bpm	Beats per minute
BW	Body weight
CP	Crude protein
DE	Digestible energy
DI	Disposition index (DI),
DM	Dry matter
ESAP	Ecole Secondaire Agro Pastorale, in Segou
FAO	Food and Agricultural Organization
FSIGT	Frequently sampled intravenous glucose tolerance test
Gb	Basal glucose
HF	High frequency, parasympathetic tone
HM	Halter method
HTM	Halter training method
HR	Heart Rate
HRV	Heart rate variability
Ib	Basal insulin
IPR/IFRA	Rural Polytechnic Institute for Training and Applied Research, University of Mali, in Katibougou
ISFRA	Higher Institute of Training and Applied Research, University of Mali in Bamako
LF	Low frequency, sympathetic tone
NDF	Neutral detergent fiber

LIST OF ABBREVIATIONS

MHR	Mean heart rate
NIRS	Near-infrared reflectance spectroscopy
rMSSD	the Square root of the interval to interval-in heart beat
SDRR	Standard deviation from interval to interval-in heart beat
Sg	Glucose effectiveness
Si	Insulin sensitivity
SM	Stick method
SPANA	Society for Protecting Animals Abroad
TSM	Traditional stick training method
US	United States
USD	United States Dollar
ZWD	Zimbabwe dollar

CHAPTER I.

INTRODUCTION

Improving Donkey (Equus asinus) Welfare through Enhanced Management

The donkey (*Equus asinus*) is believed to be the first member of the Equidae family that was domesticated (Rossel et al., 2008). Recent archeological findings in Egypt have dated donkey skeletons that were buried in tombs with pharaohs to be over 6,000 years old. They have proven to be predecessors of the modern day African Wild Ass (*Equus africanus*) (Rossel et al., 2008). Domestication of the donkey could largely be credited with changing society from a pastoral society to a contemporary urbanism. The beast of burden could carry large loads of goods on their back or pull carts while thriving on minimal nutrition, thus making transportation and trade possible between communities that were far apart. The donkey has even been attributed with making trade possible between Africa and Western Asia (Blench, 1994). Thousands of years later, donkeys are still used in agrarian societies and developing countries as a primary source of transportation for getting goods to markets, performing daily household chores, and cultivating crops (Pritchard et al., 2005). Even though these animals make major economic contributions for some of the poorest members of society and are often subjected to poor working conditions, their welfare has been largely ignored (Blench, 1994, Pritchard et al., 2005).

In industrialized nations, e.g. the United States, the value of donkeys and mules (the offspring of a male donkey and female horse) has increased and in some cases even surpassed that of horses (Burnham, 2002). The increase in value and change of owner's attitudes has in some regard changed the perception of donkeys as no longer being an inferior equine (e.g. beast of burden) (Burnham, 2002). The first donkeys imported into the U.S. were gifts to George Washington from the King of Spain (Haur, 2005). One jack was named Royal Gift and the other was the Knight of Malta (Haur, 2005). The prized animals were given to the country's new leader in hopes of producing superior mules for draft purposes, which would help develop the country (Haur, 2005). However, over the centuries as technology advanced, the use of donkeys and mules declined in the United States. They were eventually replaced with tractors and trucks until present day when their popularity has once again grown; this time for recreation as saddle animals (Taylor and Ray, 2005).

When looking at the world population of donkeys and mules there are close to 54 million, which roughly equals that of the world horse population (FAO, 2003). Approximately 6,000 years after domestication, most donkeys and mules are still kept for draft purposes in developing parts of the world. The desirable traits of donkeys and mules, e.g. being able to work and tolerate hot and humid climates, enhanced disease resistance, and their ability to survive on minimal feed inputs, make them just as important in the economic fabric of civilization today as they were thousands of years ago (Fedorski, 2004).

When comparing the typical use of donkeys and mules in industrial countries versus developing areas of the world, it has evolved in industrial countries from draft animals to show and companion animals. The demand for donkeys has risen in such countries. In addition, the need for knowledge pertaining to how to properly manage these animals such as nutrition, veterinary care, and training has also increased (Taylor and Ray, 2005). In developing countries, some animal welfare groups have focused their efforts on trying to identify welfare indicators to improve current management situations with donkeys. These groups are interested in methods to change the behavior of the donkey owners in order to improve management practices that may lead to improved welfare (Aluja, 1998, Pritchard et al., 2005).

In Mexico reports of working equids confirm that they are often abused, insufficiently fed and overall neglected (Aluja, 1998). A working donkey in a country like Mexico or Mali is often fed only corn stover (traditionally a poor source of nutrition). In contrast, a donkey in the United States is often allowed to graze lush pastures for endless hours and required to perform no daily tasks. These two situations both present poor welfare; e.g. a Malian donkey is expected to work while emaciated and fed a poor source of nutrition, and the U.S. donkey is faced with obesity and no exercise. Both issues could possibly be improved through management. Unfortunately, little research has been dedicated to improving the welfare of the donkey but some organizations, such as the Society for Protecting Animals Abroad (SPANA) and the Brooke Animal Hospital, are highly concerned about their well being. These organizations are beginning to focus on holding welfare workshops for owners while providing free veterinary care for donkeys and mules. However, treating the donkeys after they have been injured or poorly cared for is not the complete answer to improving their circumstances. Instead, implementing ways to encourage owners to prevent aversive handling and poor working conditions should be further examined.

In a donkey's natural habitat, such as a semi-arid environment like Mali, a donkey will often browse on sparse, fibrous vegetation, while wandering long distances, as well as going for long periods of time without drinking water (Mueller et al., 1998). These animals have adapted to living in harsh environments where both food and water can be scarce and seasonal changes impact food availability (e.g. feast or famine) and the condition of the donkey. Many donkeys that are used for draft purposes in developing countries are faced with this dilemma, foraging for limited food and water sources while exposed to many hours of work (Pearson, 2005). In contrast, donkeys that are kept in developed countries are often exposed to a more abundant food supply and little exercise (Crane, 2007). Donkeys kept for showing and companion purposes in such countries are often faced with health issues such as obesity, laminitis, and potential risks for metabolic disease (e.g. insulin resistance or Cushing's Syndrome) (Svendsen, 1997).

In general, donkeys are thought to be able to survive on less feed and poorer quality feed than a horse, while still maintaining body weight and even performing work (Pearson, 2005). Donkeys, when compared to horses, have been observed consuming and being fed more mature, less digestible, woodier plant material of poorer quality and still maintaining body condition. These forages tend to be high in fiber such as cellulose, lignin, and hemicellulose and low in protein (Aganga et al., 2000). Donkeys in developing countries are often being fed such a diet (Aganga et al., 2000). However, donkeys in industrialized countries are faced with the opposite dilemma; many donkey owners tend to over-feed them – especially with diets high in cereal grains, and provide little exercise (Pearson, 2005; Burnham, 2002). In horses, such high starch rations have previously been shown to promote insulin resistance, which often leads to laminitis and Cushing's Syndrome (Hoffman et al., 2003).

Some have assumed that donkeys and some breeds of horses and ponies are genetically predisposed to conditions such as insulin resistance (Jenkins et al., 1987). Insulin resistance has been described as an adaptive response when energy is limited because the animal then uses the excess fat it has stored as a source of energy (e.g. the donkey's ability to survive during a famine period) (Kronfeld et al., 2005; Taylor and Ray, 2005). The donkeys in Mali are faced with feast and famine due to the growing seasons; a dry season and a wet season. During the dry season (October through May) very little food or forages are available for the donkeys to consume, but during the wet season (June through September) more sources of nutrients are found. During the wet season the donkey will store much of its energy as fat, which is stored along the crest of the neck and the tail head. During the dry season, or famine situation, the donkey's body will utilize the fat as a source of energy when food is limited. However, when a donkey is placed in an environment with an abundant supply of lush forages, they easily gain weight and can potentially be at risk for certain metabolic conditions. Insulin resistance can be controlled by avoiding diets high in nonstructural carbohydrates and cereal grains (Jenkins et al, 1987). Collecting and analyzing feedstuffs and forages consumed by donkeys in Mali may improve our understanding about diets consumed by donkeys that are faced with feast and famine. Learning more about what donkeys in Mali eat could also help prevent U.S. donkeys from becoming obese and similar diets could be prescribed. In both cases nutritional challenges including metabolic diseases such as insulin resistance could be managed by proper diets.

Other management options for donkeys in developing countries include improving current working conditions. Common practices such as using a stick to guide and/or beat donkeys or overloading their carts are problems that could be changed through enhanced management. Improving current driving training practices may be addressed with pilot studies in the U.S. Currently little information is known in regards to applying learning theory when training donkeys (Heleski et al., 2008). Learning theory can be defined as a use of meaningful and consistent cues, reinforcing behavior fairly and quickly (e.g. the donkey moving forward when guided with a stick, refer to Appendix A for additional information on types of reinforcement, stimulus and actions). One study conducted by Heleski et al. (2008) focused on comparing negative reinforcement and luring methods to get donkeys to complete a novel task; in this case crossing a tarpaulin. The study indicated there was no difference in using negative reinforcement (applying pressure on the lead rope until the donkey moved forward and pressure was released) compared to luring (food was placed in front of the donkey throughout the trial). However, it was physically less demanding on the donkey handler to use the luring method. Further studies should examine alternative training methods for teaching donkeys new tasks such as driving to a cart.

It is essential to learn more about training donkeys to perform daily tasks such as pulling carts. The data from such a training study may offer beneficial information that could be used to improve donkey welfare by reducing the incidence of beatings. The donkey is a vital part of the Malian people's daily lives and work. The donkeys in Mali can be seen performing endless daily tasks and chores, from hauling water and charcoal, to pulling threshing machinery, hauling commodities to markets and even serving as a taxi for people. The intensity of the donkey's work can change throughout the year, as well as its access to food and water. The beginning of the growing season is when the donkey's workload is the greatest and nutrients are scarce. The combination can create decreased body conditions when they are faced with expending more calories than they are consuming. The FAO estimated in 2003 that approximately 600,000 donkeys were in Mali. However, Dr. Amadou Doumbia, a veterinarian who has worked in Mali for over a decade with SPANA as its Director, estimates there are approximately 2 million donkeys (personal communications, January 2008).

In 2007, a survey conducted in Mali by Diarra et al. (2007) with SPANA examined the current working conditions in over 2,600 donkeys in two locations: Segou and Niono. The variables and indicators which were analyzed included: the number of donkeys per owner, the intended use of the donkey, the quality of the harness, the daily distance traveled by the donkey, the nature and mass of the transport, hours worked per day, how often the donkey was fed, how often it was watered during the day, where the donkey was kept when not working, health problems treated by the owner, severity of medical problems, length of medical condition, how the owner treated the problem, conditions causing the donkey to not work, and length of time the donkey could not work. The results of this survey indicated that 76% of the harnesses were in bad condition, 79% of the donkeys traveled greater than 20 km in a day, 50% of the donkeys carried over 500 kg of goods per day, and 67% of the donkeys worked longer than 6 hours per day. The combination of poor quality harnesses along with large loads and traveling long distances could be associated with the numerous lesions seen on the donkeys' withers, shoulders, and under their tails, as well as the donkeys' inability to perform work in some cases. Furthermore, the survey reported that most donkeys were not fed appropriately (daily or an adequate amount) and medical follow up (treatment or check-up on a current medical problem) was not seen in most cases (72%).

The donkey is a valuable resource to the Malian people in that it assists in generating extra income and in performing daily household chores, such as obtaining water for the family. When donkeys are unavailable to work, the women and children bear additional chores. The death of a donkey can mean life or death for the family it serves. The average daily income for a Malian is about $1 US and the cost to purchase a donkey is approximately $150 US (2008, personal communication Dr. Amadou Doumbia). Even though the cost of donkeys continues to rise as people realize the advantages of owning a donkey, very little is being done to improve their overall well being in terms of support from the government (e.g. animal welfare policies) or research institutions. A healthy donkey enhances the well being of the family it serves and increases their opportunity to obtain many resources, as well as generate income by hauling goods to local markets.

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CHAPTER II.

LITERATURE REVIEW

Through out time the donkey, *Equus asinus*, a member of the Equidae family, has played a pivotal role in civilization especially in regards to development. According to the Food and Agriculture Organization, in 2002 there were over 44 million donkeys worldwide, 13.3 million mules and hinnies, and 52 million horses. Most of the world's donkey population is located in remote or developing countries. Industrialized nations such as the United States and Europe have far fewer donkeys than horses (Burnham, 2002). Due to increased interest and popularity of the mule, and hinny (reciprocal hybrid cross, a male horse and a female donkey) as trail mounts or show animals, donkeys have made a strong comeback in the United States (Hauer, 2005). The donkey's popularity has also grown internationally. This is especially true throughout Africa where the donkey has often replaced oxen due to extended periods of drought, deforestation, and increased human population (Blench, 1999; Pearson et al., 1999). Despite the valuable contributions donkeys make towards society, particularly in developing areas of the world, very little is known about the proper care and management of this species and their welfare is often neglected (Blakeway, 1994).

2.1. Working Donkey Management

Donkeys are considered by most farmers to be hardier than a horse, can survive on fewer resources and can adapt to harsher environments. Furthermore, they are highly heat and dehydration tolerant, making them suitable for semi-arid climates (Wold et al., 1999; NRC, 2007). Most donkeys are not individually fed; rather they are turned loose to forage on their own. Veterinarians are generally not called in to treat sick or injured donkeys in developing countries unless free veterinary services, such as the Society for the Protection of Animals Abroad (SPANA) are available (Itepu, 1999). Other management problems include primitive and ill fitting harness that is often described as cruel because it causes galling and sores (Diarra et al., 2007; Itepu, 1999). It has been hypothesized that increased support, both in financial and educational terms, to small holder farmers who own or work donkeys in developing countries could possibly increase the well being and longevity of the donkey (Diarra et al., 2007). The average life of a donkey in Ethiopia or Mali is 5 to7 years and in the U.S. 28 years. The longer a donkey lives, the longer the donkey could provide economic opportunities for smallholder farmers (Diarra et al., 2007). The use of the donkey is a very practical approach to energy limited transportation and farming in developing countries but more knowledge on the care of these animals and methods of harnessing and hitching needs to be provided to owners (Kidanmariam, 1999; Pearson et al., 1999; Wold et al., 1999)

2.2.1. Donkey Physiological Characteristics

These unique animals have proven to be popular in many cultures due to their remarkable ability to adapt to harsh and extreme conditions. Donkeys adapt to extreme environmental conditions and have less demanding feeding requirements than horses (Fedorski, 2004) but why they are able to adapt so well and thrive in such conditions is not fully understood (NRC, 2007). The donkey has provided much interest in terms of scientific curiosity, but unfortunately little research has been devoted to better understanding their superior ability to adapt and survive in harsh, resource-poor environments (Burnham, 2002). Yousef et al. (1970) reported that donkeys are able to maintain an appetite even in water deprived circumstances and maintain body weight, which may be one of the attributes to the donkey's ability to work in desert type climates such as Death Valley, Nevada and withstand temperatures of up to 48°C due to their ability to conserve blood volume and maintain an appetite while dehydrated.

2.2.2. Hematocrit, Temperature, Respiration, and Heart Rate.

Some researchers have observed that there is no difference in blood variables of donkeys from baseline to 36 hours post-water deprivation (Mueller and Houpt, 1991). When comparing blood variables, such as packed cell volume, between a donkey and pony, the donkey's value was different than that of the pony, 0.43 g/l (donkey) compared to 0.38 g/l (pony) (Mueller and Houpt, 1991). Mueller and Houpt (1991) also compared plasma protein-osmolality ratio and there are species differences for these parameters, 78 g/l (donkey) compared to 82 g/l (pony). The lower plasma protein-osmolality in the donkey suggested there is a lower amount of sodium, glucose and urea in their blood plasma when compared to a pony. Typically, osmolality will increase as an animal becomes more dehydrated.

Other hematological differences of value to identify include the resting heart rate which is has been reported by Svendsen (2005) to be 60-80 beats/min (donkey) compared to 36-40 beats/min (pony) and respiration 28-48 breaths/min (donkey) and 10-14 breaths/min (pony). On the other hand, Mueller et al. (1994) reported the resting heart rate of donkeys to be 44.6 bpm and at optimal exercise levels it is 223 bpm. Escudero et al. (2009) has examined the difference in heart rate among male and female donkeys and concluded that different donkey breeds, environment (e.g. temperature and humidity), and stress could play a role in the resting heart rate. Matthews and Taylor (2004) found the resting heart rate to be 42 bpm similar to Mueller et al. (1994) and Montes et al. (1986) (42-52 bpm). Other variations that can affect temperature, respiration and heart rate include diurnal variations (Ayo et al., 2008; Minka and Ayo, 2007). Lemma and Moges (2009) found that the donkey's age could affect the resting heart rate, temperature, and respiration. Younger donkeys (less than 4 years old) would have an average temperature of 37.4°C, heart rate 42.6 bpm, respiration 21.8 breathes/min and older donkeys (10 years or greater) would have an average body temperature of 37.4°C, heart rate 43.2 bpm, and respiration 20.5 breathes/min (Lemma and Moges, 2009).

2.2.3. Heat Dissipation.

Donkeys unlike horses have very large ears similar to that of various species of cattle that live in dessert like climates (Burnham, 2002). Their large ears aid in heat dissipation as well as having a thicker epithelia and a coarser hair coat than horses that prevents overheating or sun burn (Burnham, 2002; Taylor and Matthews, 2002a). Male donkeys tend to have large blood vessels and arteries in their scrotum and ears that aid in heat regulation (Taylor and Matthews, 2002a). Typically a donkey's body temperature is 38.9°C, which is almost two degrees higher than a horse, 37.8°C (Svendsen, 1997). Donkeys also require less water than most mammals of comparable size, but the water requirements of the hinny and mule are not well defined (Maloiy, 1973).

2.2.4. Hydration.

When donkeys are deprived of water or dehydrated, some physiological changes occur in the large intestine such as enhanced gut mucosa and fluid retention that occurs along with increased fermentation in the cecum and ventral colon (Maloiy, 1970; Sneddon et al., 2006). Enhanced fermentation capacity and absorption capacity in the dorsal colon along with retained fluid to maintain appetite are all essential adaptive qualities of donkeys working in semi-arid conditions (Sneddon et al., 2006). Their ability to conserve blood makes it possible for the donkey to maintain circulation along with heat dissipation and either increased or maintained salivary flow (Yousef et al., 1970). Furthermore, when donkeys are exposed to environmental conditions that could cause heat stress, they sweat very little (Yousef et al., 1970 and personal observations). In producing drier fecal matter and absorbing less sodium in the intestine donkeys can also conserve body water (Maloiy, 1973). Maloiy (1973) has compared the cecum of the donkey to that of the rumen of the cow in terms of acting as a water reservoir. Dill et al. (1980) suggested that donkeys might store water in their gastrointestinal tract, which leads to decreased water intake. Water intakes in donkeys have been reported from 8.4 ±

0.6 L/d (resting donkey) to 13.25 ± L/D (working donkey) in 28 to 32° C temperatures and relative humidity of 64-70 percent (Ram et al., 2004). Depending on temperature, workload, and humidity these factors may affect the amount of water consumed by donkeys (Mueller et al. 1994b). Cuddeford et al. (1995) has reported donkeys (1.93 L/kg DM) to have lower water intakes when compared to Shetland ponies (2.4 L/kg DM) and Thoroughbreds (3.87 L/kg DM). In general, donkeys are thought to consume up to 9% of their body weight when temperatures range from 25-37° C and humidity is high (Mueller et al. 1994b).

When donkeys are deprived of water or water is scarce, the donkey will fully rehydrate at the rate of 17-20% of their body weight when offered water. In general donkeys are thought to have a lower water requirement when compared to other mammals with the exception of the camel (Agnaga et al., 2000). Some researchers have found that water consumption in donkeys depends on the type of forage they are consuming (Muller and Houpt, 1991). The two donkeys used by Yousef et al. (1970) drank enough water in 5-10 minutes to restore 95-98% of what they had lost. When measuring the fecal matter of these two donkeys who defecated immediately after drinking, the feces contained 66% water content, which is only 5% less than when the donkeys were hydrated (71%) (Yousef et al., 1970). Donkeys can maintain their appetite and body weight when being dehydrated by continuing to consume feed or forages even of poor quality (Dill et al., 1980; Yousef et al., 1970). The donkey's ability to thermoregulate and still provide enough saliva for mastication is essential for its ability to consume food after being dehydrated (Yousef et al., 1970). Often times the donkey will consume hay before drinking water, even when dehydrated, to maintain feed and energy intake (Yousef et al., 1970). The donkey is a special animal that can adapt to the harsh conditions of desert heat and it can be 20% dehydrated without being exhausted and still perform work (Yousef et al., 1970). The donkey has been compared to other desert animals such as the camel due to its ability to rehydrate quickly, regulate plasma volume along with its ability to reduce water and energy turnover rates (Schmidt-Nielsen, 1964; Yousef et al., 1970). These traits make it possible for donkeys to live and survive in places such as Death Valley in the U.S. or the Sub-Saharan regions of Africa (Schmidt-Nielsen, 1964; Yousef et al., 1970).

2.2.5. Nutrition.

Other adaptive qualities of the donkey include its ability as a monogastric to consume poor quality forages and maintain weight even when dehydrated. This adaptive feature is common to other desert animals, especially ruminants such as goats or camels (Izraely et al., 1989b). Donkeys can consume poor quality forages, and still maintain body weight due to increasing dry matter intake and efficiently recycling urea (Izraely et al., 1989a). Izraely et al. (1989a) reported that donkeys were able to maintain body weight on wheat straw that was 3% crude protein. Donkeys, like ruminants, can efficiently recycle urea and can actually recycle more urea than found in the food source and reabsorb 82% of the urea filtered by the kidney when fed poor quality forage, e.g. wheat-straw (Izraely al., 1989a).

However, when the donkey was fed a diet consisting of high quality forage, such as alfalfa, the donkeys only recycled and utilized 48% of the urea when filtered through the kidney (Izraely et al., 1989a). Mules and hinnies have similar capabilities in terms of using of poorer quality forages and require less maintenance than a horse of the same size but exactly how much less has yet to be determined by research (Pearson et al., 2005 and personal observations). In general mules and hinnies consume less grain than horses of the same size but donkeys have a higher consumption rate when fed poor quality forages when compared to ponies and goats (Burnham, 2002; Brosh et al., 1987; Izraely et al., 1989a). When donkeys are fed lower quality forages, the forage percentage needed by the donkey increases from 2% of their body weight to as high as 10% depending on the source of forage, NDF content, and work level (Mueller et al., 1994b). In general donkeys have been reported to have a slower rate of passage of poor quality forages than ponies, depending on the source of forage, either alfalfa, wheat straw, or barely straw. The rate of passage a wheat straw based diet was 36.4 ±3.2 hours (Izraely et. al., 1989a). Some researchers have indicated that donkeys may be more efficient in digesting poorer quality diets than horses due to enhanced microbial digestion in the donkey's cecum (Suhartanto et al., 1992). Volatile fatty acid production was reported in this same study to be higher in donkeys (47.0-67.1 mmol/l vs. 33.6-41.9 mmol/l) when compared to ponies when fed wheat straw mixed with or without a concentrate (Suhartanto et al., 1992). It has been proposed that the donkey is more effective in microbial digestion than other equine, but more research in this area and the effects of mineral intake on digestion should be further researched (Suhartanto et al., 1992).

Energy requirements for donkeys have been compared to horses by using the same formula designed by Pagan and Hintz in 1989 for recommendations in the National Research Council feeding guidelines. The formula that is traditionally used for estimated digestible energy (DE) in horses, DE (Mcal/day) = 1.4 + 0.03 x Body Weight (BW), indicates that a small standard donkey weighing between 110 kg to 150 kg (common size found in most developing countries) would require approximately 5.9 Mcal/d depending on the quality of forage (McCarthy, 1986; Mueller et al., 1994a; NRC, 2007). The donkey's energy needs would be increased if work was performed and as work increased, the amount of energy needed would increase (Mueller et al., 1994a; NRC, 2007). However, the original equation used to estimate the dietary needs of donkeys has since been adjusted due to many who felt that the original equation over estimated the donkey's needs (McCarthy, 1986; Mueller et al., 1994a). The adjusted equation has been made for dietary energy, Mcal/day=0.61 + 0.03BW. However, when calculating other essential dietary needs, such as protein requirements, this should be taken with caution especially when considering the donkey's ability to recycle urea because the current equation may still overestimate the nutritional needs of the donkey (NRC, 2007).

Due to variations in management practices it is important to evaluate the intake of donkeys and record consumption. Donkeys consuming diets of low quality forage have been reported to consume as much as 3% of their body weight and when being fed high quality forages they have consumed as much as 2.5% of their body weight which can then lead to risks of obesity (Mueller et al., 1994b). The donkey's ability to increase intake when poor quality forage is fed is an adaptation feature that makes the donkey able to survive on poorer quality forages than a horse (Mueller et al., 1994b). Generally, a mature horse can consume approximately 1.5 to 2% of its body weight and 0.5% or less in the form of concentrates. Although some have proposed that a donkey's intake can be limited by consuming such low quality sources of feed due to gastrointestinal fill but if given the choice will consume larger quantities of better quality forages (Van Soest, 1994). There are many factors that can affect the donkey's ability to consume food. Intake of various feed ingredients and forage types can better estimate the dietary needs of the donkey.

One method that has been utilized in calculating dietary intake of the donkey is near-infrared reflectance spectroscopy (NIRS)(Kidane, et al., 2007). Manure was collected from donkeys that were fed 100 different diet combinations of forage and concentrates in order to predict the dry matter intake and the organic matter intake of donkeys using NIRS. This study showed that NIRS could be successfully used for predicting the intake of donkeys but no work has yet to be done on the intake of dry matter and organic matter for mules and hinnies (Kidane, et al., 2007). Studies have also indicated that donkeys from different parts of the world, such as donkeys from the United States compared to donkeys from Africa have shown differences in terms of intake and ability to consume poorer quality forages and maintain weight (Mueller et al., 1994b; Izraely, et al., 1989b).

Vaux et al. (1998) identified a species-specific gram-positive bacterium, *Entercoccus asini* in the cecum of the donkey. The new strain of bacteria that has only been isolated in the donkey's cecum has some unusual characteristics when compared to other microflora; since, they only produce acid from d-xylose. This new species of microflora is also able to utilize pyruvate, which negatively reacts to Arginine and enhances fermentation activity. Enhanced fermentation causes an increase in mucosal absorptive capacity even with limited food intake of poor quality as well as restricted water intake and aides the donkey in its ability to survive in such harsh conditions (Vaux, et al., 1998; Vaux, 1998; Sneddon et al., 2006). The donkey's behavior may also play an important role in the animal's efficiency (Yousef et al., 1972). Nengomasha et al. (1999) recorded that donkeys maintain their appetite throughout dehydration, which aids its ability to survive in areas that lack in water and nutrients. Other researchers have observed donkeys using their narrower lips to browse and select higher quality nutrients as compared to a horse (Van Soest, 1994).

2.3.1. Donkey Behavior

There are many misconceptions about donkeys and often fallacies are associated with their seemingly peculiar ways, such as mistaking caution for stubbornness (Burnham, 2002; Miller, 2007). In general, donkeys and mules are considered to be unique creatures with special qualities and they should not be treated like horses with long ears (Burnham, 2002). Unfortunately, their behavior has often been misunderstood and in some cases animals may have been treated more harshly than they should have been. In general, most owners have acquired a great sense of patience in dealing with donkeys and mules (Burnham, 2002; Miller, 2007). A new owner should be aware of the peculiar ways of both donkeys compared to horses and learn how to work with these behavioral differences and not against them (Burnham, 2002; Miller, 2007; Taylor and Matthews, 2002a). For example, mules and donkeys are creatures of habit and especially do not like their daily routine changed (Burnham, 2002; Taylor and Matthews, 2002a). If the donkey is used to being fed at a certain time of the day, a change in schedule can upset the animal and create abnormal behavior (Burnham, 2002). An unfamiliar person may prevent a very gentle and friendly mule or donkey from being caught until the stranger leaves (Burnham, 2002; McGreevy, 2004). Both animals tend to bond with their owner once trust has been gained, which can often be done by using treats as rewards.

2.3.2. Natural and Social Behavior

Some researchers have stated that traditional rules for horse management are detrimental to the donkey (Burnham, 2002). Often times training or even routine procedures require more patience and effort than when working with a horse (Miller, 2007).

When a dangerous situation arises, a horse, which originally developed in a plains region, is likely to bolt and run due to the strong flight mechanism, but a donkey is less likely to run away from danger as it evolved more in hilly areas with cliffs (Miller, 1998; Miller, 2007). Donkeys are described to have a “fight” behavior meaning they will not expend the energy in running away but instead will hold their ground and fight off the predator (Miller, 1998). Donkeys are commonly used as guard animals for cattle, sheep, goats, and at horse breeding facilities to protect newborn foals (Burnham, 2002). In general donkeys do not like dogs or coyotes and can be quite aggressive toward them (Burnham, 2002; Miller, 2007).

However, donkeys become accustomed to animals that live on the same grounds or property as them. Donkeys have been known to stomp predators to death with their rapid stomping abilities in the forehand. The donkey will also pick up the predator by the nap of its neck and shake it vigorously, sometimes until its death. Typically, gelding donkeys (or cut jacks) and jennets are used as guard donkeys. Intact males, (e.g. jacks), are often more aggressive and have been reported to harm newborn calves, lambs, and kids (baby goats).

The hind legs are also used for defense. Donkeys are very agile and can kick all the way in front of its shoulder (Burnham, 2002). Even in times when the donkey or mule is being restrained, such as having a front leg tied up, the animal can still balance on two legs and kick with a hind (Taylor and Matthews, 2002a). The fight mechanism also makes both of these animals perfect trail mounts because they are less likely to run from danger (Miller, 2007).

Donkeys were domesticated around 4,500 years ago but some wild herds of donkeys remain today. Some believe the success of the donkey is due to its flexible social structure (Svendson, 1997), which primarily depends on the available resources such as food or water (French, 2000). The numbers of wild Asses are few but they still exist such as the Somalian Wild Ass in Ethiopia or the Asiatic Wild Ass of Russia. Somalian Wild

Asses have been observed grazing in large groups during the day but if grazing is limited the donkeys will separate into smaller groups. During the evenings, Klingel (1998) observed the herds breaking up into even smaller groups such as only jennies and foals. The groups that break up in the evening could also include immature or young jacks, jennies with foals or a dominant male with a group of jennies that may be in estrus or close to coming into estrus (McDonnell, 1998). There are also populations of feral donkeys found around the world living in environments that are either sparse in forages or abundant in resources. These animals are able to survive in harsh semi-arid climates where water is often limited. The donkey survives on high fiber diets and spends much of its time walking in search of food.

In areas where the food is abundant, a so called lush environment, two groupings of donkeys have been observed by French (2001) that are not seen in semi-arid environments: 1) a dominant male controlling a territory with a group of jennies and their offspring, 2) a bachelor group of males. It is not uncommon to see donkey breeders keeping a group of young jacks together similar to that in the wild (personal observation). The jacks will live in peace as long as there is no female (horse or donkey) in sight or across the fence. In developing countries it is not uncommon to see jacks as well as jennies turned loose and grazing together with signs of little conflict (personal observations, 2008 and 2009). It would be interesting to determine whether this would still be the case if these working donkeys were less exhausted and had access to more resources.

The type of social group a donkey forms is dependent upon the environment in which it lives but typically it belongs to a territorial social system instead of a harem system. The harem system is more commonly seen in feral horses and some breeds of zebras. In the harem system, a dominant stallion will control a group of females and herd them everywhere (McDonnell, 1998; Klingel, 1977). Donkeys may form various social groups consisting of either a small or large group with both females and males, and immature jacks may be found either living alone or in small groups with other young jacks (Klingel, 1998). Donkeys living in arid type climates form a territorial social system meaning a dominant male (jack) will fight other donkeys coming into a specific area or trying to enter the herd (Klingel, 1998). If the donkeys are living in an environment where resources are plentiful the social system can be referred to as a natal band system or birth band (e.g. group of donkeys that are family and born into the group) (McGreevy, 2004).

Typically, in wild herds there will not be a dominant jack that maintains a harem like in horses but instead he will maintain a territory for breeding purposes called a lek (McGreevy, 2004). More commonly jennies in the wild are seen separating from herds with their foals, which later leads to a lack of social interaction or play (French, 2004) However, this is not the case in domestic donkey foals that are raised with other donkey foals (French, 2004). Play and socialization are important in domestic donkeys because it establishes a social hierarchy as well as aids in development of dominating strategies such as learning to fight, biting in key locations such as the throat latch, and knees (French, 2004; Svendson, 1997).

Donkeys often seem to become very attached to a mate or another equine (Burnham, 2000; Svendson, 1997). Donkeys have been observed showing signs of depression or distress when their mate is taken away or dies (McGreevy, 2004; Svendson, 2007). However, even in times of missing a pasture mate or companion when the donkey may bray and walk the fence line, these behaviors typically do not later develop into stereotypic behavior as is sometimes seen in horses (McGreevy, 2004). In addition, the gentle nature of the donkey and the characteristic flexibility of its social structure make it an ideal equine companion animal for other horses, foals, goats, and cattle (French, 2004; Svendson, 1997). Donkeys are often seen accompanying newly weaned foals as well as traveling partners for high dollar performance horses to reduce stress related to transportation and competition(s) (personal observations, 2005, 2008). Donkeys, especially jennies and geldings, rarely show aggression towards humans, which make them ideal pets for young children as well as guard animals for other livestock (Svendson, 1997). Jacks may show aggression towards other equine and livestock and are often recommended to be gelded if not being kept for breeding purposes (Burnham, 2002; Svendson, 1997).

2.3.3. Ingestive Behavior

Another common belief is a donkey will not over eat like a horse. In some cases this is true. More importantly one should realize that a donkey or a mule should not be fed the same ration as a horse (Burnham, 2002). Their weight should be monitored closely even if the animal is only consuming forage. Many donkeys and mules can survive on pasture alone due to their grazing skills and relatively low nutritional demands. Donkeys are considered to be both browsers, as well as grazers, and in general are “easy keepers.”

An animal that is a browser will sort out species of plants that are most appealing to its appetite instead of eating everything in sight (Mueller et al., 1998). The narrow muzzle and prehensile type lips that a donkey and mule have are believed to make them able to be more selective when grazing (Mueller et al., 1998). Often times they will consume plants that are not eaten by horses such as plants that have briars or even thistles. They will often consume plants that are high in tannins, which produce a bitter taste, and which most livestock avoid (Mueller et al., 1998).

When consuming forage, the donkey will chew its food about 10 times before swallowing (Mueller et al., 1998). They do not particularly chew faster than a horse but they are able to consume more fiber at a faster rate than most ruminants their size due to a more efficient tooth and jaw apparatus that allows them to swallow larger feed particles (Mueller et al., 1998). Even though the donkey can swallow large particles of feed, one may think this would predispose the animal to esophageal obstruction however choking in donkeys is rarely seen (Mueller et al., 1998; Taylor and Matthews, 2002a).

2.4. Management.

Management schemes vary according to location and use of the animal. Donkeys in developing countries are often faced with over work and poor nutrition but donkeys in industrialized nations are typically exposed to little or no work and abundant sources of nutrients (Pearson et al., 2005). A major problem in the United States is obesity in donkeys. These animals become extremely obese due to over feeding, yet their owners may be feeding far less amounts than to a horse of the same size (Pearson et al., 2005). These animals are also predisposed to developing fatty liver or hyperlipemia. (Pearson et al., 2005). This condition is more common in miniature and standard donkeys and mules that are of draft breeding. It is unknown if this is a problem in hinnies. Rarely will donkeys, mules, and hinnies over eat which can lead to colic and founder but donkeys *can* develop laminitis and colic (Burnham, 2002). Laminitis often occurs in all four feet of donkeys and sometimes only in the rear (Taylor and Matthews, 2002a). Often a donkey will not show signs of colic until its past the point of survival due to their high level of pain tolerance before exhibiting behavioral signs (Taylor and Matthews, 2002b). It is assumed that donkeys that go off feed for several days may be suffering from colic, hyperlipemia, or hyperlipidemia (Taylor and Matthews, 2002a). In general, donkeys are considered to be more resistant to disease such as African Sleeping Sickness and have less lamanitic issues. These animals can live longer (average 27 years) than a horse if properly maintained (Pearson, 2005). Donkeys are subject to hosting lungworms and sarcoid tumors (Taylor and Matthews, 2002a).

2.5. How Donkeys Serve Mankind

A majority of donkeys and their hybrid offspring, mules and hinnies, are still used today as beasts of burden in developing countries for traction and agricultural purposes such as tilling soil, harvesting crops, and transporting commodities to markets (Pearson et al., 1999). Owning a donkey or mule is an important asset for small shareholder farmers (Pearson et al., 1999). In terms of initial purchase prices, donkeys typically cost less than oxen or mules and hinnies. Mules and hinnies are typically highly valued and often three times the price of a donkey and twice the price of oxen (Howe and Garb, 1999). Recently, the price of donkeys has increased in many markets due to their increased use (Pearson et al., 1999). In Zimbabwe a donkey cost 80$ZWD or $0.21 US) in 1990 and in 1995 the price had risen to 600 $ZWD or $1.59 US (Pearson et al., 1999). Donkeys in Mali sell on average for $150 US. Donkeys are considered one of the cheapest forms of labor other than human power (Pearson et al., 1999).

The smallish size of a donkey can often limit the amount of work that can be performed (Pearson et al., 1999). However, a well-managed donkey can often perform the same amount of work as an ox (Pearson et al., 1999). Given the smaller amount of resources (e.g. food and water) required by a donkey, the donkey can be a more efficient labor tool than an ox. Certainly the reduced amount of resources required by donkeys makes them a more appropriate traction animal for many parts of the world. A study in Zimbabwe indicated that well managed (properly fed and harnessed donkeys) and well trained donkeys when worked as a team of four, could pull a draft force of over 1kN (a measure of force, 1kN = 1000 N = 100 kg) for a 4-hour period (Nenogomasha et al., 1995). The draft power indicates how well the donkeys can pull in heavy soil but often times donkeys are not well managed which limits their work output or longevity (Nenogomasha et al., 1995).

Another alternative to donkey power is the use of mules and hinnies. They are often stronger than both the donkey and horse parents and can withstand different climates and terrains when compared to donkeys and horses. The use of mules and hinnies are often favored in more mountainous and humid type climates such as in Columbia or the Kaffecho Zone of Ethiopia where mules are favored to haul large amounts of "black gold" or coffee. The mule and hinny have proven to be a more appropriate or productive animal for draught purposes in high altitudes that are often wetter and more humid type climates (Howe and Garb, 1999). The mule is also more suitable for carrying larger loads of goods when compared to the donkey, and they are more agile and faster than an ox on tight mountain trails (Howe and Garb, 1999). By using draught animals such as donkeys, mules and oxen, some claim that 20 billion gallons of petroleum are saved annually (Ramaswamy, 1998). When comparing the cost of draught animals to tractor usage, by using draught animals for farming versus tractors approximately 10 billion US dollars are saved (Ramaswamy, 1998).

In the past 30 years, according to the Food and Agriculture Organization (FAO), the use of donkeys and mules has increased by 10 fold each year. In countries such as Mexico and India, the poorer farmers cannot afford mules so they solely depend on donkeys (Gebreab et al., 1999). Some farmers in developing countries do not own a donkey or mule and these farmers are considered investment-poor because they are forced to borrow a donkey and trade part of their crop for its use. There is even a joke in Ethiopia “if you do not own a donkey then you are a donkey” because your workload is increased to the amount of what a donkey would work. When comparing a man and donkey’s level of work on the busiest day of trade, (e.g. Friday), at the market, in Addis Ababa, Ethiopia, there are over 1,100 donkeys each carrying approximately 100 kg of goods compared to about 450 men each carrying approximately 10 kg of goods to the market (Zenebe and Fekade, 1999). The average donkey in Ethiopia weighs approximately 250 kg and in some countries such as Mali most of the donkeys pack or transport approximately 500 kg or more (Gabreab et al., 1999).

2.6. Economic Impact of Donkeys

Many researchers state that the donkey has made a major contribution to the lives of people in developing countries (Marshall and Ali, 1999; Pearson et al., 1999; Sieber, 1999; Wells et al., 1999). For example, the excise duty for a donkey hauling rock salt from Mekele, Ethiopia is 0.75 birr ($0.06 US), in 1970 the annual revenue collected from donkeys hauling rock salt averaged at 60, 680 Birrs ($4,551 US) and mules averaged 172,093 Birrs ($12,906.98 US) (Zenebe and Fekade, 1999). In total donkeys have contributed to over 50% of the excise duty (Zenebe and Fekade, 1999). Donkeys are seen carrying as many as 15 commodities to market in Addis Ababa and during the dry season the donkey owners use the animals for packing goods such as salt (Zenebe and Fekade, 1999). The donkey can be used for many other diverse jobs to generate income. Households that own donkeys are considered to be more economically prosperous than those who do not (Sieber, 1999).

A major constraint is the initial purchase price of a donkey and many smallholder farmers do not have any extra income available (Sieber, 1999; Wells et al., 1999). One study conducted in Makette, Tanzania indicated that donkeys were both efficient for transportation and could contribute a great deal economically to the owners of the donkeys (Sieber, 1999). Moreover, when comparing the initial purchase price of oxen to a donkey, the donkey is much more affordable (Marshall and Ali, 1999). The oxen are used for only a short period (tilling) throughout the year but the donkey can be used all year (Marshall and Ali, 1999). Overall, the income opportunities provided by owning a donkey have begun to change attitudes toward owning and working donkeys (Marshall and Ali, 1999).

Many donkeys are used in Tanzania, Africa. The annual household income in Makette, a city in Tanzania, was estimated to be $120 US (Sieber, 1999). In Tanzania, on an annual basis, households that owned donkeys have saved time and money (Sieber, 1999). In terms of labor, a donkey will make on average 93 trips, which will take 133 hours and save approximately $10 annually (Sieber, 1999). Total annual benefits of households who owned donkeys in this region were estimated to range between $55-124 US per household (Sieber, 1999). Even though the donkeys proved to be an important tool for transport in this part of the world, many households could not afford the initial purchase price of a donkey at $88 US

(Sieber, 1999). Some households were able to make the big investment that lived closer to large markets such as the one found in Matamba, Tanzania (Sieber, 1999).

Those households that could not make the initial investment could rely on a government subsidy to purchase a donkey. The subsidy promoted the adoption of donkeys but once the subsidy program was over very few low-income households could purchase these animals in Makette, Tanzania (Sieber, 1999). If credit were made available, the increased use and adoption of donkeys would probably increase, according to households who were interviewed during this project (Sieber, 1999). Subsidies for purchasing donkeys have been made available in Sudan where the government provided a $50 US subsidy for purchasing a donkey (personal communication, Dr. Charles MacKenzie, 2008). According to the survey in Tanzania, the government would more likely benefit smallholder farmers by using these funds for credit for farmers to buy a donkey (Sieber, 1999). Providing credit to smallholder farmers would increase the demand for donkeys as transport animals. This survey suggested that a donkey could be economically beneficial to the smallholder farmers by allowing them an opportunity to improve their annual income by decreasing the amount of hours spent working and increase the amount of commodities taken to the road (Sieber, 1999). If more credit was made available for smallholder farmers to purchase a donkey, it is likely that in most developing countries the adoption would be even greater by the investment-poor farmers.

Many researchers have conducted surveys on the use of donkeys in developing countries and the promotion of traction animals (Pearson et al., 2000; Starkey and Starkey, 2000). Some research has indicated after looking at profitability analyses in three regions of Ethiopia (North Welo, Kobo, and Oromiya) that a donkey and a donkey cart are very important tools for generating income (Kidanmariam, 1999). For example, the price of a donkey in Ethiopia is around 240 Birrs ($18 US), a pack saddle for the donkey is 15 Birrs ($2 US) or a cart is 1144 Birrs ($181.59 US) estimated labor costs are 450 Birr/year ($71.43 US), feeding costs 730 Birrs ($115.87 US), maintenance costs of the cart 180 Birrs ($ 28.57 US) and wages working 150 days a year for the daily income of 30 Birrs/day ($4.76 US) for an annual income of 4500 Birr/year ($714.29 US) (including depreciation of the cart and donkey) (Kidanmariam, 1999). During the off-season the donkey could be used for domestic chores such as hauling water, charcoal, and building materials (Marshall and Ali, 1999; Pearson et al., 1999).

2.7. Donkeys and Gender Equity

Another important aspect to examine with working donkeys is gender equity. The donkey is labor saving for women and children, who often are responsible for such jobs as carrying water or charcoal (Marshall and Ali, 1999). In the Eastern Shewa and Eastern Hardedge regions of Ethiopia, donkeys are mainly used for carrying water, a task that entails six hours out of each day (Marshall and Ali, 1999). Sieber (1999) reported that 16% of a donkey's work in Makette, Tanzania, is helping with domestic chores. The donkey saves time for the women and children since a donkey can carry a load three times that of a human and reduce the annual transport burden by 93 trips, which equals 133 hours per year (Sieber, 1999). Therefore, women who have access to donkeys can decrease their daily labor or choose to invest those hours in alternative activities, such as marketing goods and education (Marshall and Ali, 1999; Sieber, 1999).

Even though there are many attributes to using donkeys, they are not widely adopted in all areas such as Namibia, Africa (Itepu, 1999). In Kavanga, Namibia oxen are predominately used for tilling and are occasionally worked by women (Itepu, 1999). Itepu (1999) reported that women surveyed would prefer to use donkeys because they are easier to work and are considered friendlier. For households in Namibia, the initial cost and daily maintenance cost of a donkey is cheaper than that of an ox but some households fear that owning a donkey sends a cultural message that they are poor (Itepu, 1999). Another important difference when comparing donkeys to oxen is ownership. Women in Ethiopia have reported that owning a donkey is a great asset because they can be engaged in income-generating activities as well as decreasing their domestic chores (Marshal and Ali, 1999). Due to cultural and religious norms, many women are not allowed to work or own oxen because they are considered a man's animal, but they *are* allowed to work and own donkeys (Itepu, 1999; Marshall and Ali, 1999; Sieber, 1999). Ownership can depend on the head of the household. In some female-headed households, women are allowed to own donkeys (Marshall and Ali, 1999). However, women in Muslim countries are generally never allowed to own oxen due to religious beliefs (Marshall and Ali, 1999).

Despite their social image, donkeys can prove to be very useful for women. For example, donkeys are important to women who are pregnant by serving as an ambulatory service (Pearson et al., 1999). The expecting women are loaded on the backs of donkeys, and the donkey carries them to the medical care clinics. Some studies have suggested that women who utilize a donkey and a cart to carry goods can increase the amount transported and the women's transport efficiency by 20 fold (Taha, 1999). Donkeys can also provide additional income for women. In some countries, such as Chile, China, and Belgium, women have begun milking donkeys for the purpose of milk consumption, medicinal purposes, and to make skin care items (e.g. anti-aging lotions and soaps). This could be another source of income that could be generated by women from the donkey.

2.8. Challenges Facing Working Donkeys

Changing owner attitudes toward working donkeys is a major problem in terms of how they are treated and managed (Itepu, 1999; Kidanmariam, 1999; Pritchard et al., 2005; Pearson et al., 1999). The donkey is often associated with poverty and thought of as a poor man's beast. Due to their low monetary value, donkeys and until recently have been ignored by scientists, extension workers and farmers in terms of how to properly manage and care for them (Pearson et al., 1999). Pearson et al. (1999) states that overcoming donkey prejudices offers a challenge to all involved with donkeys. Other challenges that plague working donkeys include but are not limited to poor nutrition, lack of treatment from owners (e.g. treating harness sores), overloading, using poorly designed implements (e.g. unbalanced carts) and crude harnessing methods (Itepu, 1999; Pearson et al., 1999).

Development of feeding recommendations for donkeys when working in different seasons is greatly needed by most farmers (Marshall and Ali, 1999; Pearson et al., 1999). In most cases donkeys are left to forage for themselves and no supplemental grain is given (Gebreab et al., 1999; Wold et al., 1999). Though this may not be a problem during the wet season, during the dry season most donkeys' body condition scores drop; unfortunately this coincides with when the greatest amount of work output from donkeys is expected (Feseha and Aweke, 1995; Gebreab et al., 1999; Wold et al., 1999). In terms of health and disease issues described by researchers, most originate from poorly constructed and ill fitting harnesses (Gebreab et al., 1999; Wold et al., 1999). Gebreab et al. (1999) reported that 680 donkeys (n=2020) had saddle/harness sores. Diarra et al. (2007) reported 834 (n=1256, 66.6%) donkeys had wounds from poor harness and Wells et al. (1999) also reported that 52% of the conditions present in donkeys in Eastern Cape, South Africa were harness sores. Diarra et al. (2007) reported that most owners did not know they could treat donkeys for health conditions (e.g. for harness sores) and most owners did not medically follow up on the treatment (72%). Diarra et al. (2007) reported that most donkeys were exposed to carrying heavy loads (greater than 500 kg) for over 6 hours per day for long distances (greater than 20 km) while being fitted with poor quality harness (76.4%). Another issue facing donkeys is the type and design of the implement they are hitched to (Gebreab et al., 1999; Itepu, 1999; Pearson et al., 1999). Many of the plows and carts were originally designed for oxen and are too large for the smaller donkey and often lead to decreased work output (Gebreab et al., 1999; Itepu, 1999; Pearson et al., 1999). In some cases donkeys have even been seen yoked to carts and plows; this is a common harnessing method that should only be used in oxen (Itepu, 1999). So, a major challenge is reaching extension officers, veterinarians, and engineers and educating them on the proper design and modification needed for harness and implements so donkeys can successfully work (Gebreab et al., 1999; Howe and Garb, 1999; Kidanmariam, 1999; Pearson et al., 1999).

2.9. Conclusion.

There are many answers yet to be found but the donkey can continue to serve as an important draught animal by decreasing labor for women and children in developing countries (e.g. Mali) and helping generate additional income. If donkeys are properly managed and harnessed, they have the potential to increase economic opportunities for smallholder farmers across the world. However, more research and educational tools are needed on how to properly care, feed, manage, harness and work donkeys in both developing and industrialized countries (Itepu, 1999; Sieber, 1999; Wells et al., 1999).

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CHAPTER III.

NUTRIENT MANAGEMENT FOR DONKEYS (*EQUUS ASINUS*)

INTRODUCTION

The word donkey is a common name used worldwide for members of the *Equus asinus* family. Most likely the domesticated donkey originated from two wild species of donkeys, the *Equus africanus*, *nubian* and *somalian* subspecies (Groves, 1974). Descendents of *Equus africanus somalian* can be found in the United States wandering the desert regions. These donkeys are often referred to as burros. Burro is a Spanish word for donkey (Burnham, 2002). These populations of wild donkeys, as well as many donkeys maintained as draught or draft animals in developing countries, are known to be highly adaptable feeders that will consume a variety of grasses in arid climates. The diets of the feral burros are quite similar to that of wild donkeys in Africa.

When considering nutritional requirements, it has been suggested that donkeys should not be considered a small form of a horse because of differences in the ways in which a donkey will select, consume, and digest feeds (Tisserand and Pearson, 2003). In general, donkeys are thought to be able to survive on less digestible feeds than a horse. Donkeys in developing countries are still able to survive and even perform work while eating diets high in fiber and low in nitrogen (Pearson, 2005; Svendsen, 1997).

Sneddon et al. (1997) and Sunvold et al. (1995) reported that the gastrointestinal transit time is slower in the donkey when compared to other monogastric animals, allowing for maximal fermentation and digestion of forages. A slower gastrointestinal transit time is also found in other desert mammals and could possibly increase short-chained fatty acid production, which would yield more energy and make it possible for them to thrive in these arid conditions (Izraely et al., 1989; Sneddon and Argenzio, 1997; Sunvold et al., 1995). The decreased rate of passage enhances fermentation and digestion of poorer quality forages (Pearson and Merrit, 1991; Mueller et al., 1994) as well as improves recycling of urea (Tisserand et al., 1991). The fiber source and type selected by the donkey is assumed to be improved in its nutritive value by remaining for a longer period of time in the cecum and ventral colon, as well as enhancing the water reservoir properties of the hind gut when the donkey is foraging for food for long distances without water (Sneddon and Argenzio, 1997). They can reach high levels of dehydration and still perform work while maintaining an appetite (Izraely et al., 1989; Yousef, 1991). When a donkey reaches a level of chronic or severe dehydration, morphological changes occur in the hindgut, specifically in the cecum and ventral colon, which enhance fluid retention and fermentation activity (Sneddon et al., 2006). Similar adaptations have been noted in other desert mammals that are also adapted to poor quality and high fiber diets such as camels (Buret et al., 1993; Svendsen, 1997). During times of dehydration, the donkey will maintain an appetite as well as consume its solid food before drinking water (Yousef et al., 1970). It will then consume a large quantity of water all at once - unlike the horse that will take several smaller drinks (Houpt, 1993; Yousef et al., 1972).

STUDY 1A.

INSULIN RESISTANCE IN STANDARD DONKEYS (*EQUUS ASINUS)* OF THREE BODY CONDITIONS- THIN, MODERATE, AND OBESE

3.1. INTRODUCTION

Donkeys and their hybrid offspring, mules and hinnies, have been used for thousands of years as draft animals. The domestication of draft animals has been key to the development of mankind and industrialization. Donkeys were first brought to the United States to produce mules for agriculture purposes but the population began to decline after World War II and the introduction of the tractor (Taylor and Ray2005). However, the donkey population in the United States has made a comeback. Donkeys have grown in popularity for riding animals, show purposes and for producing mules. The value of these animals has increased significantly and in some cases it has equaled or surpassed that of horses (Burnham, 2002). The increased growth in popularity of donkeys has created a demand from the donkey and mule industry, as well as the veterinarian and science communities, for increased knowledge with respect to the management and care of these animals.[1] Often the donkey has been considered as a smaller version of the horse but this is not necessarily true. For example, it has been reported that when donkeys are fed the same ration as a horse, a donkey will often become obese and increase the risk of laminitis (Burnham, 2002). The donkey, in fact, has been shown to differ in several ways in its general anatomy, physiology, pharmacokinetics, behavior, and nutrient requirements compared to its evolutionary relative the horse (Taylor and Ray, 2005; Burnham, 2002; Crane, 2007). Donkeys, like many ponies and certain types of horses, are often described as being “easy keepers,” being able to maintain or even put on weight when grazing relatively moderate pastures. Regional deposition of fat together with obesity, have been linked with an increased risk of insulin resistance in horses and ponies (Frank, 2007).

The Food and Agriculture Organization (FAO) estimates there are approximately 44 million donkeys and 10 million mules and hinnies worldwide and 95% of them live in developing countries (FAO, 2003). Many of the donkeys living in developing nations are faced with harsh environments where both food and water availability are scarce. In a donkey’s evolutionary habitat, such as a semi-arid environment (e.g. deserts in Africa), it will often browse over long distances on sparse, fibrous vegetation, and is capable of going for long periods of time without drinking water (Pearson, 2005). However, many donkey owners in industrialized nations tend to over-feed them – especially with diets high in nonstructural carbohydrates, e.g. lush pasture or cereal grains (Burnham, 2002; Hoffman et al., 2003). In horses, high starch rations have been shown to promote insulin resistance (Hoffman et al., 2003).

Overall, donkeys are thought to be able to survive on less feed and poorer quality feed (e.g. higher fiber and lower energy) than a horse while still maintaining body weight and even performing work (Pearson, 2005). Insulin resistance has been described as an adaptive response when energy is limited (Frank, 2007; Kronfeld et al., 2005; Jenkins et al., 1987). Donkeys, together with some breeds of horses and ponies, may have an adaptive ability to conserve energy, especially glucose, in times of negative energy balances due to expression of a “thrifty genotype” (Kronfeld et al., 2005; Jenkins et al., 1987; Nell, 1962). Diet and management may influence the extent of this insulin resistance (Hoffman et al., 2003; Kronfeld et al., 2005; Jenkins et al., 1987). The hypothesis for this work was: 1) that all donkeys, regardless of their body condition (BC), would be more insulin resistant than horses due to having lower nutrient requirements and a higher metabolic efficiency and 2) that BC would have a significant influence on the level of insulin resistance.

3.1.2. MATERIALS AND METHODS

Animals

Twelve standard donkeys (height at the withers from 101.6 to 142.2 cm) (Taylor and Ray, 2005) were chosen according to BC scores based on specific guidelines for body condition scoring donkeys (Pearson and Ouassat, 2000). Four donkeys were acquired for each of the three groups (thin, moderate, and obese) according to their initial BC: thin (T) BC 2.0 -3.0, moderate (M) BC 4.0 -5.0, and obese (O) 6.5 -8.5. Each group consisted of two jennies (females) and two jacks (intact males). All donkeys on day 0 were vaccinated for Influenza, Eastern/Western Encephalomyelitis, Tetanus Toxoid (Fluvac Innovator® 4, Fort Dodge Animal Health, Overland Park, KS) and dewormed with moxidectin (Quest® 2% Equine Gel, Fort Dodge Animal Health, Overland Park, KS). Donkeys were weighed with an electronic scale (Tru-Test, San Antonio, TX) on day 21. The donkeys were acclimated to a forage-based diet (pasture and hay only) for a three-week period before a frequently sampled intravenous glucose-insulin tolerance test (FSIGT) was performed. The donkeys were removed from pasture 14 to 16 hours prior to testing and individually placed in stalls. During testing, donkeys were offered ad libitum access to grass hay and water to avoid a fasting state and in order to better mimic grazing state on pasture (where they would have unlimited access to food and avoid a period where no food was available) (Hoffman et al., 2003). The study was approved by Michigan State University Institutional Animal Care and Use Committee.

FSIGT Sampling Protocol

Glucose and insulin dynamics were assessed via minimal model analysis. All donkeys were equipped with a jugular catheter 1 h prior to taking the first baseline sample at –60 min (Hoffman et al., 2003). During the 5-h duration of FSIGT, 35 venous samples were collected. Baseline heparinized samples were collected at -60, -45, 0 min, then 300 mg/kg BW glucose (Dextrose Solution 50%, Butler Animal Health Supply Co., Dublin, OH) was given I.V. within two min, and blood was sampled at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 min post (Hoffman et al., 2003; Treiber et al., 2005). At 20 min, 0.8 mIU kg/BW of insulin (Humulin® R, Eli Lilly and Co., Indianapolis, IN) was rapidly administered I.V. within 30 sec through the catheter followed by sampling at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, and 240 min. Samples were transferred immediately after collection to heparinized tubes (Vacutainer, Fisher Health Care, Chicago, IL). After each blood sample was collected, the catheter was flushed with 10 mL of heparin isotonic saline. Samples were placed in a refrigerator until centrifugation (3,000 x *g* for 10 min) within 20 to 30 min of collection. Plasma was removed immediately after samples were centrifuged and frozen at -4^{o} C. The plasma samples were later used for subsequent analysis for glucose and insulin. Throughout the procedure, blood glucose was monitored using a hand-held glucometer (MediSense

Precision Xtra; Abott Laboratories, Alameda, CA). Plasma glucose was analyzed by enzymatic assay (Autokit Glucose; Wako Diagnostics, Richmond, VA). Insulin was determined using an RIA (Coat-A-Count Insulin; Diagnostic Products Corp., Los Angeles, CA).

Minimal Model Analyses and Calculations

The basal resting insulin and glucose concentrations were used to calculate proxies of insulin sensitivity and ß-cell responsiveness for comparison with the values obtained using the minimal model (Treiber et al., 2005). The following parameters were calculated using MinMod Millennium and WinSAAM software (Bergman et al., 1997; Boston et al., 2003): insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg), disposition index (DI), basal glucose (Gb), and basal insulin (Ib). The following equation was used to determine glucose and insulin curves by using the MinMod Millennium software program and solving for three unknown parameters described below (Sg, p_2, and p_3): $G'(t)=-G(t) \times [Sg + X(t)] + Sg \times Gb$.[13] G'(t) was the net rate (mg dL^{-1} min^{-1}) of the change in plasma glucose concentration (mg/dL) at time (t or G(t)). Sg (glucose effectiveness) was described by one component of the plasma disposal rate (min^{-1}), which was the capacity of the cells to take up glucose without insulin mediation (this parameter was unknown). The parameter p_3 described the delivery of insulin to the interstitium. The parameter p_2 described the disposal of insulin from the interstitial fluid. X(t) was defined as the rate of change of the insulin action. X'(t) was the insulin action and it represented the insulin mediated component (min^{-1}) of the plasma glucose disposal rate via the acceleration of glucose uptake in response to an increment change in the insulin concentration by using this equation: $X'(t) = p_3 \times [I(t) - Ib] - p_2 \times X(t)$. The responsiveness of ß-cells to glucose was described by the acute response of insulin to glucose (AIRg, mIU/[L min]), which stood for the increase in plasma insulin above the basal concentration integrated from 0 to +10 min after the glucose bolus was administered.[14] The deposition index (DI) was determined from the product of AIRg and SI. This indicated the ß-cell response relative to the degree of insulin resistance in the tissue.

Statistical Analysis

Statistical analyses (SAS 9.0, SAS Inst., Inc., Cary, NC) were conducted using a 2-way ANOVA evaluating BC group, sex, and their interactions on AIRg, DI, Si, Sg, Gb, Ib, BW, BC, and age. Normality of the residuals was tested using Shapiro-Wilk test and by examining the normal probability plot. P-values less than 0.05 were considered to be significant.

3.1.3. RESULTS

The mean BW were 76.8, 192.5, and 213.3 ± 11.3 kg, the mean BCS were 3.6, 5.3, and 8.0 ± 0.3, and the mean ages were 9, 4, and 7 ± 3 yr for T, M, and O, respectively. During the FSIGT, donkeys did not become hypoglycemic, meaning the donkeys did not exhibit any physical signs of seizures or comas nor did their blood glucose levels monitored by the hand-held glucometer drop below basal levels (84 ± 22) at any point (Burnham, 2002). Although some donkeys did exhibit signs of increased respiration after the glucose bolus (administered at 0 min) and the insulin bolus (administered at 20 min). Using MinMod Millennium and WinSAAM software the following parameters were generated Si, Sg, AiRg, DI, Gb, and Ib from the basal glucose and insulin values to estimate insulin resistance.[14, 15] No significant effect of BC or sex was found on Sg, AIRg, DI, Gb, or Ib, and no effect of BC on Si. Mean values for Si for BC groups were: T = 4.9 x 10^{-4} $L{\cdot}mU^{-1}{\cdot}min^{-1}$ ± 0.8, M = 1.8 x 10^{-4} $L{\cdot}mU^{-1}{\cdot}min^{-1}$ ± 0.8, O = 1.5 x 10^{-4} $L{\cdot}mU^{-1}{\cdot}min^{-1}$ ± 0.8 (see Table 1.1). Despite the lack of differences between treatment groups, Si was lower (*p* = 0.03) in females versus males (1.19 x 10^{-4} $L{\cdot}mU^{-1}{\cdot}min^{-1}$ ± 0.7; 4.3 x 10^{-4} $L{\cdot}mU^{-1}{\cdot}min^{-1}$ ± 0.7) (see Table 1.2).

3.1.4. DISCUSSION

These initial results suggest that Si values for moderate and obese donkeys are within the range found in moderate and obese horses (1.28 to 3.32 x 10^{-4} $L{\cdot}mU^{-1}{\cdot}min^{-1}$) (Hoffman et al., 2003; Treiber et al., 2005). However, no literature was found to compare values for thin horses to the values found for the thin donkeys. Further work in more donkeys is required to confirm or refute the trend that obese donkeys are more insulin resistant than their leaner cohorts. Interestingly, in this study, gender had a significant effect with females having a lower Si and a higher AIRg, suggesting that they may be more prone to becoming insulin resistant. In addition, diet may affect the insulin sensitivity and glucose effectiveness when comparing this study to others where donkeys were fasted or fed grain when testing glucose tolerance (Forehead et al., 1997; June et al., 1992).

In general, donkeys are assumed to have a lower incidence of over-eating grain due to differences in glucose metabolism when compared to horses and ponies (June et al., 1992). During hypoglycemia or a negative energy balance in donkeys, the tissue is often reduced in insulin sensitivity (Forehead et al., 1997). The reduction in insulin sensitivity is likely to be a homeostatic mechanism that is responsible for maintenance of normoglycemia during a famine or fasting period (Forehead et al., 1997). One study reported that donkeys, when compared to horses and ponies, fed a diet consisting of hay and sweet feed had higher plasma glucose levels (114 mg/dL) for a longer period of time (June et al., 1992). In contrast, donkeys in this study were not fed grain but only forage. The results for this study reported basal glucose (Gb) values closer to the horses and ponies in the study (87 and 82 mg/dL) (June et al., 1992). Glucose rapidly declined in the donkeys over time. However, the insulin concentration, unlike previous reports in horses, peaked twice for all BC groups of donkeys (Hoffman et al., 2003; Treiber et al., 2005). Insulin aids in glucose transport activity by stimulating glucose transport proteins. However, when a donkey is in a famine state, the tissue becomes less sensitive to insulin and glucose is preserved for energy for vital organs and tissues. The dose of insulin used for this study was determined in pre-trial testing using two doses of insulin (0.4 mIU kg/BW versus 0.8 mIU kg/BW) on 2 donkeys. The objective was to use a dose of insulin that would provide a physiological dose and not a pharmacological dose that could induce hypoglycemia. A previous study had used a higher dose of bovine insulin (0.4 IU kg/BW) on fasted donkeys but some donkeys did become hypoglycemic (Forehead et al., 1997). Others had recommended 20 mIU kg/BW of human rDNA insulin for horses; however, no recommendations were found for donkeys (Treiber et al., 2005).

In conclusion, all donkeys, regardless of body condition, may have an adaptive ability to conserve energy when compared to horses. Donkey owners should consider the nutritional value and quantity of diets being fed to donkeys, especially female donkeys, to avoid obesity and metabolic conditions that could lead to adverse conditions such as hyperlipemia or laminitis (Burnham, 2002; June et al., 1992).

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Table 3.1.1. Glucose effectiveness (Sg), insulin sensitivity (Si), acute insulin response to glucose (AIRg), disposition index (DI), basal glucose (Gb) and basal insulin (Ib) reported as arithmetic means (± SEM) in donkeys of various body condition.

Variable	**Thin**	**Moderate**	**Obese**
Sg, x 10^3 min^{-1}	0.013 ± 0.002	0.011 ± 0.002	0.017 ± 0.002
Si, x 10^{-4} $L·mU^{-1}·min^{-1}$	4.9 ± 0.8	1.8 ± 0.8	1.5 ± 0.8
AIRg, mU/L·10 min	125 ± 53	193 ± 53	314 ± 53
DI, x 10^4	652 ± 239	182 ± 239	510 ± 239
Gb, mg/dL	89.9 ± 8.5	82.8 ± 8.5	97.4 ± 8.5
Ib, mU/L	3.3 ± 6.2	8.6 ± 6.2	17.8 ± 6.2

Table 3.1.2. Glucose effectiveness (Sg), insulin sensitivity (Si), acute insulin response to glucose (AIRg), disposition index (DI), basal glucose (Gb) and basal insulin (Ib) reported as arithmetic means (± SEM) in male and female donkeys.

Variable	**Males**	**Females**
Sg, x 10^3 min^{-1}	0.01 ± 0.002	0.017 ± 0.002
Si, x 10^{-4} $L \cdot mU^{-1} \cdot min^{-1}$	1.19 ± 0.7	4.3 ± 0.7
AIRg, mU/L·10 min	176 ± 43	245 ± 43
DI, x 10^4	246 ± 195	650 ± 195
Gb, mg/dL	87.2 ± 6.9	92.8 ± 6.9
Ib, mU/L	8.5 ± 5.1	11.2 ± 5.1

STUDY 1B.

COMPOSITIONAL ANALYSIS OF FEEDSTUFFS COMMONLY FED TO WORKING DONKEYS IN MALI, WEST AFRICA

3.2.1. INTRODUCTION

Donkeys in Africa live on sparse forages, whose nutrients will vary according to rainfall throughout the year. For example, donkeys in Botswana have been observed grazing native species of forages such as *Boscia foetida* (a native grass) and *Acacia* (native shrubs) even during the dry season when the nutrient value is at its lowest (Aganga and Tspospito, 1998). When compared to the horse, the donkey has shown itself to be more of a browser, and it uses different feeding strategies depending on the quality of the feed (Mueller et al., 1998). Donkeys have been observed eating bark when forages are scarce and even pulling away the bark to reach the more succulent part of the tree. The same is true when grazing in a pasture; the donkey will first consume the highest quality forages and then feed on lower quality forages. In general, donkeys are thought to be able to survive on less feed and lower quality feed than a horse, even when compared on a pound for pound basis. Donkeys will often consume more mature, less digestible, woodier plant material of poorer quality when compared to a horse. These forages are typically high in fiber and low in nitrogen. Donkeys are able to survive on poor quality forages and even perform substantial amounts of work (Pearson et al., 2005; Svendsen, 1997).

Donkeys used for draught animals in Mali, West Africa are fed a variety of feedstuffs including: cow pea bean hay, bourgoui grass, corn-sorghum stover, rice bran, rice hulls, rice straw, and sorghum grain. Additional information on each feedstuff could possibly lead to improved feeding recommendations for donkey owners. Improved feeding recommendations may help donkeys maintain a higher BCS during the dry period or during periods of extreme work such as the beginning of the wet season when crops must be planted. During this time period the donkeys are often in the worst body condition and deprived of energy but are faced with planting crops. Typically, the donkeys will not resume moderate body condition until the middle to end of the wet season. However, additional information about feedstuffs commonly fed to donkeys could lead to better recommendations on what to feed donkeys during this time period (planting season and the beginning of the wet season) to avoid a negative energy balance.

Compositional analysis of forages consumed by donkeys in Mali may result in a better understanding of nutrient content of these feedstuffs. Furthermore, increased knowledge about what the donkeys are eating may help connect the theory of adaptive traits, such as insulin resistance, and possibly lead to a better understanding of how donkeys can live through a feast and famine situation on such poor nutrition. The objective of this study was to analyze feedstuffs commonly consumed by donkeys in Mali and based on the results, develop recommendations for donkey owners to optimally utilize available feedstuffs.

3.2.2. MATERIALS AND METHODS

The following feed samples were collected and analyzed: 2 samples of cowpea bean hay (one sample from the SPANA office in Bamako and the other from the village of Segou); bourgoui grass from Mopti, Niono, and Djenne; rice bran, rice hulls, and rice straw from

Niono; and two varieties of sorghum ("stay green" and regular). All samples were collected in Mali and taken to the laboratory of Boubacar Dembele at the IPR/IFRA campus in Katibougou. The samples were then chopped to smaller pieces and double packed, labeled and placed in plastic bags. The samples were then brought back under APHIS permit for importation to Michigan State University Animal Science Department by Dr. John Staatz, Department of Agriculture Economics for analyses. These feed samples were analyzed for dry matter (DM), acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP) using the methods of Goering and Van Soest (1970). Alfalfa and timothy hay grown in Michigan were also analyzed for crude protein. These samples were not received during the time of the other analysis and therefore only crude protein was run on these samples. The samples were all ground to 2 mm using a Wiley Mill and stored in plastic sample cups for further testing. Duplicates were run on each sample. A total of 22 samples were tested in each trial with the exception of the CP trial where 26 samples were run.

NDF Analysis:

When testing for NDF, 0.50 grams of each feedstuff sample was placed in a 1,000 mL beaker. The samples were treated with 4 mL of amylase solution. Each sample was also treated with an additional 4 mL of solution consisting of 14 grams of concentrated Teramyl (10%) plus 126 grams of distilled water. Next, 0.5 g of sodium sulfite was then added to the samples in order to assist in breaking down the di-sulfide bonds. By breaking the di-sulfide bonds this can aid in the filtration process. The samples were then treated with an additional 2 mL of amylase solution. The feedstuff samples were rinsed with 100 mL of neutral detergent solution and then placed on a condensing heating rack for 1h after each sample reached the boiling point.

After the samples reached a boil, and if any residues were left on the sides of the beakers, the residue was rinsed with detergent. After boiling for 1h, the beakers were removed from the heating racks. The samples were then filtered through crucibles and treated with 2 ml of amylase solution to assist in degrading fiber. The samples left in the crucibles were then thoroughly rinsed with hot water to wash away the neutral detergent solution. Samples that were difficult to filter were also treated with water and acetone. When the detergent was washed thoroughly, the crucibles were placed overnight in a drying oven. The next day the crucibles were weighed for dry matter. Measured dried samples of ash and crucible resulted in the neutral detergent fiber values (NDF).

ADF Analysis:

Beakers (1,000 mL) were filled with 0.50 grams of each sample and treated with acid detergent solution. Acid detergent solution is made of sulfuric acid, reagent grade standardized to 1 N (49.01 g/L), cetyl trimethylammonium bromide (CTAB) technical grade (20 g/L) and sodium sulfite (anhydrous regent grade) (Goering and Van Soest, 1970). Each beaker was placed on a condensing heating rack and brought to a boil for 1h. After each sample was brought to a boil, the samples were removed from the heating wracks. The samples were then filtered through crucibles. Each sample was washed with acetone and hot water. The samples were then placed in a drying oven overnight. The samples were weighed the next day for acid detergent fiber values.

ADL Analysis:

Half a gram of each sample (0.5 grams) was weighed and placed in beakers. Samples were placed on heating racks and brought to a boil. After samples were boiled for 1h the samples were taken off of the heating rack. Samples were filtered through crucibles using hot water and acetone. Then samples and crucibles were placed in a drying oven overnight. Samples were weighed the next day for acid detergent lignin values.

CP Analysis:

Each sample, along with the inclusion of domestic alfalfa and timothy hay for comparative purposes, were analyzed for crude protein content (CP). All samples were weighed to 0.25 grams placed in hock flasks. A total of 4 mL of sulfuric acid (H2 SO4) was added to each sample and then the samples were allowed to digest overnight. After digesting overnight, each hock flask with the sample was placed on Dijesdahl burners (heating units) and vacuum system, which was heated to 440 degrees Celsius for 6 minutes. At 6 minutes, 10 mL of hydrogen peroxide (50%) was added to each hock. The hock flask were then continued to be heated until the solution turned clear and no residue was seen. Once the solution was no longer black in color and completely clear, each hock was then removed from the burner and allowed to cool. The heating and cooling process destroys the carbon bonds. When the hock flasks were cool to touch with bare hands and no gloves, distilled water was then added to the meniscus line and then shaken.

The samples were then left overnight. The following day each sample was filled to the meniscus with more distilled water or diluted to 100 mL, which reaches the "state of same volume." Then each sample was once again shaken and placed into small vials. The number on the hock flask was then written on the corresponding vial. Samples were moved to the small vials to decrease the difficulty of pipeting. From the small vials, 0.80 mL of each sample was transferred to centrifuged tubes. Next, 0.20 mL polyvinyl alcohol (PVA 0.1g/L) was added to each centrifuge tube and vortexed. The PVA adds protein in the solution as a carrier for the reagent and helps dilute the solution more. After this step each sample was then pipetted (0.80 mL) and placed on a plate and read by computer analysis. The line equation and y-intercept were recorded from computer-generated data from the plate. The information was used on a spreadsheet along with average dry matter, sample weight, sample dry weight (average dry weight multiplied by the sample average) information from the equation of the line to calculate crude protein and nitrogen percentages.

3.2.3. RESULTS

When comparing the dry matter content amongst feedstuff samples from Mali, rice hulls from Niono, were the greatest in dry matter (95.75%, 95.88% refer to table 1.1.2.). Dry matter was least in sorghum "stay green" variety (92.51, 93.78% refer to table 1.1.2.). In terms of NDF values for each feedstuff sample, rice hulls from Niono, Mali were the highest in neutral detergent fiber (71.04%, refer to table 1.2.2.) and sorghum grain "stay green" variety was the lowest (15.93%, refer to table 1.2.2.). Rice hulls from Niono, Mali were also the highest in ADF and ADL values (8.42%, refer to table 1.3.2.) when compared to the other feedstuffs and sorghum grain "stay green" variety was the lowest in ADF and ADL (1.31%, refer to table 1.3.2.). Cowpea Bean hay was the highest in crude protein (15.78, 16.05%, refer to table 1.4.2.) followed by sorghum grain "stay green" variety (10.86, 11.89%) and Bourgoui grass from Mopti, Mali was the lowest in crude protein (2.08, 2.10%, refer to table 1.4.2.).

3.2.4. DISCUSSION

After reviewing the commonly fed feedstuffs to donkeys in Mali, the data suggests that the feedstuffs vary greatly according to the type of forage and location where it is grown. Although at this time no statistical analysis has been conducted to say the results are significantly different. It has been suggested that the amount fed and type of forage fed can influence digestibility of forages by donkeys (Pearson et al., 2001). When looking at the NDF (neutral detergent fiber), this method first developed by Van Soest in the late 1960's separates the neutral detergent soluble from the nutrient available fibers (Goering and Van Soest, 1970). The NDF proportion contains cellulose, hemicellulose, and lignin, which are the structural carbohydrates of the plants. These components contribute to the plants being more fibrous and often times more mature. Donkeys are often observed consuming more fibrous forages when compared to horses.

For future studies, it may be beneficial to also analyze the nonstructural carbohydrate or water-soluble carbohydrate content in forages for interest in feeding donkeys with metabolic issues such as insulin resistance or Equine Cushing's Syndrome. Some researchers have suggested that donkeys have higher digestibility coefficients for crude fiber, crude protein, and organic matter, as a result of more effective microbial digestion in the hindgut (Tisserand et al., 1991; Izraely et. al, 1989; Suhartanto et al., 1992). The microbial cellulolytic activity in the cecum has been reported to be 13 percent higher in donkeys when compared to ponies when fed a diet of alfalfa hay or wheat straw (Suhartanto et al., 1992).

In reviewing the results for the various Malian feedstuffs, several such as cowpea bean hay, bourgoui grass from Djenne and rice bran appear to offer a better source of nutrition for the donkey when considering the crude fiber, crude protein and percent dry matter content. Currently, the NRC (2007) states there is a lack of information on protein requirements for donkeys. Izraely et al. (1989) was able to feed donkeys a diet that contained only 3% crude protein and the donkeys were able to maintain body weight. Also, the amount of nitrogen recycled into the blood stream exceeded the amount that was fed. The donkey's ability to retain and recycle urea contributes to its ability to live off of poor quality forages in arid environments. Mueller et al. (1994) suggested that crude protein requirements for the donkey range from 3.8 to 7.4 percent. In addition, donkeys may be able to better survive in desert-like climates, due to their ability to store water in their gastrointestinal tract, which may enhance water-holding capacity when consuming high fiber diets (Dill et al., 1980). However, additional research is needed in the area of high fiber diets and intestinal water holding capacity of the donkey to further support this hypothesis.

It has also been suggested that donkeys that are working such as those in Mali, who weigh on average 200 kg, should consume approximately 1.6 kg a day of poor quality forage and 2.4 kg of concentrate (NRC, 2007). However, the closest feedstuff to a concentrate that was being fed to donkeys in Mali was millet, wheat or sorghum bran, and sorghum grain. Unfortunately, millet and bran was not collected or sampled. Donkeys that were being rehabilitated at the SPANA donkey clinic were fed approximately half a kilo of millet and half a kilo of wheat bran per day along with free choice bean hay and corn stover. Other estimated nutrient intakes for adult donkeys consuming forage-based diets suggest that a 200 kg donkey should consume 2.5 kg of dry matter intake and receive approximately 9.43 Mcal/d (NRC, 2007). To better determine the best source of nutrition for donkeys in Mali, a study measuring dry matter intake (which has previously been reported to vary in donkeys from 1.75 to 3.1% body weight, NRC, 2007), apparent digestibility of nutrients such as using a marker to measure gastrointestinal transit time and even measuring energy needs and requirements of working and nonworking donkeys would be beneficial. Another important factor to consider when making feeding recommendations to Malian donkey owners is the season of the year and forage availability at that time of the year and location.

3.2.5. FUTURE STUDIES

There is still a need for additional research in nutrition and metabolism to better provide more information regarding nutritional needs and requirements of donkeys for owners worldwide. Additional studies could look at these commonly fed feedstuffs at different stages of maturity as well as collection of feedstuffs available during the dry period would be worthy of analysis.

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Table 3.2.1. Dry matter content of forages fed to donkeys in Mali.

Samples	% Dry Matter
Cowpea Bean Hay	94.78
Cowpea Bean Hay SPANA	94.34
Bourgoui Djenne, Mali	94.55
Bourgoui Mopti, Mali	94.91
Bourgoui Niono, Mali	94.64
Rice Bran Niono, Mali	92.63
Rice Hulls Niono, Mali	95.75
Rice Straw Niono, Mali	95.88
Sorghum grain	93.78
Sorghum grain "stay green"	92.51
Standard (Corn Silage)	92.33

Table 3.2.2. Neutral Detergent Fiber (NDF) values of forages fed to donkeys in Mali.

Sample Name	*% NDF*	*Average % NDF*
Cowpea Bean Hay	31.15	**32.20**
	33.24	
Cowpea Bean Hay SPANA	48.19	**47.85**
	47.50	
Bourgoui Djenne, Mali	38.46	**37.26**
	36.05	
Bourgoui Mopti, Mali	66.78	**67.00**
	67.21	
Bourgoui Niono, Mali	62.98	**63.10**
	63.19	
Rice Bran Niono, Mali	57.15	**55.98**
	54.80	
Rice Hulls Niono, Mali	71.04	**71.04**
	71.04	
Rice Straw Niono, Mali	67.49	**67.54**
	67.59	
Sorghum grain	20.80	**20.44**
	20.07	
Sorghum grain "stay green"	16.13	**15.93**
	15.71	
Standard (Corn Silage)	45.33	**45.02**
	44.71	

Table 3.2.3. Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) values of forages fed to donkeys in Mali.

Sample Name	% ADF	% ADL	Average % ADF	Average % ADL
Cowpea Bean Hay	12.5	2.83	12.31	
	12.11	2.95		2.89
Cowpea Bean Hay SPANA	19.37	3.98	19.33	
	19.29	4.19		4.09
Bourgoui Djenne, Mali	13.35	2.62	13.31	
	13.27	2.38		2.5
Bourgoui Mopti, Mali	16.94	2.57	17.28	
	17.62	1.99		2.28
Bourgoui Niono, Mali	16.36	2.75	16.36	
	16.36	2.68		2.72
Rice Bran Niono, Mali	20.61	6.11	20.71	
	20.81	6.19		6.15
Rice Hulls Niono, Mali	26.62	8.49	26.71	
	26.8	8.35		8.42
Rice Straw Niono, Mali	20.06	2.48	20.32	
	20.58	2.94		2.71
Sorghum grain	4.96	2.25	4.63	
	4.3	1.58		1.92
Sorghum grain "stay green"	3.75	0.94	3.88	
	4	1.68		1.31
Standard (Corn Silage)	12.9	1.41	12.71	
	12.52	1.37		1.39

Table 3.2.4. Percent nitrogen and percent crude protein values of forages fed to donkeys in Mali

CP Measurements	%N	%CP
Cowpea Bean Hay	2.53	15.78
	2.57	16.05
Cowpea Bean Hay SPANA	1.39	8.66
	1.38	8.63
Bourgoui Djenne, Mali	0.53	3.33
	0.53	3.33
Bourgoui Mopti, Mali	0.33	2.08
	0.34	2.10
Bourgoui Niono, Mali	0.52	3.25
	0.58	3.64
Rice Bran Niono, Mali	0.73	4.55
	0.73	4.54
Rice Hulls Niono, Mali	0.58	3.60
	0.69	4.32
Rice Straw Niono, Mali	0.47	2.91
	0.45	2.79
Sorghum grain	2.02	12.62
	1.96	12.24
Sorghum grain "stay green"	1.74	10.86
	1.90	11.89
Standard (Corn Silage)	1.28	8.02
	1.29	8.03

CHAPTER IV.

EVALUATING LEARNING THEORY IN DONKEYS (*EQUUS ASINUS*) WHILE MEASURING HEART RATE VARIABLITY AND BEHAVIOR WHEN TEACHING DONKEYS TO DRIVE TO A CART*

4.1. INTRODUCTION

Since equine have been domesticated there has existed a need to direct and manage them, but often the methods to train and harness their power have been inhumane (Sevilla and Leon, 2007). The donkey is believed to be the first member of the *Equidae* family to be domesticated around 3,000 B.C. in Egypt (Ruder, 2004). There are an estimated 44.3 million donkeys worldwide and 95% of them are kept for work in developing countries (FAO, 2005). Many working equids are often faced with overloading, beatings and whiplashes (Ramaswamy, 1998). Methods used to train equine throughout the world are often reflections of a culture's attitude toward animal welfare (Waran et al., 2007). The term "training" can be defined as the processes where human handlers introduce the equine to new situations and associations (Waran et al., 2007). Many researchers consider the donkey to be the most neglected and abused draught animal in many countries and attempts to learn more about training donkeys to drive without the use of a whip or stick have not previously been researched (de Aluja and Lopez, 1991; Blakeway, 1994). Donkeys and mules are considered to be unique creatures with special qualities and different behavior when compared to horses. Some claim they should not be treated like horses with long ears (Burnham, 2002). Many times their stoic and cautious behavior has been misunderstood as being stubborn (Burnham, 2002; Taylor and Matthews, 2002).

In many cases these animals have been treated more harshly than necessary. Such treatment can be observed among many donkey owners in both industrial and developing countries. In Mali, West Africa, veterinarians that work for the Society for Protecting Animals Aboard (SPANA) treat many donkeys for lacerations caused by drivers overusing sticks to encourage the donkeys to move forward. The lacerations sometimes become infected and often become scars that are direct results of harsh treatment (Diarra et al., 2007; Svendsen, 1997).

For thousands of years, donkeys have been used as a means of transportation (Svendsen, 1997). Some claim that their willingness has been badly exploited (Svendsen, 1997). Donkeys tend to make good driving animals due to being less “flighty” than most horses (Miller, 2007; Weaver, 2008). In general, donkeys are thought to be easy to train and to work (Nenogomasha et al., 2000). These characteristics make the donkey a likely choice for many children and women to use in developing countries (Nengomasha et al., 2000). However, little research has focused on the behavior of the donkey or its acceptance of training methods. Research and implementation in these areas would likely improve working conditions for donkeys throughout the world.

In a pilot study evaluating learning theory in donkeys, we evaluated how donkeys would respond to learning a novel task, crossing a tarp, with negative reinforcement, positive reinforcement and/or luring (Heleski et al., 2008). That study’s objectives were to examine the application of learning theory to donkeys and to record their ability to complete a novel, potentially frightening task and compare different training methods for accomplishing this (Heleski et al., 2008). The study’s rationale was that increased knowledge about training donkeys could improve current training techniques. The enhanced training techniques could then be shared with donkey owners and handlers. Post-hoc testing also provided an alternative method for motivating donkeys to cross a novel object. Donkeys that failed to cross the tarp with positive reinforcement, negative reinforcement or luring were successfully encouraged across when a “donkey motivator” was used. A donkey motivator was a longe whip with a piece of plastic sheeting attached to the end. The motivator was shaken behind the donkey when approaching the tarp but physical contact with the donkey was not made.

The current study focused on comparing two training techniques for teaching donkeys to drive, Halter Training Method (HTM), and Traditional Stick Method (TSM). Cardiac response was measured during this study to measure a physiological response to perceived stress. The objective of the study was to compare the two training methods and then switch the treatments after ten days to measure if donkeys could be retrained to drive with the opposite method. It was expected that donkeys trained with HTM would show more favorable responses to the behavioral assessment measures, especially the human approach test, have less variability in their heart rate, and would perform the driving test with fewer mistakes generating a higher score in a shorter amount of time when compared to the donkeys trained by the TSM. A second objective was to use the data collected in this study as a tool for teaching donkey owners, drivers, students, and veterinarians in Mali, West Africa an alternative training method for teaching donkeys to drive.[1]

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4.2. MATERIALS AND METHODS

2.1 Animals and Training Protocols. The study tested two training methods with 10 standard donkeys (measurement at the wither 101.6 to 142.2 cm tall, Taylor and Ray, 2005) from Cross Roads' Donkey Rescue in Clare, Michigan. The sex, age, and body condition score were recorded for each donkey. The donkeys were all geldings and kept at two separate farms (Farm 1 and Farm 2) 4.8 km apart. The average age for all donkeys was 7.3 yrs; 8 yrs for HTM (ranging from 3 to 13 yrs) and 6.6 yrs for TSM (ranging from 3 to 10 yrs). The average body condition score for all donkeys was 6. The donkeys were randomly divided into two groups, Group A, (n=5) and Group B (n=5). Each group was assigned to a treatment, Treatment HTM or Treatment TSM (see figures 1 and 2). The daily order for each training session was randomly drawn. After day 10, all donkeys were moved to HTM due to safety reasons (i.e. with these healthy, well-fed donkeys, the TSM donkeys showed too much inclination to run away when hitched to the cart; from a handler perspective, it was simply unsafe to continue with the TSM treatment).

Group A was initially assigned to HTM and Group B was initially assigned to TSM. Each group of donkeys was individually worked for approximately thirty minutes for ten days (five consecutive days in a row and then two days off, then five more consecutive days). Both groups were worked in a round pen completing novel ground handling tests (such as learning to stop and move forward with voice commands, learning to longe at the walk and trot) for the first five days. Days six through 10 the donkeys were hitched and driven to a two-wheel cart (see figure 3). Days 11 through 14 all donkeys were worked on the ground with HTM. Days 15 through 18 the donkeys were hitched and worked to the cart using only HTM.

Treatment 1, HTM. Group A were equipped with a halter and a set of driving lines (two cotton ropes attached on either side of the halter). The donkeys were guided by gently tugging on the lines in the direction in which the driver wished to go (right line was pulled for going to the right and left was pulled for going to the left). The voice command “Gee” was used when turning to the right and “Haw” was used when turning to the left. To encourage the donkey to move forward, both lines were tapped on the donkey’s sides (approximately in the rib cage area) and the voice command “walk” was used. When stopping the donkey, both lines were pulled back and the voice command “Whoa” was used. The pressure was released when the donkey stopped (see Figure 1 and 2, Treatment 1, Halter Training Method, HTM).

Treatment 2, TSM. Donkeys in Group B were equipped with a halter for safety, as well as a place to place the heart rate monitor watch, and a stick with plastic sheeting tied to the end (referred to as a donkey motivator) was used. After day 1 two driving lines were attached to each side of the halter (for safety) and were used along with the “donkey motivator.” Initially, though, the halter and lines were not used; they were simply attached in case of an emergency. The donkey motivator was used as a guiding tool. The donkey was gently tapped with the motivator on his hindquarters from the point of hip to the rump and occasionally the rib cage. The donkey was encouraged to move right by shaking the motivator on the opposite side, the left side, and the voice command “Gee” was used. When asked to move to the left, the motivator was raised and shaken on the right side and the voice command “Haw” was used. In order to get the donkey to move forward in a straight line, taps were applied to the croup area to encourage the donkey to move forward and the voice command “walk” was used. In some instances the motivator was raised directly over the dorsal area of the donkey and shaken and contact was avoided when possible. The voice command “whoa” was used when asking the donkey to stop and the stick was lowered to the driver’s side (see Figure 3 and 4, Treatment 2, Traditional Stick Training Method, TSM).

2.2 Behavioral Assessment and Heart Rate Variability Assessment.

On day 1, behavior parameters were recorded for each donkey. The following parameters were measured, recorded and videotaped: 1) general attitude (e.g. alert, apathetic, avoidance), 2) response to observer approaching the donkey’s neck (e.g. no response, friendly approach, avoidance, aggression), 3) walk around the donkey (e.g. no response, moves away, tucks tail, aggressive), 4) ear test (e.g. allows ear to be touched, tolerates, avoids ear touch), 5) response to unfamiliar person (e.g. approaches, no approach, spooks) (Based on previous work by Burn et al., 2008, Hausberger et al., 2008, Pritchard et al., 2005).

The measurements were repeated on days 10, 11, and 18 with the exception of test 5) response to unfamiliar person. Test 5 was replaced with test 6) response to a familiar person (e.g. approaches, no approach, spooks) (Hausberger et al., 2008). During the training session, the heart rate variability was measured for the duration of the session. Heart rate samples were taken on days 1, 3, 7, 10, 11, 13, 15, and 18 (Matthews et al., 1998) using a Polar Equine RS800 G3 heart rate Wearlink W.I.N.D. Transmitter (Polar

Electro Europe BV, Fleurier, Switzerland). The monitor was placed around the heart girth of the donkey. Ultrasound transmission gel (Aquasonic 100, Parker Laboratories, Inc., Fairfield, NY) was applied to the belly and withers of each donkey near the probe on the heart rate wearlink. The heart rate watch receiver (Polar Watch Receiver, Polar Electro Europe BV, Fleurier, Switzerland) was attached to the left side of the halter. Each training session lasted approximately 30 min (Matthews et al., 1998). The heart rate data stored on the heart rate watch receiver was downloaded via a Polar Interface (Polar Electro OY, Polar Electro Europe BV, Fleurier, Switzerland) to a laptop computer. We elected to use the same HR measurements (MHR, SDRR, LF, etc.) that other equine researchers have found useful (Rietmann et al., 2004, Visser et al., 2002.)

Two observers reviewed videotapes of each donkey during the behavioral response tests on day 1. Observer 2 reviewed videotapes of each donkey for day 10 and day 18 during the behavioral response tests. Inter-observer reliability was assessed via recommendations of Martin & Bateson (1993).

2.3 *Driving Test.*

On days 10 and 18, the driving test was given to each donkey in each group (see Table 1 Driving Test). The donkeys were video taped and scored accordingly for their performance using a 5-point scale (see Table 2 Driving Test Scoring System). During the driving tests depending on the donkey's training progress, some were driven to the cart and others were hand-line driven (driven without the cart, and guided by the driving reins attached to the sides of the halter) depending on the amount of progress the individual had made over the training period. If it was deemed unsafe to drive the donkey while in the cart, the subject was hand line driven. The driving tests were administered at two different farms due to the location of the donkeys. This study was approved by the Michigan State University Animal Care and Use Committee (#04-09-067-00), East Lansing, Michigan.

2.4 Statistical Analysis

Statistical analysis was conducted using SAS V9.2. The logistic regression model, PROC GLIMMIX, was used for statistical analysis when comparing the behaviour parameters in relation to the treatment group, Group A (always Treatment 1, HTM) and Group B(Treatment 2, TSM). This model was also used for comparing time (before day 11 and after day 11) for testing behaviour responses for group A (always Treatment 1, HTM). The Spearman rank correlation test was used when testing behaviour responses for between-observer reliability, observer 1, observer 2 and first author's scores for day 1, 10, and 18 were compared. A logistic regression model was used when studying the behaviors for donkeys in Group A (HTM) before day 11 and after day 11 to test for a time effect. ANOVA analysis was used to analyze the heart rate variability parameters before day 11; mean heart rate (MHR), standard deviation of the average RR intervals calculated over short periods (SDRR), square root of the mean squared difference of successive r-r interval (rMSSD), low frequency (LF) and high frequency (HF). Time effect, before day 11 and after day 11, was measured for the HR responses for Group A (HTM). Time was modeled as the fixed factor in the ANOVA model for MHR, SDRR, rMSSD, LF, and HF. Heart rate responses for all donkeys in both groups (A & B) before and after treatment change in Group B (Treatment 2, TSM changed to Treatment 1, HTM after day 10, Group A remained in the same treatment, HTM for all 18 days) for all test days (1, 3, 5, 7, 10, 11, 13, 15, and 18) were compared using the ANOVA model. The residuals for each model (treatment/sequences) were not normally distributed and log transformation was used for MHR, rMSSD, LF and HF. The driving test times and test scores were analyzed using ANOVA analysis. The treatments were fitted as a fixed effect while farm 1 and 2 were fitted as a random effect in the ANOVA model. When analyzing Group B donkeys driving tests on day 10 and 18 (after treatment changes), normality assumption was met but there was an unequal variance for each treatment. A heterogeneous variance ANOVA model was used and tested for treatment effect on the driving times for donkeys in Group B (testing time before and after treatment change from TSM to HTM after day 11). Test times for Group A (treatment never changed from HTM) on day 10 and day 18 were compared using a heterogeneous variance ANOVA model. Normality assumption was met but there was an unequal variance for treatments. Pearson correlation was used to test the score and driving times for all donkeys. When comparing scores for Group A and B as a fixed effect and farm as a random effect, the logistic regression model was applied.

4.3. RESULTS

3.1 Effect of Behavioural Responses among Treatment Groups

When measuring behavioural responses for Group A (always HTM) and Group B (initially TSM) on day 1, the results indicated that there was no significant difference in treatment effect for all behaviours responses; response to unfamiliar person (p = 0.99), to observer approaching neck (p = 0.88), walking around donkey (p = 0.41), and ear test (p = 0.40). When measuring the relationship of behavioral response scores over time for

Group A (always HTM) before day 11 and after day 11, there was no significant difference in response to unfamiliar person (p = 0. 30), to observer approaching neck (p = 0. 09), walking around donkey (p = 0.12), and ear test (p = 0.97).

3.2 Observer Reliability among Recording Behavioral Responses

Observer 1 and 2's observations were compared for each behavioral response on day 1. A significant correlation was found for observer 1's and 2's scores for behavior response to unfamiliar person (p = 0.02). When comparing observer 1's and 2's scores for response to observer approaching the neck, the correlation was significant (p = 0.01). For the behavioral response to walking around the donkey, a high correlation was found for both observers' scores (p = 0.01). Both observers' scores and the first author's scores were compared for day 1 (see Table 3). Observer 2 and the first author's scores were compared for day 10 and 18. When comparing scores from observer two and the researcher for response to familiar person, the scores were highly correlated (p = 0.006). Both observer 2 and the first author had highly correlated scores for walking around the donkey (p = 0.001). There was a low correlation for both the observers' and researcher's scores when recording the ear test response (p = 0.40).

3.3 Effect on HR variables among Treatment Groups and Time

Heart rate variability readings were taken on day 1, 3, 5, 7, 10, 11, 13, 15, and 18. When comparing heart rate variability responses for both treatments before day 11 no significant differences were found (see Table 4). When comparing both groups of donkeys for all test days, significant differences were found for rMSSD and HF (p-value=0.01, 0.02 respectively) (see Table 4). When measuring time effect for Group A (donkeys that always remained in Treatment 1, HTM for the entire study) no significant differences were found among HRV responses for this group before or after day 11 (see Table 4).

3.4 Driving Test Performances and Times among Treatment Groups

The driving tests were conducted at two farms, Farm 1 and Farm 2 on day 10 and day 18. There was no significant difference in the test time for driving test 1 at farm 1 or farm 2 ($p = 0.57$) on day 10. When studying the treatment effect and driving test times for donkeys in Group A, always treated with HTM (Treatment 1), there was no significant difference between driving test time 1 or 2 ($p = 0.62$). When measuring the random effect of farm 1 versus farm 2 for donkeys in Group A (always treated with Treatment 1, HTM), there was no significant difference ($p = 0.64$). Driving times before and after the treatment change for donkeys in Group B (Treatment 2, TSM from day 1 to 10, then switched to Treatment 1, HTM from day 11 to 18) were checked and no significant treatment effect was observed ($p = 0.40$). When comparing test scores for all driving tests on both dates at each location there was no significant difference found (correlation coefficient= 0.11, $p = 0.63$). When only testing test scores for Test 1 there was no significant differences ($p = 0.17$) and no significant treatment effect found for Group B (TSM) ($p = 0.11$) or Group A (always HTM) ($p = 1.00$) for test scores on both days donkeys completed the driving tests.

Our most significant finding, though, was that in just ten days, 7 of our 10 initially naïve donkeys were trained to drive to the cart. By day 18, an additional donkey (8 of the 10) was driving. Two of our donkeys, though, still had strong fear responses to driving to the cart. One of these two did work well in the round pen and may have made improvements if being worked in a more controlled arena type environment. The other donkey would have required more work and time before being able to be hitched and safely driven to the cart. Due to our interest in testing a training theory that can be implemented in developing parts of the world, this quick response to training for the majority of our animals was an important finding.

4.4. DISCUSSION

This study suggests that donkeys can be taught to drive with only a halter and reins and/or a halter with reins plus a donkey motivator in a relatively short time frame. Seven of the ten subjects were successfully hitched and driven to the cart within the first ten days and 8 donkeys were hitched and driven to the cart within 18 days. In general, most donkeys after day 3 in the round pen seemed to move forward less willingly and required more encouragement from the handler. This might be considered a habituation response. The donkeys appeared to be desensitized from moving away or forward from the first author moving towards their shoulder and encouraging forward movement from the hindquarter. Most donkeys on day 6 were quiet to hitch for the first time and displayed little signs of flight. The temperament of each donkey may have affected his or her success to drive. Temperament is defined as an individual's basic stance towards continuing changes and challenges in its environment (Mason, 1984). Future studies should consider temperament evaluations as this study noted donkeys that displayed more signs of flight proved to be more difficult to hitch and drive safely. Furthermore, donkeys that were exceptionally flight orientated, meaning they would often bolt and run in fear versus stopping and refusing to go, proved to be less trainable in the short amount of time. Temperament has also been used when attempting to place donkeys in foster homes (French 1992). Not all donkeys will work in certain environments and situations, so French (1992) designed a test to evaluate temperament before placing donkeys in their new homes. Temperament can relate to management and the animal's ability to achieve a specific goal, e.g. a donkey driving to a cart (Visser, 2002).

At both farms a pasture was used for training and conducting the driving test. It is possible that the donkeys' training may have improved in a more controlled environment such as an arena, however, 8 of the 10 donkeys within 18 days were pulling a cart. In addition, the idea was to drive donkeys in Group B (TSM, Treatment 2) with no halter and only guided by a stick as seen in Mali, West Africa. The conditions present in Mali could not be replicated in Michigan in the short period of time and within the appropriate ethical constraints of an IACUC. Many of the donkeys seen driving without halters or bridles in Mali are traveling down a straight path, e.g. a road that they travel on a routine basis, and they are often over loaded, undernourished and dehydrated (Diarra et al., 2007). The donkeys in Michigan were all in good physical condition and well fed. Also, we did not know the length of time it takes a Malian donkey driver to train a donkey to work with only a stick. It is not uncommon to see additional donkeys tied with a rope around their necks to the shaft of a cart. Some claim this is a training exercise and others claim the donkey is hitched for additional pulling power. However, this could aid in training and teaching the donkeys to drive without a bridle or halter.

In order to measure stress associated with the treatments, both behavioral measurements and heart rate readings were taken. This study showed a highly correlated observer reliability score between the first author, observer 1 and 2 when measuring most behavioral responses. Other studies have also used observer reliability tests to test repeatability of behavior scoring (Burn et al., 2009). In this study there were some differences between observer 2 and the researcher's scores when scoring the ear test. The ear test is applicable to the mule and donkey industry as often times an ear shy equine can be dangerous to work with and the shyness is often blamed on abusive type treatment (e.g. twitching or harshly twisting ears). However, in order to measure such a response a stronger correlation on scoring needs to be developed.

Heart rate readings were taken during the training session to attempt to measure stress associated with the two training methods. Stress can be defined as a threat, real or implied, to the psychological or physiological integrity of an individual (McEwen, 1999). To accurately measure stress responses proves to be more difficult as different physiological systems are involved, and it requires multiple simultaneous responses measured at once (Heard, 1991). Heart rate variability parameters have been used when measuring behavioral responses and to make correlations among stressors in horses (Rietmann et al. 2004). Donkeys that drove well tended to have an increase in their heart rate when first placed in the cross ties before being hitched. Though we often perceive an increase in heart rate as representing a stress response, perhaps the increased heart rate actually represented a positive anticipation response (von Borrell et al., 2007). Behavioral responses of our donkeys would support this possibility. When the donkeys' heart rate would increase to well over 150 beats per minute when first placed in the cross ties, it tended to decrease once brushing began or the harness was placed on the donkeys. Other studies in horses support the concept that brushing tends to decrease heart rates of nervous animals (Feh and Mazieres, 1992). No physical signs of increased respiration or signs of fear, such as attempting to bolt, were noted. No differences in heart rate variability parameters MHR, SDRR, LF and HF were found between the two groups with the exception of the square root of the mean squared difference of successive beat-to-beat interval (rMSSD, p= 0.04). Visser (et al. 2002) compared mean HR to SDDRR and rMSSD at base line and in response to various stimuli. In this study baseline heart rate values were not taken separately from training sessions and physical activity can create a variation in HR and nonmotor HR (Rietmann et al., 2004). The resting heart rate in donkeys has been reported to be similar to that of horses, approximately 41 bpm (Matthews et al., 1998; Mueller et al., 1994; Rose et al., 1991). Rietmann et al. (2004) has indicated that measuring LF and HF allows for a more precise measure of mental stress at low-level exercise than relying simply on mean HR (average of the beats per minute over time being tested), SDDR or rMSSD (which focus on beat to beat intervals or the log of the beat to beat interval). rMSSD estimates the high-frequency activity of the parasympathetic nervous system (Visser et al., 2002). The vagus vein is a parasympathetic nerve and if an animal is stressed, this nerve may reduce in activity therefore, causing the animal to be less likely to cope with the stressor (Rietmann et al., 2004). Visser (et al. 2002) has reported that horses that have a low degree of parasympathetic nerve activity are more likely to be mentally stressed compared to those who have high activity. Therefore, by measuring the heart rate, which measures the net effect of the vagus and the variability between the beats, the vagal and sympathetic activity can be measured. Since the donkeys did show a difference in rMSSD this could suggest a difference in temperaments as well as a change in their environment that increased stress or excitement (e.g. new person watching or donkeys moving close by, or other donkeys being fed treats). Visser (et al. 2004) suggested that the difference in the rider's response to a change in the environment could have played a role in the horses that had a lower rMSSD. Therefore, a change in environment could have influenced the researcher's response and possibly played a role in the donkeys (primarily in Group B, TSM) who had a lower rMSSD over time.

When donkeys were first handled, e.g. caught and haltered or placed in cross ties before hitching, the handler did observe that the heart rate would increase to 60 to 180 bpm and then after initial brushing or placing the harness on the donkey(s) the heart rate would drop within a few seconds to 30 to 35 bpm for most donkeys. Visser (et al. 2002) reported in her study that horses would also show an increase in MHR due to sudden excitement or fear up to 110 bpm and this was accredited to a decrease in parasympathetic nerve activity. Donkeys that tended to exhibit signs of being tense or bolted with the cart or when being hand line driven (driven only with the reins attached to the halter and not hitched to the cart) had a constant heart rate of 80 to 100 bpm throughout the grooming, harnessing, and training process. Similar findings with horses suggest that slow alterations in heart rate are mediated by both the sympathetic and parasympathetic nerve activity and may be associated with a prolonged fear response (Friedman et al., 1998; Visser et al., 2002).

In order to quantify which training method HTM vs. TSM was more successful in teaching donkeys to pull a cart, each donkey performed a driving test. The test was administered twice to account for the change in treatment for Group B (TSM) to the HTM after day 10. The test used to evaluate driving performance was modeled after a driving course commonly used in donkey driving classes at donkey and mule shows in the U.S. The three donkeys that scored the highest scores for the driving tests were in Group A (always HTM). Even though the driving test scores or times were not significantly different for the two training methods, it is still encouraging that donkeys can be trained to pull a cart with only a halter with reins and successfully complete a driving test. It is not uncommon to see harsh bits and extreme methods being used when teaching donkeys and mules to drive or ride. This study suggests that donkeys can be taught to drive and even perform a skillful driving test without harsh bits or training methods in a short amount of time.

4.5. CONCLUSION

This study suggests that naïve donkeys can be trained in a short period of time (less than 30 days) with proper application of learning theory to pull a cart while using only a halter and driving lines. Despite the fact that two donkeys (n=10) did need more time and training before successfully being hitched to the cart, this could be related to these donkeys' individual temperaments and/or their past history with humans. Visser et al. (2002) suggests that findings in horses according to the individual's temperament may affect HRV and possibly suitability to be trained to perform novel tasks such as riding or the ability for a donkey to learn to pull a cart. Thus, a donkey's temperament, social system and past interactions with humans may influence their overall ability to be trained to drive as well as their HRV. However, considering the majority of the donkeys did learn to drive in a short period of time, this study has the potential for application in developing countries when teaching and training donkeys, donkey owners, and para-professionals alternative methods for training donkeys to pull carts.

Footnote:

[1]Information and data collected in this study regarding donkey behavior and training was later used in a study in Mali, West Africa examining ways to improve donkey driving and training methods. Donkeys are still used as a primary source of draft power in Mali but they are often subjected to harsh training techniques and working conditions.

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FIGURES

Figure 4.1. and 4.2. Treatment1, Halter Training Method (HTM). The donkey is equipped with a halter and two driving lines are attached to either side of the halter.

Figure 4.3. and 4.4. Treatment 2, Traditional Stick Training Method (TSM). The donkey is also equipped with a halter and two driving lines attached to the side and a donkey motivator (a longe whip with plastic sheeting attached to the end) is being used to help guide the donkey.

TABLES

Table 4.1. Driving Test Procedures (NASMA, 2007-8). This was a standardized test that was used as a way to assess each donkey's driving ability and as a method to compare driving ability among treatment groups. Each test was video taped, timed, and scored. Times and scores were compared for each treatment group in order to establish which method was more successful in teaching a donkey to drive.

Obstacles:	***Directions***
Serpentine 	Donkey walked through 3 cones, the cones were set 152.4 cm apart, each cone had a tennis ball on top to ensure that when the donkey drove around the cone, it was steering and was responsive as per the driver's instructions and did not bump a cone thereby knocking off the tennis ball
U-Turn 	Six poles in a "U" shape, one pole in the middle, the sides were 457.2 cm and the middle was 609.6 cm long and pole in the middle was 243.8 cm long to be performed at a walk, 3 tennis balls were placed on the middle pole
Straight and Narrow 	2 parallel poles set 30.48 cms apart and 762 cm long, three tennis balls placed on each board

Table 4.2. Driving Test Scoring System. This system was used to numerically score each driving test and then compare test scores according to treatment groups and which method was more successful in teaching a donkey to drive.

Score	Performance	Description
1	Poor	Being led by handler, not hitched to cart, relies on guidance from handler when being driven by driver with only the reins, and the handler must keep a tight lead on the donkey while remaining close to its head and shoulders.
2	Fair	Being driven and guided by the driver with only the driving lines attached to the halter, not hitched to the cart, but still being led by handler with slack in the lead line, no guidance from handler, handler is positioned behind shoulder or hip, or hitched to cart but tight lead, driver not in cart
3	Average	Hitched to cart but lead line still attached, handler walking with slack in line and behind shoulder or hip
4	Good	Hitched to cart, no one leading, but handler walking close by, donkey turns and stops with little resistance when asked by the driver.
5	Very Good	Moves out freely when hitched to cart, no assistance from handler, no one leading, responds to voice commands, turns and stops easily with no resistance.

Table 4.3. Observer Reliability for observer 1, 2, and first author's Behavioral Response Scores on Day 1 to video taped responses for unfamiliar person, observer approaching neck, walking around the donkey, and ear test.

Behavior Response	**Correlation Coefficient**	***p* - Value**
To unfamiliar person	-0.64, -0.64	0.04[a], 0.4[b]
To observer approaching neck	0.80, 0.91	0.008[a], 0.000[b]
Walking around donkey	0.32, 0.51	0.35, 0.12
Ear Test	0.82, 0.09	0.006[a], 0.80

[a] Significant correlation ($p < 0.05$) for observer 1 and First Author's behavioral response scores

[b] Significant correlation ($p < 0.05$) for observer 2 and First Author's behavioral response scores

Table 4.4. Heart rate and heart rate variability responses for Group A (always HTM, Treatment 1) and Group B (TSM, Treatment 2) before day 11, before and after day 11 with Group A, and all test days (1-18) for both Groups A and B, treatments 1, 2, and 1,1.

Time	**Group**	**Treatment**	**MHR[a] (bpm)**	**SDRR[b] (ms)**	**rMSSD[c]**	**LF[d] (n.u.)**	**HF[e] (n.u.)**
Before Day 11							
	HTM	1	97.2	272.2	178.54	13066.65	11627.51
	TSM	2	66.6	298.7	194.46	14648.49	13488.42
***p* - Value**			0.22	0.13	0.08	0.22	0.21
Before and After Day 11							
Before Day 11	HTM	1	94.8	269.1	109.2	4561.37	4473.31
After day 11	HTM	1	72.9	210.4	93.4	4198.01	4350.53
***p* - Value**			0.74	0.13	0.73	0.98	0.69
All test days (1-18)							
	HTM	1, 2, 1, 1	83.8	239.8	101.3	4379.6	4411.9

	TSM	1, 2, 1,1	77.5	252.4	81.1	3764.6	3764.6
***p* - Value**			0.39	0.22	0.01[f]	0.06	0.02[f]

[a] Mean Heart Rate (MHR)

[b] Standard Deviation from Interval-Interval (SDRR)

[c] the square root of the interval to interval (rMSSD)

[d] Low frequency (LF)

[e] High frequency (HF)

[f] Significant difference ($p < 0.05$) for all test days and treatments between: rMSSD and HF.

CHAPTER V.

IMPROVING WORKING DONKEY (*EQUUS ASINUS*) WELFARE AND MANAGEMENT IN MALI, WEST AFRICA*

5.1. INTRODUCTION

The Malian economy depends heavily upon agriculture and animal husbandry (Diarra et al., 2007). Mali is considered one of the ten most resource-poor countries with the per capita gross domestic product (GDP) averaging $420. Agriculture comprises 32.9% of their annual GDP ($7.3 Billion) and occupies over 70% of the workforce. Small shareholder farmers dominate the agricultural sector with subsistence farming of cereals, sorghum, millet and maize covering over 1.4 million hectares (3.4 million acres) (http://www.state.gov/r/pa/ei/bgn/2828.htm). The agricultural economy depends heavily upon the donkey. Donkeys are used especially for traction (96 %) and occasionally plowing (Diarra et al., 2007). They perform many duties from transporting commodities and people to the market, hauling garbage or simply carrying water and fire wood (Diarra et al., 2007). Despite their importance, the donkey has received little attention in terms of research (Starkey, 1994). In some cases the poorest of farmers cannot afford a donkey and must rent one or work for someone who does own donkeys.

Worldwide there are an estimated 52 million donkeys, mules, and hinnies[1]; this is similar to the number of horses, which is estimated at 55 million (FAO, 2006). A majority of these animals are found in developing areas of the world (Herbert, 2006; Starkey and

Starkey, 2000). Approximately 27 million working equids are found in Africa and nearly 2 million of these are Malian donkeys (Herbert, 2006; Personal communication with Dr. Amadou Doumbia).

The donkey has increased in popularity in West Africa due to extended periods of drought that have made it harder to feed and care for oxen. However, many of the same implements once used for oxen are now being used with donkeys, and this has created problems such as oversized carts and improper harness (Starkey, 1994). Unfortunately, due to a myriad of problems, donkeys are often unable to work or unable to work at their potential. Diarra et al. (2007) reported that most donkeys receive little medical care and problems go largely untreated (n =100; 72%). The loss of a donkey or the time that a donkey cannot work creates many hardships for the people it serves.

Diarra et al. (2007) carried out a survey with over 2,500 donkeys to identify reason(s) why donkeys cannot work. The survey indicated that most donkeys were equipped with poor harness (n=2,0330; 76%), traveled long distances (> 20 km/day) (n=2,086; 79%), worked many hours (> 6 hrs/day, n = 1,782; 82%), carried/pulled loads over 500 kg (n=1,344; 51%), and were provided with inadequate nutrition (Diarra et al., 2007). Pearson et al. (1999) has also indicated a need for further understanding on donkey management and working practices (e.g. harnessing and training); furthermore, she indicates that problem areas can often be overcome through better training and education. For example, harsh training methods can be avoided when training donkeys to perform novel tasks from crossing a tarp to pulling a cart (Heleski et al., 2008; McLean et al., in preparation). The decrease in harsh and abusive type training methods plus improved nutrient management has great potential to improve the welfare and longevity of working donkeys. The correct application of learning theory, such as appropriately using training techniques like negative reinforcement (e.g. applying pressure on the right driving rein and releasing the pressure when the donkey responds and turns right) that has been used by Heleski et al. (2008) could be taught to donkey owners/drivers. Correctly using a halter or bridle for driving may decrease the use of sticks and subsequent beating of donkeys that is commonly observed in developing countries.

An earlier study conducted with ten donkeys in the U.S. showed that donkeys could be trained to pull a cart with only a halter and reins or a halter, reins and a donkey motivator in a short amount of time (in less than 20 days)[2]. The motivator was shown to work effectively in previous studies to motivate the donkey to move forward without adverse affects (Heleski et al., 2008, McLean et al., in preparation). Half of the donkeys in this study were driven with a halter, reins and donkey motivator, instead of a stick as seen in Mali (McLean et al., in preparation). Within just ten days, most of the donkeys (70%) were pulling a cart (McLean et al., in preparation).

In the current study we conducted three experiments and a welfare assessment. When examining how to train donkeys to pull carts, it was our hypothesis that donkeys trained to drive with a halter and reins would exhibit less signs of behavioral and physiological stress. Our second experiment focused on assessing another major problem plaguing many working donkeys, inadequate harness and carts. We hypothesized that donkeys with a good quality harness hitched to a well-balanced cart would have less pressure applied to their withers/back and therefore be subjected to fewer lesions of the withers/back. The third experiment focused on testing the donkey management knowledge of students enrolled in an agricultural preparatory high school and an agricultural college. We hypothesized that increasing education and awareness to paraprofessionals and professionals (e.g. "train the trainers" workshops) who work closely with donkeys and their owners would be important to improving long term working donkey welfare. In addition, a welfare assessment was taken in order to measure current management practices and to assess the welfare of working donkeys in conjunction with a SPANA monthly mobile clinic in Segou, Mali. This is an area with a large number of donkeys on market days.

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5.2. MATERIALS AND METHODS

5.2.1. Assessing the driving method, halter (HM) versus stick (SM), related to behavioural assessment, heart rate variability (HRV), and pressure associated with harness and cart quality (see Fig. 1, Fig. 2, and Fig. 3).

5.2.2. Animals. Ten Malian donkeys owned by two owners and driven by 10 hired drivers were tested. Donkeys were driven with both a halter and line(s) (HM) or only with a stick/donkey motivator (SM). Four donkeys were driven in the HM manner and six were driven in the SM manner. All donkeys were sexed and aged, and this information was recorded. Any signs of scars and lesions were noted. The average age for

HM group was 8.6 years and SM group 9.25 years. All donkeys were intact males (jacks).

5.2.3. Materials and Methods. Before beginning the driving test, each donkey was given a behavioural assessment test. The following parameters were measured 1) general attitude (alert or apathetic), 2) response to observer approaching the donkey's neck (no response, friendly approach, avoidance, aggression), 3) walk around the donkey (no response, moves away, tucks tail, aggression), and 4) ear test (allows ear to be touched, tolerates, avoids ear touch), 5) response to unfamiliar person (approaches, no approach, spooks), (Burns et al., 2008, Hausberger et al., 2008, Pritchard et al., 2005). After the behavioural assessment, each donkey was then fitted with a Polar Equine RS 800 G3 heart rate monitor (Polar Electro Europe BV, Fleurier, Switzerland). Ultrasound transmission gel (Aquasonic 100, Parker Laboratories, Inc., Fairfield, NJ) was applied to the girth and whither areas of the donkey near the location of the electrode strips on the heart rate monitors. Pressure film (Extreme Low Pressure 4 LW Fuji Film, Tokyo, Japan), approximately 21 cm x 31 cm, was placed over the withers of the donkey, underneath where the harness back pad would be placed. The donkeys were then harnessed and hitched to their respective carts. The donkeys were driven for five minutes. The harness and cart were graded as satisfactory or unsatisfactory based on the following criteria (criteria developed by the authors along with information from Chadborn, 2008; Davis, 2008; Davis, 2006; Jones, 2008): satisfactory harness had soft-nonabrasive material for the back padding and collar, unsatisfactory harness had abrasive material for back padding and or collar; a satisfactory cart had balanced shafts, shafts that came to the point of the donkey's shoulder, inflated tires and was balanced over the axles;

an unsatisfactory cart had one or more of the following problems: uneven/unbalanced shafts, shafts that were shorter than the point of the donkey's shoulders, flat tires, unbalanced over axle (placing a majority of the weight on the shafts). Some donkeys were equipped with double padding under the saddle/backband and this was noted. When the donkeys were finished driving, the harness was removed and the film was photographed, and labeled: right, left, and front, then removed. After returning to the U.S., the film was scanned (Imager Scanner II, Amersham Bioscience, Piscataway, NJ) and analyzed for intensity of the dots by using ImageQuant TL Software (Amersham Bioscience, Piscataway, NJ). Film was photographed as a precautionary step since it was not known how the high temperatures and humidity would affect image stability.

5.2.4. Welfare Assessment of current working conditions of donkeys in Segou, a representative village in Mali with a large working donkey population

Survey Protocol. A welfare assessment was held in Segou in conjunction with a monthly scheduled SPANA mobile veterinary clinic at the Ecole Secondaire Agro Pastorale (E.S.A.P.), a technical school for teen aged students studying agriculture (see Figure 5.7 and 5.8). The clinics are held traditionally on market day so donkey owners are already coming to town and SPANA can serve the most owners at once. The assessment was taken during the clinic to record the number of donkeys that were being driven with halters versus driven with sticks, donkeys' body condition scores (1 to 5, Donkey Sanctuary 2007), color, sex, age, branded or not, with or without lesions/lacerations, severity of lesions/lacerations (superficial, subcutaneous, severe/bone or multiple layers of tissue showing) location of lesions/lacerations, location of scarring and lameness (0= sound, 1=irregular gait, 2= not bearing weight on limb) and hydration status. A behavioral assessment test measured the responses to the following parameters: 1) general attitude (alert or apathetic), 2) response to observer approaching the donkey (no response, friendly, spooks, aggressive), 3) response to observer approaching the donkey's neck (no response, friendly approach, avoidance, aggression), 4) walk around the donkey (no response, moves away, tucks tail, aggression), and 5) ear test (allows ear to be touched, avoids ear touch) (Burns et al., 2008, Pritchard et al., 2005). The Michigan State University Animal Care and Use Committee, number 04-09-067-00, East Lansing, MI approved all experiments and testing procedures.

5.2.5. Testing Para-professional's Knowledge and Skills on Donkey Husbandry

Materials and Methods. Two "train the trainer" sessions were conducted in Mali. The first session took place in Segou at the Ecole Secondaire Agro Pastorale (E.S.A.P). Fifty-four students (males = 45, females = 9) were included in the study and varied in their level of study from first year to fourth year students (see Figure 5.9 and 5.10). Each student was given a pre-assessment exam about donkey management and welfare. A 45 min seminar was then presented to the students and translated into French by a local translator. Our translator, Boubacar Dembele, is an animal science professor at the Rural Polytechnic Institute for Training and Applied Research (I.P.R./I.F.R.A.) of Katibougou, University of Mali. A 30 min hands on demonstration with a donkey was conducted after the seminar (see Figure 5.11). The students were shown how to tell the donkey's age, how to correctly use methods of restraint, how to identify parts of the hooves, how to clean the hoof, importance of grooming and cleaning the area where the harness is placed, and how to properly harness and hitch a donkey. The students were then re-tested with the same exam. After completing the session each student was presented with a certificate of completion.

A second instructional session was conducted in Bamako, Mali at the Higher Institute of Training and Applied Research (I.S.F.R.A.), University of Mali, with 28 college students (27=males, 1=female) enrolled in the Animal Science program (see Figure 5.12 and 5.13). These students were given a longer pre-assessment exam prior to the presentation. The presentation was written in French and verbally translated by Professor Boubacar Dembele. A practical demonstration was conducted after the seminar. The students at the college were shown how to age donkeys (by viewing their teeth), restraint methods, how to pick up and clean a hoof, importance of cleaning the back and belly of the donkey prior to harnessing, and instructions on hitching and cart design, and how to use the donkey motivator (see Figure 5.14 and 5.15). A driving demonstration was also conducted while measuring the heart rate (Polar Equine RS800 G3, Polar Electro Europe BV, Fleurier, Switzerland) of the donkey. The students were then shown the heart rate variability results after the driving demonstration. Lastly, the students were re-tested with the earlier exam. These students also received certificates of completion at the end of the day's sessions. At both schools, students were allowed a question and answer period. Also, each student received a copy of the Basic Husbandry Manual for Donkeys, written by the authors, which had been translated into French. Electronic copies of this manual (in English or French) are available by request.

5.2.6. Statistical Analysis. The logistic regression model, PROC GLIMMIX, was used for statistical analysis to model the relationship between various behaviors and driving treatment (halter, HM or stick, SM). The ANOVA model was used when testing the relationship between treatment and heart rate variability data. Normality of the residual and equal residual variance was checked. The logistic regression model was used when testing the pressure data in relation to the type of harness, cart, and weight. The average intensity was the predicted variable. The Pearson Correlation Coefficient was used to test body condition score and age. The logistic regression model was used to test the relationship between body condition score and age, sex, lameness, scarring, hydration, color and lesions. Body condition score, behavior responses, and hydration correlation were tested using PROC GLIMMIX logistics regression model. The mean and standard deviation were obtained for each sex and variable: age, brand, body condition score, number of lesions, and number of scars. A generalized linear model approach (Proc GLIMMIX, SAS, V9.2) was used to determine the significant effects of body condition score, age, and hydration in relationship to general attitude, response to unfamiliar people, and ear test. Normal probability plot and Shapiro-Wilk test were used to test normality for the exams administered at both schools. Since the scores were not normally distributed, Wilcoxon signed rank test was used to test the difference in scores in the two schools. The *p*-value for significance was *0.05.*

5.3. RESULTS

5.3.1. Effect of behavioural responses among driving treatment groups

When measuring behavioural responses for HM group and SM group the results indicated that there was no significant difference in treatment effect for all behaviours (see Table 1).

5.3.2. Effect on heart rate variability among driving treatment groups (HM vs. SM)

Heart rate samples were taken on the day of testing at the SPANA clinic in Bamako, Mali. The donkeys had been either trained to drive with the halter method (HM group) or with the stick method (SM group). There was no significant difference in HRV parameters for either group (see Table 2).

5.3.3. Testing back and wither pressure associated with harness and cart quality in working donkeys

A significant difference was found when measuring the average intensity of pressure placed on the donkeys' withers when equipped with unsatisfactory quality harness and carts with 400+ kg of weight (harness type: $p = 0.02$, weight: $p = 0.009$). Unsatisfactory quality harness and heavier loads resulted in increased pressure as verified by the Fuji pressure film. There was no significant difference in average pressure intensity when testing extra back pad padding or type of cart (satisfactory vs. unsatisfactory) (extra padding: $p = 0.23$, cart type: $p = 0.27$) (see Table 3, Fig. 4, and Fig. 5).

5.3.4. Assessment results of general condition of working donkeys in Segou

During the welfare assessment, we recorded many conditions related to donkey welfare, such as the donkey's age, body condition score (BCS), coat color, hydration status, number of lesions, scars, and lameness. A behaviour assessment test was conducted with each donkey examining its general attitude, response to an unfamiliar person, response to the observer walking around the donkey and an ear test. In general, most donkeys appeared alert (44 of 46), 25 approached the unfamiliar person (9 did not and 20 spooked out of 54), and 34 allowed their ear's to be touched during the ear test (12 tolerated touch and 7 avoided their ears being touched out of 53). When examining age in relationship to behavioral responses there was no correlation among general attitude ($p = 0.49$), response to an unfamiliar person ($p = 0.56$) and the ear test ($p = 0.60$). There was no correlation to body condition score and behavioral responses with the exception of the ear test ($p = 0.03$).

The mean and standard error were calculated for body condition score (BCS), age (years), and hydration status. In general, most donkeys were considered thin (BCS 2.3± 0.70, n = 41), averaged 6.9 ± 3.9 years of age (n = 53), and considered to be hydrated (1.29 ± 0.46, n = 44, 1 = hydrated, 2= dehydrated). When comparing the relationship of hydration to age and body condition score, there was no significant effect when comparing hydration status to age ($p = 0.07$) but there is a significant effect when comparing hydration status and body condition score ($p = 0.01$).

The results indicated that donkeys that were in better body condition tended to be less dehydrated than those who were thinner. When testing the relationship between the body condition score and hydration, the results suggest it was more likely for a donkey with BCS 1 to be dehydrated than hydrated (95% confidence interval for the odds ratio, 0.013, 0.623, $p = 0.01$). When comparing the relationship between body condition scores to age, sex, lameness, scarring, color, and lesions there were no significant effects.

The results suggest that there maybe a relationship between age and hydration but not a significant effect ($p = 0.07$). The data suggests that the older a donkey was, the less likely it would be dehydrated.

5.3.5. Measuring current donkey management knowledge among para-professionals

The pre- and post-examination results from both schools, the Higher Institute of Training and Applied Research (I.S.F.R.A.), in Bamako and at the Ecole Secondaire Agro Pastorale E.S.A.P in Segou did not show a significant increase in test scores, even though there was a numerical increase in each case. In Segou, the test scores were not normally distributed and when using the Wilcox Signed Rank Test, there was no significant improvement in test scores ($p = 0.15$). The distribution of tests scores was normally distributed at the I.S.F.R.A. Annex in Bamako. When using the t-test, the increase in average test scores at the Annex did not significantly increase when using the significance level at 0.05 ($p = 0.06$). The average exam scores did numerically improve (E.S.A.P. pre-exam average = 80.72, post- exam average= 84.18; I.S.F.R.A. pre- exam average = 88.11, post-exam average = 92.70).

5.4. DISCUSSION

5.4.1. Effect of behavioural responses among treatment groups

We had expected to see greater differences in behavioral responses between donkeys driven with the halter method versus donkeys driven with the stick method. However, it should be noted that only two individuals owned the donkeys used in this training study (n=10) and these owners hired drivers for each donkey. These donkeys were in good physical condition, as compared to the general Bamako population, and no lesions or scarring were observed. It is possible that this population was above average in care and physical condition and subsequently indicated less signs of behavioural stress associated with either treatment.

5.4.2. Effect on HRV among driving treatment groups HM vs. SM

Some researchers have claimed that monitoring and identifying stress in equine can be difficult to assess (Herd, 1991, Miller, 2001). The difficulties arise in measuring the response in several physiological systems, e.g. neuroendocrine and cardiovascular systems, along with behavioural responses. Stress can be defined as a threat, real or implied, to the psychological or physiological integrity of an animal (McEwen, 1991). Animals dealing with stressors exert behaviors known as “coping” but when an animal can no longer cope with the stressor, then stress-related behaviors and problems are often exhibited (Stauffacher, 1992). If an animal is stressed for a long period of time, then the sympathetic nervous system along with chronically elevated or depressed adrenocortical functions can become harmful to the animal (Kelling and Jensen, 2002). Heart rate variability can provide more information of both individual stress and the magnitude of an actual stress response (Porges, 1995). Our study suggested that no significant differences were found in heart rate variability when looking at mean heart rate, standard deviation from beat to beat interval (SDRR), the square root of interval to interval (rMSSD), high frequency (HF, parasympathetic tone) or low frequency (LF, sympathetic tone) when comparing two different training methods, HM versus SM. Several studies have shown a decrease in heart rate variability in horses during exercise on a treadmill (Thayer et al., 1997; Physick-Sheard et al., 2000; Voss et al., 2002). Since our study was measuring heart rate during exercise this could have reduced the HRV. Also, it is possible a difference may have been noticed in these parameters if taken over multiple sampling periods and ideally taken at rest samples. Researchers have shown that heart rate can vary according to diurnal variations (morning versus afternoon), environmental conditions (e.g. ambient temperature, relative humidity, and wind speed), physical conditions of the donkey (e.g. age, breed, nutrition status), and fatigue (Ayo et al., 2008; Matthews et al., 1998; Minka and Ayo, 2007; Yousef and Dill, 1969). Ayo et al. (2008) indicated that donkeys in Nigeria during the rainy season had a mean resting heart rate of 36 to 72 bpm over a ten-hour period. The peak in heart rate was noted around 15:00 hrs (Ayo et al., 2008). Ayo et al. (2008) reported the temperature varied from 34.7 to 38.7°C and the relative humidity was 76.0% at 15:00 hours. The conditions in the Ayo et al. (2008) study were similar to the conditions in this study, 14:00 hrs, 32.7°C and 89 % humidity with the exception of measuring the heart rate while donkeys were exercising. Minka and Ayo (2007) reported the mean heart rates for donkeys (n = 10) after packing 93.9 ± 1.5 kg for 19.1 ± 0.6 km to be 71.0 ± 3.4 bpm. Our study recorded similar mean heart rates for donkeys exercising at 14:00 hrs, 73 ± 4.04 (HM group) and 71.75 ± 9.87 (SM group) bpm.

Another factor when comparing the different driving treatments (HM vs. SM) with HRV was the sample of donkeys used for this test were in good physical condition as compared to many of the other working donkeys we observed in Mali. More variations in HRV may have been seen in the SM group if application of the stick was used differently. Traditionally, it is not uncommon to see drivers hitting the donkeys with the stick repeatedly, which often leads to lesions. However, what we observed with our SM group was the drivers often waved the stick over the hindquarter of the donkey to encourage him to move forward and contact was avoided. Though several possibilities exist, it is likely that the drivers did not want to actually hit the donkeys while we, the researchers, were watching. It is possible that testing more donkeys from a broader background over several sampling periods may have shown more differences in heart rate variability.

Further studies looking at HRV as an indicator of stress should consider the donkey's conditioning history as well as monitoring fatigue. Matthews et al. (1998) examined the physiological response of donkeys driving in a hot (29 to 34°C) and humid climate and the effect of conditioning. They found that the heart rate while working after a month of conditioning was lower. Also, the resting heart rate values decreased significantly after donkeys had been conditioned.

5.4.3. Pressure response of harness and cart quality on the withers of working donkeys

Harness development and design have long been a problem for working equids in developing parts of the world (Connan, 2008; Davis, 2008b; Herbert, 2006). It is widely accepted that most debilitating injuries are due to poor harnesses (Davis, 2008b). Many researchers have reported a high proportion of poor harnesses, which cause lesions and scarring, found amongst donkeys working in Africa (Chadborn, 2008; Diarra et al., 2007; Herbert, 2006, Pearson et al., 1999; Starkey, 1994). Veterinarians may treat as many as 60% of their total caseload that has been caused by ill-fitting or poorly constructed harnesses (Davis, 2008a). Veterinarians are then left to make harness recommendations, and many claim that they lack the knowledge to properly do so (Davis, 2008b). Davis (2008a) claims that work production can be increased 20-25% with a properly fitting harness. Other work related problems include the design of the cart and weight of the load being carried by the donkey.

This study suggested that pressure is associated with the type of harness and weight carried by the donkey. Part of the mission of many organizations and researchers is to educate owners and drivers on how to properly harness working equid. During this study, the owners and drivers were shown the pressure film after each driving test as evidence of the pressure response to harness and cart type (see Figs. 4, 5, 6). In addition, this information was shared with students at both schools. Improving harnessing methods, such as padding used under the saddle of the harness, has the potential to decrease pressure applied on the withers and back and increase the longevity of a working donkey. In addition, not overloading donkeys and maintaining the carts, such as by keeping the tires inflated, can also decrease pressure placed on the withers. Many researchers have engaged in harness making workshops throughout Africa to show owners how to make affordable harnesses (Davis, 2008a; Connan, 2008; Chadborn, 2008; Jones, 2008b). Teaching veterinarians and paraprofessionals how to build low cost harnesses as well as well-balanced and well-maintained carts has tremendous potential for enhancing the welfare of working equids. Part of this study examined the current knowledge of paraprofessionals and professionals in Mali on donkey management including how to properly harness, train and work donkeys. Future workshops and research should focus on these key individuals who can share the knowledge with many owners for years to come.

5.4.4. Welfare assessment of working donkeys in Segou

The assessment provided some insight into current management conditions of donkeys in the Segou area of Mali. When SPANA first began working in Mali, the average work life for a donkey was 2 years (personal communication, Dr. Amadou Doumbia, 2008). The mean age for donkeys in the Segou area during this study was 6.9 ± 3.99 years. Therefore, it appears that through the efforts of SPANA's treatment and education programs that the longevity of the donkey has increased. Diarra et al. (2007) found only

9.9% (n = 73 of 736) of the Malian donkeys to show signs of lesions, but this survey indicated 39.5% (n= 19 of 48) of the donkeys had lesions. In terms of lameness, this survey indicated that 15% (n= 8 of 52) of the donkeys exhibited an irregularity in gait and 1.9% (n=1 of 52) showed signs of not being able to bear any weight on a limb. Diarra et al. (2007) had found 43% (n = 320 of 736) of the donkeys to be lame. The survey suggests that lameness has possibly decreased and the number of lesions has increased. This study also provided some insight into current donkey welfare indicating that lower body condition scores were often associated with donkeys that were dehydrated. Body condition score also had a significant affect on the behavioral test, ear touch. The donkeys with a BCS of 2 and mostly 3 avoided having their ears touched where donkeys that scored a 1 allowed touch.

5.4.5. Measuring current donkey management knowledge among para-professionals

Starkey (1994) has reported an increase in the use of donkeys in sub-Saharan Africa in the last ten years. However, Starkey (1994) reports there is a shortage of information relating to donkeys in this area. Diarra et al. (2007) also reported that many owners were unaware that donkeys could be treated for injuries or disease, or at least were unaware of the free treatment options at local SPANA clinics. To our knowledge, there is just one donkey science course taught at I.S.F.R.A. in Bamako, Mali by Dr. Amadou Doumbia, Director of SPANA, and this may be the only course taught throughout Mali, despite the prevalence of donkeys throughout the region. Dr. Doumbia and his SPANA team work on a monthly basis with E.S.A.P., a secondary school in Segou, Mali. Thus both populations of students we worked with and tested were more likely to be exposed to donkey husbandry knowledge than the average student enrolled in an institution in most developing countries (or elsewhere in Mali). Many groups advocate training owners on how to properly harness their donkeys as well as care for them. However, we believe reaching those who will continue to work as professionals and para-professionals has tremendous potential to reach even more owners versus concentrating efforts on only training owners. Based on the starting test scores of the two groups of students tested in this study, their baseline donkey knowledge appeared to be fairly high and therefore significant increases in test score averages were not seen. Nonetheless, students were enthusiastic about attending the seminars and the hands-on workshops and followed up with many excellent questions on the material. It is important to point out that very few women were enrolled in either school and yet women often work the donkeys. There was only one-woman student (out of 28) in the I.S.F.R.A. training session and nine (out of 54) at E.S.A.P. in Segou. The females at both schools had less practical knowledge than the male students; for example, the females could not tie a common knot referred to as a slip or safety knot, yet all of the male students knew how to do this. Therefore, reaching more women and children who are often responsible for working donkeys would likely be tremendously beneficial.

5.5. CONCLUSION

This multi-part study examined several key areas that have the potential to improve overall working donkey welfare in developing parts of the world: 1) testing donkey driving methods and their relationship to donkey stress, 2) measuring pressure associated with harness, carts, and cart load 3) surveying current conditions and assessing welfare of working donkeys in Segou, Mali, and 4) implementing donkey management and welfare education in colleges and secondary schools in an effort to test the efficacy of "train-the-trainer" workshops. Improved training methods were explored and the adoption of the donkey motivator was well received by the SPANA veterinarians, drivers, and students at the two schools. Follow up work to assess whether the motivator is continuing to be used will be important. The study suggested increased use of the halter for driving donkeys could potentially improve donkey-owner interaction.

This study, as with previous studies with working equids, showed that improved harnesses are greatly needed.   The welfare assessment provided more insight about general donkey management and conditions in the Segou area. In regards to the final experiment, assessing the knowledge of donkey management among agricultural and veterinary technical students, even though a significant difference in test scores was not shown at each school, the enthusiasm displayed by all involved indicated a keen interest in donkey management and welfare. Many of the students were impressed with the introduction of the donkey motivator as well as with learning how to properly handle and restrain donkeys. Many nutrition and management questions were asked at both workshops. Knowing in advance the current level of knowledge when designing the tests would have been beneficial. However, continuing to train those who will live and work in developing countries about working equids is vital to the well being of working equids. These individuals have the potential to reach many donkey owners for many years,

5.6. FUTURE IMPLICATIONS

It is likely that donkeys will continue to be very important resources in developing parts of the world. Enhancing their welfare subsequently improves the well being of the families that the donkeys provide for and should be emphasized. There is a severe lack of information on how best to implement proactive strategies for enhancing working equid welfare. New strategies and sharing of information can all be implemented with minimal or no cost increases to resource-poor donkey owners and drivers. Future nongovernmental organizations and research institutions should focus on training individuals who will continue to live and work in these developing countries. Adding curricula to secondary schools, agricultural colleges and veterinary institutions on donkey husbandry and management could have a major impact on donkey welfare.

Footnotes

[1] Hinnies- a hybrid cross between a male horse (stallion) and female donkey (jenny).

[2] Donkey Motivator- a longe whip or driving stick with plastic sheeting or a plastic bag attached to the end used to encourage the donkey to move.

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FIGURES

Figure 5.1. Halter Method. Donkey is being driven with the halter method. The halter is the rope device placed around the donkey's head and nose. This is being used as a method to control and guide the donkey when pulling the cart.

Figure 5.2. Stick method. Donkey is being guided and driven with the stick method. The donkey moves away from the contact made by the stick.

Figure 5.3. Donkey motivator method. Donkey is being driven with no halter and the donkey motivator. The donkey motivator is a stick with a plastic bag tied to the end. It is a low cost implement that can replace the stick. The donkey responds to the sound of the motivator and moves away versus moving away from the contact made by the traditional stick method.

Figure 5.4. Extreme Low Pressure 4 LW Fuji Film showing low intensity results. This film was used with a good cart and harness. The film shows very little pink coloring. The pink color is a response to pressure placed on the film. The less pink indicates less pressure placed on the withers and back of the donkey. This film sample is from a donkey equipped with good harness and a good cart.

Figure 5.5. Extreme Low Pressure 4 LW Fuji Film showing higher intensity results. This film was used with a cart with 400 kg of maize (corn) loaded on it. The film shows pink concentrated in the front, near the withers.

Figure 5.6. Wither lesion caused by unsatisfactory harness and padding.

Figure 5.7 and Figure 5.8. Donkey owners participating in welfare assessment in Segou.

Figure 5.9. E.S.A.P. student class that participated and completed the donkey husbandry and welfare teaching seminar and demonstration.

Figure 5.10. Students from E.S.A.P. in Segou participating in the teaching workshop lecture session.

Figure 5.11. Students from E.S.A.P. in Segou participating in the teaching workshop hands on demonstration.

Figure 5.12. and Figure 5.13. Students from I.S.F.R.A. participating in the in classroom lecture on donkey husbandry and management

Figure 5.14. and Figure 5.15. Students from I.S.F.R.A. in Bamako participating in the practical demonstration.

TABLES

Table 5.1. Behavioural Response Group A (HM, n = 6) and Group B (SM, n = 4) in Malian donkeys at the SPANA clinic in Bamako, Mali.

Behavioural Response	*p* -Value
To unfamiliar person	0.94
General Attitude	0.97
To observer approaching neck	0.97
Ear Test	0.97

Table 5.2. Heart rate variability (HRV) responses for donkeys (n=10) in HM group or SM group on test day at the SPANA clinic in Bamako, Mali, West Africa (significant at *p*-Value < 0.05).

	MHR (bpm)[a]	**SDRR (ms)**[b]	**rMSSD (ms)**[c]	**LF**[d] **(n.u.)**	**HF**[e]**(n.u.)**
HM group **(n = 6)**	73 ± 4.04	176.41 ± 42.50	60.43 ± 32.82	2078.14 ± 1476.88	1377.14 ± 1568.50
SM group **(n =4)**	71.75 ±9.87	198.4 ± 86.03	63.52 ± 7.74	1420.92 ± 958.18	1019.04 ± 515.91
***p*-Value**	0.81	0.64	0.49	0.45	0.84

[a] Mean heart rate in beats per minute (MHR, bpm). The average beat to beat per minute over a period of time reflecting both sympathetic and parasympathetic nerve activity/responses (von Borrell et al., 2007, Rietmann et al., 2004, Visser et al., 2002).

[b] Standard deviation from beat interval to interval (SDRR) is used to quantify the overall heart rate variability (von Borrell et al., 2007, Rietmann et al., 2004, Visser et al., 2002).

[c] Square root of mean beat interval to interval (rMSSD). rMSSD reflects short term variations in heart rate related to parasympathetic nervous responses, e.g. breathing, physical activity (von Borrell et al., 2007, Rietmann et al., 2004, Visser et al., 2002).

[d] Low frequency in normalised units (LF, n.u.) measures the sympathetic nerve response/tone (2007, Rietmann et al.,2004, Visser et al., 2002)

[e] High frequency in normalised units (HF, n.u.) measures vagal activity (parasympathetic activity) such as breathing or stress. A positive emotion can increase HF or negative emotion can decrease HF (von Borrell et al., 2007, Rietmann et al., 2004, Visser et al., 2002).

Table 5.3. Comparing average intensity of Fuji pressure film when testing harness and cart type, cart with/without added weight (400 kg), and extra saddle padding over the withers with Malian donkeys at the SPANA clinic in Bamako, Mali.

Variable	***n = Satisfactory***	***n = Unsatisfactory***	***p*-Value**
Harness type	4	1	0.02[a]
Cart type	9	6	0.27
	n = no weight	**n = weight**	
Cart with Weight	3	2	0.009[a]
	n = no extra padding	**n = extra padding**	
Extra-saddle padding	6	4	0.23

[a] significant at p-Value < 0.05

CHAPTER VI.

CONCLUSION

Improving donkey welfare through enhanced management such as nutrition, alternative training methods and education may improve the overall well being of donkeys in developing and industrial countries. The nutrient management study focused on insulin resistance, and suggested that female donkeys are more likely to be sensitive to insulin resistance, which maybe due to reproduction status. However, if all donkeys are insulin resistant regardless of sex or body condition score due to an adaptive trait or “thrifty” genotype this has yet to be determined. However, we do know that donkeys in developing countries such as Mali are able to survive and work on sources of feedstuffs that are high in fiber and low in protein content. This may suggest that these donkeys do have the ability to readily store energy as adipose tissue and later use this as a source of nutrients when they are scarce such as during the dry season. The second nutrition study, the forage analysis did indicate that many common sources of feedstuffs fed to donkeys in Mali are high in fiber and low in protein. Donkeys in the U.S. other industrialized countries are typically exposed to diets richer in carbohydrates and proteins and little to no exercise, which often lead to obesity issues and possibly metabolic syndromes like insulin resistance. However, donkeys that are worked on a daily basis are able to survive on high fiber diets but are not always able to maintain a moderate body condition score. It still could be possible that donkeys are genetically predisposed to such conditions as insulin resistance especially when considering how easily they become obese on grass diets alone and fat deposition is generally located in key areas such as the crest of the neck and the tail head, which is common to horses with metabolic syndromes (e.g. insulin resistance). More testing with donkeys in developing countries may reveal additional information about this disease. Proper nutrient management of donkeys in both developing and industrialized countries can contribute to improved welfare for these animals such as supplying additional forage to donkeys in developing countries to maintain body condition or less grazing time to donkeys in industrialized countries. Careful nutritional management and providing proper nutrients are part of the key to a healthy donkey and improved donkey welfare.

In addition to over and under feeding donkeys the well being of donkeys is often jeopardized when donkeys are being trained. Traditional methods of over using harsh techniques can be altered. The training study in Michigan showed that donkeys could be trained in a short amount of time to pull a cart with only a halter and driving lines. Donkeys can also be driven and guided with this technique plus the donkey motivator. The donkey motivator is an economical and effective training tool that can be utilized by donkey owners and drivers in industrial and developing countries instead of the stick or riding crop. Even though the studies did not suggest one training method was more or less stressful, a difference in heart rate variability over time was noticed in the donkeys originally trained to drive with a donkey motivator and halter with driving lines. These donkeys did show a difference in parasympathetic activity after changing from the donkey motivator method to the halter training method. The parasympathetic activity measured by High Frequency (HF) was decreased after training methods changed and over the period of 18 days. This suggests the donkey becomes less stressed with the halter training method versus the donkey motivator method over time. However, when we tested the ten donkeys in Mali no difference was seen when comparing the halter method to the traditional stick method. It may have proven beneficial to have tested the same group of donkeys over a period of time to detect differences in the driving methods as heart rate variability measurements are often more reliable when tested more than once.

In terms of enhancing donkey welfare, it is also important to consider the harness, harnessing method and the implement (e.g. cart) that the donkey is being driven to. If a donkey is not properly harnessed or hitched to a cart, this can affect it's ability to pull the cart as well as potential harm it can cause the donkey. The amount of weight a donkey is pulling as well as the condition of the cart (e.g. inflated tires) can also affect the donkey's ability to pull the load. This study indicated that poor quality harness and excessive loading of the cart could all negatively affect the donkey's welfare by increasing pressure placed on the withers of the donkey. Increased pressure and improper harness and weight can then lead to severe lesions that increase the difficulty of the donkey's ability to work. Last but not least the Malian study suggested that it's important to educate those who work with or around donkeys. For example, the welfare assessment suggested that many donkeys that were dehydrated were often in poorer body condition. In addition, donkeys that were in good body condition (body condition score 2 or 2.5 on a 1 to 5 point scale) were more likely to be hydrated and more likely to resist having their ear's touched. Even though reactive care was provided to these donkeys via SPANA clinics, little proactive information was provided to the owners or drivers on how to prevent the ailments or improve the condition of their animals. The study found that most paraprofessionals had some basic knowledge on donkey husbandry but we did not have the opportunity to find out what the base level of knowledge was for the owners/drivers of the donkeys. Due to the limited research that has been done on donkey husbandry and behavior, these studies contribute important information to the areas of donkey husbandry, behavior and welfare. Remembering that there are approximately 44 million donkeys in the world, with most of them providing work in developing countries, it is clear that more research in this area is needed.