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thesis entitled

AN OBJECTIVE METHOD OF ASSESSING SAND
ACCUMULATION ON EQUINE ABDOMINAL RADIOGRAPHS

presented by

Nathan James Keppie

has been accepted towards fulfillment
of the requirements for the

Master of Science	degree in	Large Animal Clinical Sciences

Diana S. Rosenstein DVM

Major Professor's Signature

Date

AN OBJECTIVE METHOD OF ASSESSING SAND ACCUMULATION ON EQUINE ABDOMINAL RADIOGRAPHS

By

Nathan James Keppie

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal Clinical Sciences

2005

ABSTRACT

AN OBJECTIVE METHOD OF ASSESSING SAND ACCUMULATION ON EQUINE ABDOMINAL RADIOGRAPHS

By

Nathan James Keppie

Abdominal radiography has been utilized as a primary method of diagnosing sand colic; however, assessing such radiographs is currently highly subjective. Variations in interpretation by this subjective method influence the diagnosis, treatment and potentially the outcome of affected horses. In order to improve the diagnostic utility of radiography in cases of sand colic, a more objective assessment method was designed and tested by retrospectively examining previous clinical cases of sand colic. The objective method was determined to be more reproducible and accurate for diagnosing and assessing sand colic than current subjective methods. This objective method was then tested on 10 horses that were experimentally administered known quantities of sand over a period of time and radiographed. The newly developed objective method of assessing sand accumulation again proved to be accurate and was able to differentiate different quantities of sand whereas subjective means could not. The objective assessment method provides an ordinal scale ranging from 0-12 that attributes a relative quantity/severity of sand accumulation with a score of 7 or higher having an 83% likelihood of being associated with a diagnosis of sand colic. This method is recommended to be used when assessing equine abdominal radiographs upon initial examination for sand colic and for re-evaluation of the patient following treatment.

ACKNOWLEDGMENTS

I gratefully acknowledge the following people who helped me throughout this research project. First and foremost, I acknowledge my advisor, Diana Rosenstein, who helped me everywhere along the way and always provided much needed enthusiasm and mental and physical support. I extend my appreciation for my committee members Sue Holcombe and Hal Schott for their interest, expertise and guidance throughout. Dr. Carleton provided me with the eight mares for the experimental portion of the study and is gratefully appreciated. Dr. Schott and Dr. Stick provided me with 2 more horses to complete the study and are acknowledged. Hammond Farms graciously donated the much needed sand for the research. The Department of Plant and Soil Sciences analyzed the sand samples and are thanked. I could not have completed this project without the help from the Stats Help Desk, Department of Plant and Soil Science for the statistical analysis. I deeply thank everybody in the Radiology Department and the Large Animal Clinic who put up with me and helped me out by answering to my beckoning call. And Danielle Eifler, who put in tremendous time helping me with the experimental procedure and supported and lived with me throughout the entire time with a smile.

TABLE OF CONTENTS

TABLE OF CONTENTS (CON'T).

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CON'T).

LIST OF FIGURES (CON'T).

CHAPTER 1

Retrospective study and development of an objective assessment for sand accumulation on equine abdominal radiographs

Introduction

Chronic ingestion of sand can cause non-strangulating large intestinal intraluminal obstruction (colic) or chronic diarrhea in horses. Sand ingestion occurs most commonly in areas with loose sandy soil, with California, Florida, Michigan, Arizona, Colorado, and other coastal regions reported as having the highest incidence. Sand impactions alone account for 2.4% of all colics reported (White and Lessard, 1986). Another epidemiological study in 1997 (Kaneene *et al.*, 1997) found that in the state of Michigan alone, sand colic represented 5% of the total colics investigated. The sand found in clinical cases of colic and/or diarrhea is fine beach sand or clay, but gravel or bluestone shale has also been implicated as a cause of sand-related disease. The volume of sand required to produce clinical signs is not known but it has been reported that greater than 20 kg is needed to cause clinical problems in horses of small size (Ragle, Meaghner, Schrader and Honnas, 1989). However, sand found at surgery in cases of sand colic weighed between 8-70 kg (Ragle, Meagher, Lacroix, and Honnas, 1989; Ford and Lokai, 1979).

Sand may be present in the hay or may be ingested from the environment. Environmental sand ingestion may be due to ground feeding if stabled in a sandy pasture, grazing of grass covered by silt after flooding, grazing of roots and attached soil of plants uprooted in short or overly grazed pastures, or drinking from shallow or muddy pools at times of fresh water unavailability. Horses, especially young foals, may deliberately consume sand (pica) from boredom or salt deficiency. Horses may also scavenge more frequently and ingest sand if their diet is too low in roughage.

It is debatable whether or not sand of different particle size accumulates in different regions in the gastro-intestinal tract. Coarse sand has been reported to accumulate in the right dorsal colon, transverse colon and pelvic flexure while fine sand accumulates in the left and right ventral colon, and associated sternal and diaphragmatic flexures. Hammock *et al.*, (1998) reported that both fine and coarse grain sand was found equally in all areas of the large intestine. Sand may collect in the large colon because it is a fermentative area of reduced flow and the sand consequently settles out of the ingesta. The pelvic flexure and transverse colon become obstructed when masses of accumulated sand become lodged in these narrowed segments. Pathophysiologically, the sand obstruction results in gas, secretions, fluid and ingesta accumulation in the intestine proximal to the obstruction (Freeman, 2002). This is followed by secondary bowel distension and mesenteric traction causing pain (colic). Obstruction induced spasm (increased motility) occurs in the intestine proximal to the obstructed site, immediately after it occurs, and is caused by localized intestinal irritation rather than distension, and also causes pain (Allen, 1990). The intestinal wall veins are compressed as luminal pressure increases, causing a rise in the capillary hydrostatic pressure and filtration rates. This results in tissue edema and a net secretion of fluid into the intestine, leading to mucosal ischemia, cellular necrosis, loss of epithelium and villi, and mucosal hemorrhage. As the ischemia and inflammation destroys the integrity of the intestinal epithelium, the outer wall of enteric gram negative micro-organisms gain access to the circulation causing an acute phase response and fever (endotoxemia). Prolonged ischemia, edema and distension atony can cause loss of intestinal contractility. Paralytic ileus may also result from pain and over-stimulation from the sympathetic nervous system that complicates the proximal intestinal distension. Long standing sand impactions may result in a weakened and devitalized colonic wall that may predispose to rupture of the intestine. The weight of the impacted mass can also displace and/or twist the impacted segment of bowel resulting in a strangulating large intestinal obstruction.

Clinical signs of horses presenting with sand impactions are usually mild to moderate in severity and may be intermittent. The obstructions are usually incomplete and allow the passage of small amounts of dry, scant fecal material that is dehydrated fecal balls covered in mucous due to delayed passage. Dehydration is mild at first because water is still passed and absorbed in the cecum. The obstruction may become complete resulting in rapid accumulation of gas and ingesta with subsequent distension and more severe signs of colic.

Sand ingestion may also cause diarrhea where the horses present with signs or a history of inappetance, weight loss, mild dehydration, and chronic-recurrent colic. Intestinal motility may be increased or decreased. Chronic diarrhea in adult horses is almost always associated with large intestinal disease caused by either physical damage to the colonic wall or physiological disturbances of colonic function (Mair, 2002). Sand enteropathy (enteritis) is caused by sand-induced irritation to the colonic mucosa disrupting the normal motility leading to malabsoptive diarrhea. The abrasiveness of sand can cause mucosal damage leading to bacterial and endotoxin absorption. Damaged mucosa also results in a decreased amount of fluid absorption, so fluid then accumulates in the lumen. There may also be increased vascular permeability that causes fluid to leak into the lumen. The subsequent fluid distension results in pain and diarrhea.

Sand impaction can be difficult to differentiate clinically from feed impaction, and the diagnostic tests for sand impaction are prone to a high incidence of false positives and false negatives (Snyder, 1992). To aid in the diagnosis for sand impaction, any historical findings that suggest ground-feeding practices in an environment that has sandy soil or historical signs of diarrhea followed by mild-moderate intermittent colic may suggest current sand enteropathy.

Rectal palpation may reveal firm sand impactions in the colon, however, sand accumulation in the dorsal right colon or transverse colon may be difficult to detect with palpation. The weight of the impaction may drop the site of accumulation out of reach, so rectal palpation is often not conclusive (Doran, 1993). As well, it is difficult to differentiate sand impaction from firm digesta impaction via rectal palpation.

The history or presence of sand in the feces can indicate exposure to sand, but does not indicate clinical disease as small amounts of sand can be found frequently in the feces of healthy horses. Coarse sand may be visible or felt in the feces, however, fine sand often requires sedimentation to be identified. Tests for the presence or volume of fecal sand do not correlate well with the presence or volume of sand in the colon (Snyder, 1992). Presence of sand does not indicate sand obstruction, and absence of fecal sand does not necessarily indicate that there is no colonic sand.

Auscultation of the ventral abdomen may reveal characteristic sand sounds. This is described as a friction-like rub that is compatible with sand borborygmi. However, in order to produce these sounds, there must be intestinal motility, and contact of sand-filled bowel with the ventral abdominal body wall. Therefore, if sand is not in the ventral colon, or if there is mechanical or paralytic ileus, these friction rubs will not be ausculted (Ragle, Meaghner, Schrader and Honnas, 1989).

Abdominal radiography has proven to be a valuable tool in the diagnosis of sand impaction (Korolainen and Ruohoniemi 2002). Sand accumulations are often seen in the ventral colon as opaque masses that lie in the cranioventral abdomen that tend to conform in shape to the dependent surface of the colon (Figure 1-1). Therefore, the ventral margin of the mass may show sacculations, and the dorsal margin of the mass may be horizontal if the sand has settled out from the ingesta, irregular if it is still mixed with ingesta, or if severe, filling the entire colon (Fischer, 1997). Sand accumulations can be difficult to distinguish from enteroliths if the shape of the accumulation is spherical, such as occurs in the transverse colon (Fischer, 1997). There is currently, however, no established criteria for assessment of visualized sand accumulations and correlating with a clinical diagnosis. Presence and/or volume of visualized sand does not correlate well with presenting clinical signs, presence of fecal sand, abdominal auscultation, or with surgical findings from exploratory laparotomies (Bertone *et al.*, 1988). Even large enteroliths have been missed on abdominal radiography (Rose, Rose and Sande, 1980) as obstructive changes may obscure radiopaque material. It is difficult to identify the exact location of the sand accumulation (Hammock *et al.*, 1998) and to differentiate mechanical from functional ileus based on abdominal radiographs (Butler *et al.*, 2000). Serial radiography may be utilized to monitor the passing of intestinal sand following therapy, however, the radiographic findings do not correlate well with clinical findings (Ruohoniemi *et al.*, 2001).

In order to produce abdominal radiographs of diagnostic quality in adult horses, there is a requirement for large stationary radiographic equipment with an exposure range of 90-140 KVp and 180-600 mAs. High speed rare-earth screen/film combinations should be used with a grid that has a grid ratio of 8:1 – 10:1 and 80-130 lines/cm. It is difficult, if not impossible to obtain diagnostic abdominal radiographs from horses with an abdominal width greater than 70 cm, and significant scatter radiation is produced from any adult horse abdomen. So, the size of the patient and the capabilities of the radiographic equipment available will determine what radiographs may be obtained. It is recommended that the entire abdomen be radiographed as sand accumulation is not always in the cranioventral region. If right to left lateral radiographs were taken, left to right lateral radiographs are recommended in the specific area of concern as lesions may become more or less prominent. Ingesta may obscure sand accumulation so it is recommended that horses be fasted at least 6 hours prior to abdominal radiography to avoid false negative conclusions.

Abdominal ultrasonography may aid in the diagnosis of sand accumulation, however, no structures deep to a gas-bowel wall interface can be imaged because of the resultant acoustic shadowing. It may be difficult to differentiate intraluminal gas from sand accumulation with ultrasonography as both cause attenuation of the soundwave. There is also limited penetration of the sound beam to about 20-25 cm, and there is loss of resolution at increasing depths.

A transducer with a low frequency range (5 MHz) may reveal sand impactions in the ventral colon. The colon appears flattened against the ventral body wall with loss of the normal sacculations (Reef, 1998). There is little peristaltic activity of the large colon detected because it is weighed to the floor of the abdomen by the accumulation of sand. The large colon appears as if it is adhered to the ventral body wall owing to the lack of motility between these two structures. The sand grains on the mucosal surface of the large intestine appear as small, pinpoint, hyperechoic structures casting small acoustic shadows in varying directions. They may also be seen as starburst spicules that appear to be floating due to their suspension in the ingesta. Korolainen and Ruohoniemi (2002) concluded that abdominal ultrasound was a good screening method for horses with signs suggestive of sand accumulation with a specificity and sensitivity of 87.5%.

Once diagnosed, sand impaction can be treated medically where therapy is aimed at controlling pain, maintaining hydration, and administering laxatives such as mineral oil, dioctyl sodium sulfosuccinate, psyllium, and magnesium sulfate. These therapies may also stimulate colonic motility via the gastrocolic response when instilled in the stomach. Surgical intervention is indicated if systemic deterioration or peritoneal fluid changes occur, or if pain cannot be controlled. Surgical intervention involves exploratory laparotomy with enterotomies and removal of the impacted sand.

To prevent sand impactions (or diarrhea) from occurring, intermittent administration of psyllium or bran to the feed has been advocated as a method of reducing the risk. Feeding off of the ground or away from sandy soil and grazing in pastures where there is adequate growth that would prevent horses from picking up sand would also help reduce the risk. Horses that actively seek out sand may require a muzzle or stall confinement.

Despite frequent occurrence of sand colic in certain geographic areas, there is a paucity of published manuscripts that provide scientific information of its clinical signs, diagnosis, treatment and prognosis. Most of the information available is anecdotal or based on a limited number of cases (Ragle, Meagher, Lacroix, and Honnas, 1989).

Upon review of the literature, it is evident that there is poor correlation between clinical, diagnostic and surgical findings, which causes problems in diagnosing sand colic (Specht and Calahan, 1988). A prospective study that examined surgical findings of sand accumulation found that intestinal sand was diagnosed before surgery in only 58% of cases, and accidental enetrocentesis was the most common method of detecting it Ragle, Meagher, Lacroix, and Honnas, 1989. Doran (1993) also states that because of the "insidious onset and vague clinical signs associated with the problem, many cases are diagnosed and treated surgically".

There also appears to be a discrepancy in the reports as to the most common locations of sand accumulation. Most reports state only examining radiographs of the cranioventral abdomen for sand, where Ragle, Meagher, Lacroix, and Honnas, (1989) found a higher percentage of sand impactions in the right dorsal colon, transverse colon, and pelvic flexures as compared to the left or right ventral colons or associated cranial flexures (sternal or diaphragmatic). This discrepancy would affect the sensitivity of radiography in the diagnosis of intestinal sand.

It is important to diagnose sand colic accurately in order to prompt the correct therapeutic regimen (medical versus surgical). Therefore, it should be a goal of clinicians to obtain more accurate methods of diagnosis that provides better correlation with the disease.

The objective of this study is to improve the sensitivity and specificity of abdominal radiography as a diagnostic tool for intestinal sand accumulation in horses.

More specifically, the goal of this study is to develop an objective method of radiographic interpretation of intestinal sand accumulation on equine abdominal radiographs. Currently, abdominal radiographs are reviewed and a purely subjective assessment is made as to whether the opaque material seen within the gastro-intestinal tract is sufficient to cause colic, and as to the approximate quantity or severity of sand accumulated. This method appears highly variable between reviewers and as such, can result in differing opinions and different recommendations for therapy. A more objective method of assessment would be more reliable, reproducible, and accurate. The approximated quantity of sand accumulation should be more representative of the actual intestinal contents that will be more useful in assessing severity of disease and selecting appropriate methods of treatment. This goal will be accomplished in two studies:

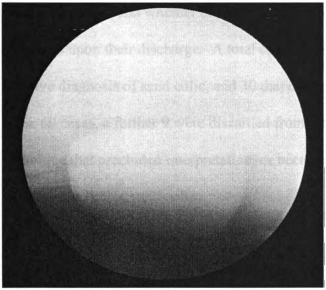
1. Identify criteria for assessing or measuring sand accumulations. This will be a retrospective study of equine abdominal radiographs with known diagnoses. Horses with both positive and negative diagnosis for sand colic will have their abdominal radiographs reviewed and the criteria that have the highest correlation with a positive diagnosis will be utilized to create an objective scoring scheme that will be tested between four different reviewers. The results of this objective radiographic assessment will then be compared to the results of the same four reviewers interpreting the same radiographs in a more subjective manner that is currently utilized in abdominal radiographic interpretation. It is hypothesized that subjective scoring is inaccurate and variable, depending on the interpreter, and that the objective

scoring would provide a more reproducible, repeatable, and more accurate assessment of the relative size and severity of the sand accumulation as compared to current subjective assessment methods.

2. Implement the new objective interpretive scheme into a prospective study to test its accuracy and radiographic sensitivity to known amounts of experimentally introduced sand in healthy horses. It is hypothesized that different known amounts of administered sand can be detected on radiographs using this objective assessment scheme that would not be apparent using subjective scoring.

Figure 1-1

An example of an abdominal radiograph showing a sand accumulation in the colon of a horse. The accumulation is of bone opacity and has a convex ventral margin and a horizontal dorsal margin.



Materials and Methods

A search through the medical records of the Large Animal Clinic of the College of Veterinary Medicine at Michigan State University was performed through the years 1996-2003. The search included horses admitted to the clinic that had abdominal radiographs performed during their stay. These results were further refined by searching for the diagnoses and whether or not it was sand colic that was established as a final diagnosis upon their discharge. A total of 60 different horses were selected, 30 with a positive diagnosis of sand colic, and 30 that did not have a diagnosis of sand colic. Of these 60 cases, a further 9 were discarded from the study due to poor radiographic technique that precluded interpretation, or because the horse had multiple abdominal disorders including sand colic.

The radiographs were initially reviewed by one observer to select different criteria to use to construct a scoring system. Each set of abdominal radiographs was reviewed in random order with the reviewer blinded towards the patient's history, prior radiographic interpretation, and final diagnosis. Radiographs were reviewed in random order without knowing whether it was the first or subsequent presentations of that horse to the hospital. The radiographs were examined for the following criteria with the descriptors used for evaluation of each parameter in parentheses:

- whether or not the entire abdomen was included on the radiographs (complete study: yes/no)
- whether adequate exposure techniques were utilized to interpret the radiographs (yes/no/partial)
- whether sand (radiopaque material) was present (yes/no)

- whether the entire accumulation(s) of sand was (were) visible (yes/no)

- the number of sand accumulations

- the length of the accumulations (cranial-caudal dimension- mm)

- the height of the accumulations (dorsal-ventral dimension- mm)

- opacity of the accumulation (mineral/soft tissue/mixture)

- homogeneity of the accumulation (homogeneous/heterogeneous/areas of both)

- predominant location of accumulation (cranioventral, midventral, other)

- width (mm) of a rib (caudal rib, mid-body, side closest to film)

- rib width : length of sand accumulation (ratio)

- rib width: height of sand accumulation (ratio)

- evidence/degree of secondary changes from obstruction (intestinal distension, significant gas accumulation, No = 0, Yes = 1-3)

- final diagnosis (sand colic, other)

In order to create the objective scoring method to evaluate radiographic sand accumulation, these parameters were tested against the final diagnosis (sand colic or other) using a Pearson correlation test (significance level set to $p < 0.05$) to see if there was a linear relationship between these parameters and a positive diagnosis (ie. sand colic). Those parameters with a statistically significant ($p < 0.05$) correlation ($r > 0.5$) were selected for use in the objective scoring assessment. Logistic regression analysis was performed on the standardized length and height dimensions of the accumulations (standardized to the rib width) to see if a cause-effect / prediction association could be made with the final diagnosis. Statistical significance was set at $p < 0.05$.

The parameters that were chosen for use in the objective scoring assessment were assigned a score that was relative to their degree of correlation to the diagnosis. For example, the number of sand accumulations was found to have a statistically significant strong correlation ($r = 0.9$) with a positive diagnosis. The location of the accumulation was also found to have a statistically significant correlation, but not as strong ($r = 0.5$). Therefore, on the objective assessment, the score assigned to the number of accumulations ranged from 0-3, with 0 representing no sand visible, 1 representing 1-2 separate accumulations visible, and 3 representing 3 or more accumulations. The score for the location of the accumulation, subsequently, ranged only from 0-1 due to its lower significance, with 0 representing sand accumulating in areas other than the cranioventral abdomen, and 1 for sand located in the cranioventral abdomen.

An objective scoring system radiographic assessment sheet for abdominal radiographs was then created to provide a more accurate interpretation for sand accumulation (Figure 1-2). It was designed so that an interpreter would evaluate the radiographs for each criterion, assign a score for each one according to the instructions, and then add up these scores to obtain a final total. The final total is a value out of a possible 12 points, representing an ordinal scale of relative severity and quantity of sand accumulation.

A subjective radiographic assessment sheet was also created. The subjective radiographic assessment sheet was formed on the basis of utilizing current systems of interpreting sand accumulation. The interpreter indicated whether or not they felt that the sand accumulation was sufficient / severe enough to cause sand colic. If the interpreter felt that there was sufficient evidence for sand colic, a score was assigned ranging from 1-5 depending on the severity of sand accumulation. Therefore, a score of 0 does not necessarily mean that no sand is present, but that it is not likely enough to cause clinical disease.

Four reviewers independently assessed all abdominal radiographs of the 51 cases. These reviewers included a radiology resident, a board certified radiologist, a board certified large animal internist, and a board certified large animal surgeon and emergency and critical care clinician. These people were selected to assess inter-observer variance among veterinarians with different areas of expertise that frequently deal with cases of sand colic and abdominal radiographs. Each reviewer was blind to the final diagnosis, the prior radiographic interpretation, and the patient's signalment and history. Each reviewer was asked to assign a score to the sand accumulation by subjective assessment first, followed by assigning a score by the objective assessment.

To assess intra-observer variance, one of the reviewers, the radiology resident, reviewed all abdominal radiographs three separate times, each time separated at least by 2 week intervals to decrease the chance of recognition of particular cases.

Statistical analysis:

The results of the subjective and objective scores of all four reviewers were tabulated and tested to see if they were normally distributed. The subjective scores obtained from the reviewer who assessed the radiographs in triplicate were compared to each other to assess intra-observer variance using an analysis of variance (ANOVA) that was modified by a Tukey-Kramer adjustment. Level of significance was set at $p < 0.05$.

A similar statistical test was done on the three objective scores for each case. The subjective scores for all three interpretations were then averaged for use in the inter-observer variance tests stated below. A similar pooling was done for the 3 objective scores.

The subjective scoring inter-observer variance was tested with an ANOVA modified by a Tukey-Kramer adjustment. The level of significance was set at $p < 0.05$. The subjective scores of each case were then compared to the final diagnosis in the patient's medical record to see that if, using this method, one may accurately predict a case of sand colic. The observed and expected results were compared to each other with a Pearson Chi-Square test of independence with a level of significance set at $p < 0.05$. The objective scores of the observed sand accumulations were tested for inter-observer variance using an ANOVA modified by a Tukey Kramer adjustment. Level of significance was set at $p < 0.05$. The objective scores of each case were then compared to the true diagnosis using a logistic regression model to see that if, using this method, may one accurately predict a case of sand colic and its relative severity.

Figure 1-2

Objective radiographic assessment scoring sheet for sand accumulation.

Case Number	Location	Number of Accumulations	Opacity	Homogeneity	Thickness of Sand: Width of Rib	Length of Sand: Width of Rib	Total
	Other=0	0=0	Soft tissue=0	Heterogeneous=0	1-3x=0	<10x=0	
	Cranioventral=1	1-2= 1	Mix=1	Mix=1	4-5x=1	10-20x=1	
		3 or >3 =3	Mineral=2	Homogeneous=2	>5x =2	>20x=2	

Results

A total of 51 different cases were used for the retrospective study (Table 1-1). Of these, 42 (82%) had single admission, while 9 (18%) had multiple admissions. Twenty-three (55%) of the horses admitted only once had a diagnosis other than sand colic, while 19 (45%) had a positive diagnosis of sand colic established. There were a total of 67 different admissions where the abdominal radiographs were reviewed. Thirty-nine (58%) did not have a diagnosis of sand colic, while 28 (42%) did have a positive diagnosis of sand colic.

Within the 51 horses, there were 6 (12%) intact males, 17 (33%) geldings and 28 (55%) females. There were 10 (20%) foals (up to 1 year of age), and 41 (80%) were adults with age ranging from 2 to 30 years. The different breeds represented in this study included: 13 (25%) Quarterhorses, 5 (10%) Standardbreds, 4 each (8%) of Arabians, Thoroughbreds, Ponies, and Grades, 3 (6%) Miniatures, 2 each (4%) of Hanovarians, Friesians, and Tennessee Walker Horses, and 1 each (2%) of Clydesdale, Spanish Mustang, Donkey, Warmblood, Haflinger, Pony of America, Appaloosa, and Paint.

Of the 24 horses that had a diagnosis of sand colic made, 3 (13%) stallions, 8 (33%) were geldings, and 13 (54%) were females. There were 8 (33%) foals, and 16 (67%) adults with age ranging from 2 to 25 years. The different breeds diagnosed with sand colic included: 8 (33%) Quarterhorses, 2 (9%) each of Standardbreds, Arabians, and Grades, and 1 (4%) each of Miniature, Pony, Hanovarian, Tennessee Walker Horse, Clydesdale, Friesian, Warmblood, Haflinger, Pony of America, and Appaloosa.

Correlation coefficients of the measured radiographic parameters to the final diagnosis are presented in Table 1-2. Those determined to have a significant relatively strong correlation with a positive diagnosis of sand colic ($p < 0.05$, $r > 0.5$) were selected for use in the objective scoring assessment scheme. The parameters used included number of sand accumulations, the opacity and homogeneity of the accumulations, the location of the accumulation, the rib width to length of accumulation ratio, and the rib width to height of accumulation ratio. It was determined that standardized height and length of the sand accumulations, standardized to the width of a rib, on their own did not have a significant cause-effect relationship with the diagnosis, but when put together in a logistic regression analysis a significant relationship was evident. There was a significant negative correlation with a complete study and a diagnosis, and a significant negative correlation with the radiographic quality and a diagnosis. The simple presence of sand and whether or not the accumulation was entirely visible on the radiographs did not have any significant correlation with a diagnosis of sand colic. Radiographic changes that could be attributed as secondary to obstruction were not found to have any significant correlation with a positive diagnosis of sand colic.

Table 1-3 summarizes the results of the subjective and objective scores determined by the 4 reviewers for each case. The data was determined to be normally distributed. Significant differences were found between the subjective radiographic assessments between the 4 reviewers ($p = 0.004$), and within the one reviewer who examined the radiographs in triplicate ($p = 0.02$). Using the objective method of radiographic assessment however, no significant differences were found between the scores of the 4 reviewers ($p = 0.127$), or for the scores assessed by the reviewer who examined the radiographs in triplicate ($p = 0.24$).

The subjective score of each case of all reviewers was compared to the final diagnosis of each case. Using a Pearson Chi-Square test of independence, a significant difference was found between the observed results and the final diagnosis ($p = 0.05$, $X^2 = 4.86$). This means that based on radiographs alone, subjective assessment of sand accumulation resulted in significant proportion of the cases being misdiagnosed.

Since good reproducibility was found using the objective scoring method among the reviewers, a logistic regression model was created. This was done to determine if the objective score could predict sand colic with accuracy. The logistic regression model created is as follows:

$$\ln P/1\text{-}P = -4.0845 + 0.5477 \text{ x score} = 0$$

It was found that an objective score of 7.01 or higher had an 83% chance of being correlated with a true positive diagnosis of sand colic.

Figure 1-3 demonstrates a small accumulation of sand in the diaphragmatic flexure of one of the horses. This particular case was scored between a 0 and 1 subjectively, indicating that some observers felt that this was enough sand to cause mild colic. Using the objective method however, scores ranged from 4-5 clearly indicating that this was not enough sand to cause clinical disease. Figure 1-4 demonstrates a moderate sand accumulation in the ventral colon of another horse. Subjectively, scores ranged from 0-4 indicating some reviewers felt that this was not enough sand to cause clinical disease, while others felt that this was enough to cause colic of moderate severity. Figure 1-5 demonstrates a large sand accumulation in the ventral colon of another case examined. Although both subjective and objective assessments indicated that this was enough sand to cause severe clinical signs, the range of scores was much less varied objectively compared to subjective means.

Figure 1-3

An example of an abdominal radiograph showing a small sand accumulation in the diaphragmatic flexure of a horse.

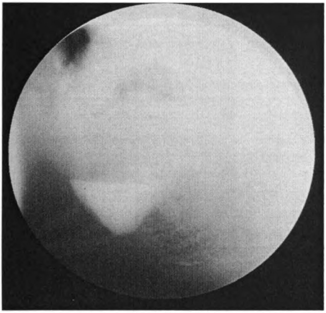


Figure 1-4

An example of an abdominal radiograph showing moderate sand accumulation in the ventral colon of a horse.

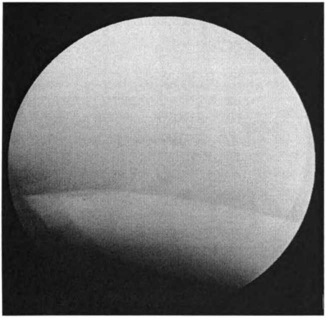


Figure 1-5

Two consecutive abdominal radiographic images demonstrating an example of a large accumulation of sand in the ventral colon of a horse.

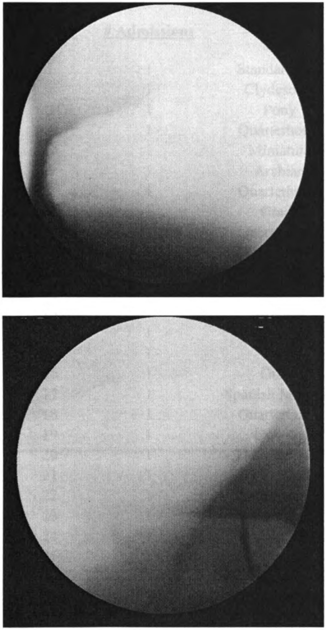


Table 1-1

Signalment and diagnosis (sand colic or other) distribution of horses used for the retrospective study. A diagnosis of sand colic and other is given to those horses that presented more than once that resolved an initial presentation of sand colic.

Horse #	# Admissions	Breed	Sex	Age (years)	Diagnosis
1	1	Standardbred	Male	11	other
2	1	Clydesdale	Gelding	17	sand colic
3	1	Pony	Gelding	27	other
4	1	Quarterhorse	Gelding	30	other
5	1	Miniature	Female	8	other
6	1	Arabian	Male	3	other
7	1	Quarterhorse	Female	7	other
8	1	Grade	Female	6	sand colic
9	1	Quarterhorse	Gelding	8	sand colic
10	1	Thoroughbred	Gelding	3	other
11	1	Tennessee Walker Horse	Female	5	other
12	1	Hanovarian	Gelding	14	other
13	1	Arabian	Female	9	other
14	1	Pony	Female	2	other
15	1	Pony	Gelding	11	other
16	1	Grade	Gelding	12	other
17	1	Spanish Mustang	Female	10	other
18	1	Quarterhorse	Gelding	10	other
19	1	Donkey	Female	6	other
20	1	Thoroughbred	Female	14	other
21	1	Quarterhorse	Gelding	5	other
22	1	Thoroughbred	Female	Foal	other
23	1	Standardbred	Female	2	other
24	1	Standardbred	Female	3	other
25	1	Friesian	Female	2	sand colic
26	1	Warmblood	Gelding	18	sand colic
27	1	Pony	Female	17	sand colic
28	1	Quarterhorse	Gelding	8	sand colic
29	1	Arabian	Gelding	16	sand colic
30	1	Haflinger	Male	Foal	sand colic

Table 1-1 (con't).

Horse #	# Admissions	Breed	Sex	Age (years)	Diagnosis
31	1	Quarterhorse	Female	3	sand colic
32	1	Grade	Male	Foal	sand colic
33	1	Quarterhorse	Male	Foal	sand colic
34	1	Miniature	Female	Foal	sand colic
35	1	Hanovarian	Female	Foal	sand colic
36	1	Arabian	Gelding	7	sand colic
37	1	POA	Gelding	25	sand colic
38	1	Appaloosa	Female	2	sand colic
39	1	Grade	Female	15	other
40	1	Quarterhorse	Gelding	11	other
41	1	Standardbred	Female	Foal	sand colic
42	1	Standardbred	Female	Foal	sand colic
43	5	Paint	Male	Foal	other
44	3	Quarterhorse	Female	2	sand colic
45	2	Quarterhorse	Gelding	2	sand colic, other
46	2	Miniature	Female	6	other
47	4	Tennessee Walker Horse	Female	12	sand colic, other
48	2	Thoroughbred	Female	18	other
49	2	Quarterhorse	Female	9	sand colic
50	3	Quarterhorse	Female	Foal	sand colic, other
51	2	Friesian	Female	2	other

Table 1-2

Correlation of abdominal radiographic parameters to a diagnosis of sand colic.

Radiographic parameter	Correlation coefficient (r)	Significance (p value)	Logistic regressional p value
Complete study	-0.61	0.034	
Radiographic quality	-0.59	0.049	
Presence of sand	0.21	0.031	
Visualization of entire accumulation	0.39	0.093	
Number of sand accumulations	0.85	0.005	
Opacity of accumulation	0.69	0.042	
Homogeneity of accumulation	0.60	0.041	
Location of accumulation	0.48	0.014	
Evidence/degree of secondary obstructive changes	0.01	0.966	
Standardized height of accumulation			0.085
Standardized length of accumulation			0.124
Standardized height of accumulation combined with standardized length of accumulation			0.031

Table 1-3

Subjective and objective scores assigned to abdominal radiographs for sand accumulation as observed by four reviewers. The three separate assessments and the averages by reviewer 1 are also provided. Subjective scores are from 0-5, with 0 representing not enough sand accumulation to cause sand colic, 1-5 representing the subjective severity for the clinical cases of presumed sand colic. Objective scores are from 0-12, which represent a relative increasing severity/volume of sand accumulation based on various objective radiographic signs. The final clinical diagnosis (sand colic or other) is also provided. X = no score provided.

Horse # (Admission#)	Subjective scores Reviewer #							Objective scores Reviewer #							Diagnosis
	1 (1st)	1 (2nd)	1 (3rd)	1 (avg)	2	3	4	1 (1st)	1 (2nd)	1 (3rd)	1 (avg)	2	3	4	
1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	other
2	3	2	1	2	3	1	0	7	7	6	7	5	6	7	sand colic
3	2	1	0	1	1	1	0	4	3	3	3	3	4	4	other
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	other
5	0	2	0	1	1	0	2	3	3	3	3	4	4	4	other
6	1	0	2	1	2	3	0	6	6	5	6	5	6	5	other
7	0	0	0	0	0	0	0	0	0	0	0	0	2	0	other
8	1	0	0	0	1	3	0	7	6	7	7	6	6	6	sand colic
9	2	1	3	2	5	3	4	7	8	7	7	8	8	10	sand colic
10	0	0	0	0	0	0	0	0	0	0	0	2	0	0	other
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	other
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	other
13	0	0	0	0	0	0	0	3	3	3	3	5	4	6	other
14	2	0	0	1	1	1	2	5	5	5	5	4	5	6	other
15	0	0	0	0	0	0	0	0	0	0	0	0	2	0	other
16	0	0	0	0	0	0	0	3	3	3	3	4	4	4	other
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	other
18	1	0	1	1	0	2	0	4	4	4	4	4	2	3	other
19	2	2	2	2	1	0	3	3	4	4	4	5	3	4	other

Table 1-3
(con't).

Horse # (Admission#)	Subjective scores Reviewer # 1 (1st)	1 (2nd)	1 (3rd)	1 (avg)	2	3	4	Objective scores Reviewer # 1 (1st)	1 (2nd)	1 (3rd)	1 (avg)	2	3	4	Diagnosis
20	3	0	2	2	1	0	2	5	4	4	4	4	5	6	other
21	1	3	4	3	2	3	1	6	6	6	6	6	6	6	other
22	0	1	0	0	0	2	0	5	4	5	5	6	6	6	other
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	other
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	other
25	2	2	4	3	5	3	2	9	9	10	9	9	9	10	sand colic
26	0	0	0	0	0	0	0	6	6	7	6	7	7	8	sand colic
27	4	0	2	2	1	3	0	10	9	9	9	8	10	9	sand colic
28	3	2	0	2	4	1	3	9	7	8	8	9	8	9	sand colic
29	0	0	0	0	1	0	0	4	5	6	5	5	6	6	sand colic
30	2	3	4	3	1	1	5	10	10	10	10	11	12	12	sand colic
31	0	0	0	0	0	1	0	6	7	7	7	6	7	6	sand colic
32	2	1	2	2	0	4	1	9	7	8	8	8	9	7	sand colic
33	3	4	3	3	1	3	0	9	7	9	8	7	9	8	sand colic
34	0	2	0	1	0	2	0	7	6	7	7	6	7	6	sand colic
35	3	0	2	2	0	3	1	9	7	7	8	9	8	8	sand colic
36	2	2	1	2	1	3	4	11	10	9	10	10	11	12	sand colic
37	3	5	3	4	5	4	3	10	10	10	10	10	10	12	sand colic
38	4	2	3	3	5	3	2	10	10	11	10	11	10	11	sand colic
39	1	3	0	1	3	2	1	5	6	5	5	5	6	6	other
40	0	2	0	1	0	2	0	5	5	4	5	5	4	5	other
41	4	2	4	X	0	3	1	10	9	10	10	9	9	10	sand colic
42	3	5	3	4	5	4	2	12	11	11	11	10	11	11	sand colic
43 (1)	2	0	0	1	2	1	0	4	4	4	4	5	4	5	other

Table 1-3
(con't).

Horse # (Admission#)	Subjective scores Reviewer #							Objective scores Reviewer #							Diagnosis
	1 (1st)	1 (2nd)	1 (3rd)	1 (avg)	2	3	4	1 (1st)	1 (2nd)	1 (3rd)	1 (avg)	2	3	4	
43 (2)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	other
43 (3)	1	0	0	0	1	0	0	0	0	0	0	0	0	0	other
43 (4)	0	1	0	0	0	1	0	0	0	0	0	0	0	2	other
43 (5)	1	0	0	0	0	1	0	0	0	0	0	0	0	2	other
44 (1)	0	1	2	1	1	3	0	7	7	7	7	8	8	X	sand colic
44 (2)	2	0	0	1	1	1	0	6	7	7	7	6	8	X	sand colic
44 (3)	0	2	0	1	1	1	X	8	7	7	7	6	7	X	sand colic
45 (1)	4	1	2	2	3	4	0	9	8	8	8	8	9	9	sand colic
45 (2)	0	1	2	1	0	1	3	2	2	0	1	2	2	4	other
46 (1)	2	1	3	2	2	3	X	5	4	4	4	6	5	6	other
46 (2)	2	0	0	1	0	1	X	2	2	2	2	4	4	4	other
47 (1)	5	2	4	4	5	1	3	12	10	10	11	10	10	12	sand colic
47 (2)	4	2	4	3	5	1	3	10	9	9	9	10	9	10	sand colic
47 (3)	3	2	1	2	3	2	0	5	6	6	6	5	9	8	other
47 (4)	0	2	3	2	1	2	0	6	5	6	6	6	7	7	other
48 (1)	2	0	1	1	1	1	0	6	4	4	5	6	5	6	other
48 (2)	0	0	2	1	0	1	0	0	0	0	0	2	3	0	other
49 (1)	3	2	0	2	3	2	0	7	8	8	8	9	9	9	sand colic
49 (2)	1	0	3	1	1	1	X	8	7	4	6	6	4	X	sand colic
50 (1)	3	1	3	2	1	3	1	9	8	8	8	10	10	12	sand colic
50 (2)	0	2	0	1	1	2	0	6	5	5	5	6	7	6	other
50 (3)	2	1	0	1	2	2	0	4	3	4	4	6	6	6	other
51 (1)	0	0	2	1	0	2	1	0	0	0	0	2	4	0	other

Discussion

These results demonstrate that the designed objective scoring system does work in creating a more accurate interpretation of the severity of the sand accumulation, and that it is more reproducible than current subjective methods of assessment.

In designing the objective scoring system, it was important that the cases used were random representative samples of cases admitted to the Michigan State University College of Veterinary Medicine. Of the cases selected, there was a 42% sand colic to 58% "other disease" distribution. These cases do not represent all of the horses that had abdominal radiographs performed over the 7 years searched, or all of those that had sand colic diagnosed, so prevalence and incidence of sand colic at Michigan State University cannot be determined from this sample. It was not part of this study to examine what other conditions these other horses were diagnosed. It was sufficient to recognize that they were not diagnosed with sand colic. Other indications for abdominal radiographs include horses presenting for colic where the radiographs would help rule out sand accumulation as the cause if the history suggests that it may be present (living in coastal areas, Michigan, or Arizona, feeding off ground, chronic diarrhea). Abdominal radiographs of adult horses are also indicated in horses presenting with colic to identify enterolithiasis, pneumoperitoneum, or intra-abdominal radiopaque foreign bodies. Abdominal radiographs are indicated in foals to identify small or large intestinal obstruction, meconium impaction, or pneumoperitoneum from gastro-intestinal rupture (Butler *et al.*, 2000).

Foals were included in this study as the intention was to design a system for application in horses of all ages presenting with sand accumulation. Foals represented

1/5 of the horses examined and most of these radiographs were presented on standard 14" x 17" radiographs as compared to adult horses that are radiographed using digital radiography with the images presented using a 6 on 1 format onto a 14" x 17" film. Using standard radiography should actually improve the interpretation of sand accumulation since most to all of the accumulation is visible on the radiograph. Using a 6 on 1 digital format, there is risk of overlap of the images, or there may be some portion of the abdomen that is not radiographed which will hamper the interpretation of the accumulated sand. However, disadvantages of routine radiographs include the radiation exposure required, and the possibility of motion unsharpness from an unstable film holder. A significant advantage of digital radiography is the availability of post-image acquisition data manipulation that can improve interpretation.

There was approximately an even representation of gender in the horses used in this portion of the study with Quarterhorses representing the breed majority at 25%. Of the horses that were diagnosed as having sand colic, these trends continued with males and females being equally affected and Quarterhorses representing the breed majority with 1/3 of the cases. The breed with the highest representation in this study that did not have a positive diagnosis of sand colic made was the Thoroughbred. Although the number of Thoroughbreds examined is small (n=4), this may represent different management styles compared to other breeds. A 5-year search through the medical records of the Large Animal Clinic of horses diagnosed with colic demonstrated a similar distribution of gender, age, and breed to that seen in these selected cases. These cases can therefore be assumed to be a good representation of the horses admitted to the Large Animal Clinic that could be used for the creation of this model. White and Lessard

(1986) examined the signalment of horses presenting with colic and found that Quarterhorses, Thoroughbreds, Arabians, and Standardbreds had the highest reported incidence, which also adds credence to the use of the selected cases in this study.

In order to create a more objective method of assessing radiographic sand accumulation, several radiographic signs and parameters were examined on the abdominal radiographs. These were determined at random, then after comparing the results with the final diagnosis, those radiographic findings that had the highest correlation to a positive diagnosis of sand colic were selected for use in the final assessment scheme.

Upon examination of the abdominal radiographs, it was found that there was an abundance of cases that did not provide complete views of the entire abdomen. The majority of these cases only included images of the cranioventral abdomen. This parameter was examined to see if, retrospectively, a complete abdominal series is required to provide a relatively accurate assessment of sand accumulation, and to provide a diagnosis of sand colic with relative severity. A significant negative correlation was found between a complete abdominal radiographic study and the established diagnosis. This means that as the abdominal radiographic series became more complete, a trend for a negative diagnosis for sand colic was found. This is most likely explained as during the radiographic examination, if sand is found, radiographs of the accumulation are taken only of those areas, while the rest of the abdomen might not be radiographed or all acquired images may not be printed. While if none to little sand accumulation was seen, most to the entire abdomen was radiographed to document this fact and that the entire abdomen was examined with digital fluoroscopy. A complete study should not therefore suggest that sand colic is not present, but rather that studies are more likely to be complete when preliminary views of the cranioventral abdomen do not show sand accumulation. This finding allowed the use of all of these cases without complete radiographic studies for the creation of the objective radiographic assessment scoring scheme.

Another aspect that affects the interpretation of radiographs is the radiographic quality. This includes the exposure technique, gray scale window and level of the printed images, presence of artifacts such as motion unsharpness and film/screen artifacts, and points of reference within the abdomen if many views are obtained. The radiographic quality, focusing on exposure techniques, was critiqued while examining the radiographs. A significant negative correlation was found between radiographic quality and the established diagnosis. The poorer quality radiographs, such as those that were underexposed or had poor radiographic contrast, may have hindered the interpretation and not allowed an accurate diagnosis of sand colic if such was the case. This finding should increase awareness of the importance of good quality radiographs, and how it may impact interpretation, possibly providing a false negative result (Fischer, 1997).

The simple presence of sand observed on radiographs did not correlate with clinical disease. This is not surprising since many horses may have gastro-intestinal sand but not show any clinical signs. It has been discussed that the presence of fecal sand is a common finding in healthy horses (Colahan, 1987). It is not known how much sand is required to produce signs of colic or diarrhea, but the mere presence or absence of sand is not an adequate indicator to support sand colic. This parameter was therefore not included as a source for points in the objective assessment scheme, since this method assumes that sand is present. A separate category for presence or absence should not be used since there is no statistical correlation between this finding and sand colic.

Whether or not the full accumulation of sand was present on the radiograph did not seem to affect the diagnosis. There was no correlation with the presence of the entire accumulation versus part of the accumulation to a positive diagnosis. This may seem surprising since, if part of the sand accumulation is missing from the radiograph, then the measured size of the accumulation, its homogeneity and opacity, and its entire location within the abdomen would be obscured. It is assumed that most of the sand of the accumulation was radiographed, providing enough information to establish a diagnosis.

The number of sand accumulations observed was found to have a significant linear relationship with a positive diagnosis for sand colic. This is supported by Kaikkonen and Ruohoniemi (2000) who demonstrated a significant relationship between number of radiographic sand accumulations and signs of colic. Most cases in the present study had 1 large accumulation, or few very small ones. However, several cases were seen to have 3-4 fairly large size accumulations which were always associated with a positive diagnosis. It is acknowledged that the numbers visualized may not be a true representation of the actual number of accumulations. Sand accumulations located in the mid-abdomen, or in areas that were not properly exposed may have been missed. Sand typically accumulates and obstructs the ventral colon and the right dorsal colon, and these areas are usually penetrated well enough to visualize sand accumulation. The radiographic contrast, however, may not be adequate enough to visualize distinct but overlying accumulations that may be seen in the sternal and diaphragmatic flexures in the cranioventral abdomen.

The overall radiographic opacity and homogeneity of the accumulation was also taken into account. It was clear that some sand accumulations appeared more opaque than others, and some appeared more homogeneous than others. It was assumed that the more opaque and homogeneous accumulations represented more compact, solid, and pure sand accumulations and most likely had associated sand colic. The more heterogenous and less opaque accumulations were considered to be accumulations of mixed contents (sand and ingesta), with some water content that should indicate a less severe sand accumulation and not likely causing obstruction. The size of the sand grains may also affect the radiographic opacity, with coarse sand being more opaque than fine sand. It has been described that fine sand settles out from the ingesta and accumulates more often in the ventral colon, and that coarse sand does not settle out until the right dorsal colon, but Hammock *et al.* (1998) found that coarse and fine grain sand was found in various parts of the large intestine. Both homogeneity and opacity were found to have a significant linear relationship with a diagnosis of sand colic.

The location of the sand accumulation was examined to see it had a relationship with a diagnosis of sand colic. Most reports state that sand causing colic is located in the left or right ventral colon, sternal or diaphragmatic flexures, the pelvic flexure, or in the right dorsal colon. Radiographically, left from right side is difficult to distinguish, unless both sides of the abdomen are radiographed, which was not the situation in any of the 67 studies. Sternal and diaphragmatic flexures may be distinguished (Campbell, Ackerman and Peyton, 1984) if the size and weight of the accumulation does not distort the normal anatomy and position of these flexures. Both flexures are in the cranioventral abdomen, with the sternal flexure more caudoventral than the diaphragmatic flexure that is more craniodorsal. The ventral colon (left or right) can also be identified due to its ventral location, size, and presence of sacculations (haustra) that commonly indent onto the impacted sand. These sacculations may not be visible if they are distorted by the weight or size of the sand. The dorsal colon is more difficult to identify specifically, but the general dorsal location and size of the intestine (visualized by the intra-luminal sand) help identify it as dorsal colon. The pelvic flexure may be identified by its caudoventral location and the presence of a convex caudal aspect. Since accurate identification of sand accumulation is difficult, it is best to describe the location based on abdominal quadrants, unless a distinct structure such as the stomach, cecum, sternal flexure, or diaphragmatic flexure is visualized. Upon retrospective examination of the abdominal radiographs, it was found that there was a weak relationship ($r = 0.5$) between cranioventral sand accumulations and a diagnosis of sand colic. Cranioventral distributions include both sternal and diaphragmatic flexures, and the cranial aspects of the left and right ventral colons. Other areas of sand accumulations were characterized by smaller or wispy inhomogeneous accumulations, of varying opacity.

Evidence for and degree of radiographic signs that are associated as being secondary from colic or obstruction were evaluated and tested to see if any relationship existed with a diagnosis of sand colic. There was no correlation found, as many other causes of colic can cause the same radiographic changes and are non-specific. These include intestinal fluid distension, significant gas accumulation, and loss of serosal margin detail. These secondary changes may also obscure the visualization of radiopaque material resulting in a false negative diagnosis for sand colic (Rose, Rose and Sande, 1980).

The length and height of the sand accumulations were directly measured and then standardized to the measured width of a rib, which also removed any effect of magnification, providing a relative size of the sand accumulations. It was felt that of all of the radiographic parameters examined, the standardized length and height should have a cause and effect association on the diagnosis, and that perhaps a prediction of sand colic could be made based on the size of the accumulation. For this reason, logistic regression analysis was performed on the measurements against the diagnosis as opposed to correlation analysis. On their own, standardized accumulation length and height did not have any significant association with a diagnosis of sand colic. When combined, standardized length and height had a significant cause-effect relationship with a diagnosis of sand colic. Kaikkonen *et al.,* (2000) states that the relationship between clinical signs of colic and the size of the radiographic sand accumulation was statistically significant. Many of the sand accumulations visualized in this study were located primarily to be trailing along the ventral floor of the ventral colon without any significant height. This most likely represents some sand that has settled out from the ingesta but has not accumulated enough to cause clinical signs. This shows that both measurements must be considered together to provide a two-dimensional perspective of the size of the accumulation. The standardized volume of the accumulation would be an ideal parameter to obtain, however the third dimension cannot be measured due to the single projection of equine abdominal radiographs and the lack of orthogonal views.

Based on the results of the preliminary retrospective examination, it was decided that those parameters with a significant association with a diagnosis of sand colic would be used to create the objective scoring scheme. This included: the number of sand accumulations, their opacity and homogeneity, their location and standardized two-dimensional size. A score was assigned to each category (Figure 1-2) dependent on their statistical strength of correlation. The four reviewers then each assigned a score to the sand accumulation that should be a reflection on its combined relative size and severity. These scores were then compared to a purely subjective score judged based on the overall appearance of the abdominal radiographs. The goal was to see if the current subjective assessment of abdominal radiographs for sand colic differs than that of the objective assessment, and to compare the accuracies of the scores to the final diagnosis.

The observed results support the proposed hypotheses. Significant differences between the subjective scores of the four reviewers indicate that subjective scoring provides a high degree of inter-observer variance. A high degree of intra-observer variance was also found based on the results of the three trials that one observer performed, indicating poor reproducibility of the assessment even within the same person. The Pearson Chi-Square test also indicated that subjective scoring has poor accuracy in terms of determining or predicting sand colic.

Subjective assessment is the current method of interpreting abdominal radiographs for sand colic. These results show that this method is inaccurate, has poor reproducibility and high inter-observer variance. This establishes a need for a more accurate and reproducible test that will better quantify and assess the severity of sand accumulation. If such a test can be created, a more clinically accurate assessment will be established, providing the correct therapeutic regimen to follow. This will also improve the current poor correlation between clinical signs, diagnostic findings and surgical findings that plagues horses with sand colic (Specht and Colahan, 1988).

The objective scoring assessment scheme was created to improve the diagnosis of sand colic and to provide clinicians a more accurate interpretation of the sand accumulation. Results from the retrospective study indicate that the objective scores assigned to each case from all four reviewers did not differ significantly from each other. This means that inter-observer variance is improved and given the interpretive criteria (Figure 1-2); others assessing the radiograph should obtain similar results. This method also proved to be reproducible, based on the fact that the results of the three trials of the one reviewer did not differ significantly. The objective scoring assessment is also fairly accurate in predicting sand colic. A case with an obtained score of 7 out of 12 yielded a diagnosis of sand colic with 83% precision. This still allows some false positives if a score of 7 or greater is attained, however, it is unlikely. An objective assessment score of under 7 means that there is still some sand present, but it is unlikely that the sand is causing the clinical signs, and that other diagnostics may have to be performed to determine the cause of the colic. The amount of sand present cannot be accurately determined, but this ordinal scale provides a relative comparison. For example, a score of 5 represents more sand than a score of 4, but less sand than a score of 7, and is most likely not causing signs of colic.

Limitations of this study include:

1). Although foals were included, they were not separately examined to see whether or not they fit the same statistical results as compared to adult horses. Foals represented a fairly significant proportion of those cases that were diagnosed with sand colic (33%). Although most of their radiographs were a single 14" x 17" film, this should not affect the method of objectively examining the sand accumulation as compared to the many views seen with the adult radiographs. They might actually provide a more accurate assessment as the entire abdomen is included and there is no overlap of images and there are no areas of the abdomen that are not radiographed.

2). Although this method of assessing sand accumulation provides a more uniform conclusion amongst interpreters, it still may not provide additional clinical utility. As discussed earlier, exact volumes or weights of sand accumulation cannot be determined, so values under 7 simply provide a relative scale to compare other accumulations. This method may have clinical application for assessing sand accumulation for horses that re-present to the clinic with signs of chronic colic to see if a previously observed sand accumulation is changing in size. Also, this method may be useful in examining the success of medical therapy, where one could compare scores on subsequent radiographs after an initial therapeutic trial. It has been discussed that one cannot rely on clinical signs and evidence of fecal sand to establish a conclusion of the gastro-intestinal sand accumulation, and previous radiographic assessment post-therapy simply provided a subjective assessment that has been proven by this study to be variable and inaccurate.

Although this objective method has proven to be more accurate and more reproducible than subjective assessment, it needs to be tested prospectively to determine its sensitivity to varying quantities of sand. This would help to validate this objective assessment tool.

Conclusion

Current methods of assessing radiographic sand accumulation tend to be inaccurate and inconsistent among readers. The devised objective assessment scoring scheme is more reproducible with improved accuracy for determining the presence of significant sand accumulation. It provides an ordinal score ranging from 0 to 12 that attributes a relative quantity/severity of gastro-intestinal sand accumulation, with a score of 7 or higher having an 83% of being associated with a diagnosis of sand colic. This objective scoring system will be helpful in applying radiographic findings to clinical diagnosis and management of equine colic.

CHAPTER 2

Prospective study and testing of the developed objective assessment on experimental horses

Introduction

Based on the results of Chapter 1 where it was determined that the developed method of assessing sand accumulation on abdominal radiographs was more reproducible and accurate than current subjective methods, a prospective study was designed. The purpose of this prospective study was to utilize and test the newly developed objective assessment method on experimental horses that have been administered known amounts of sand into their gastro-intestinal tracts. The study was designed to evaluate whether or not this method could differentiate 2 different quantities of gastro-intestinal sand, and to evaluate its potential usefulness in determining clinical disease.

Materials and Methods

Ten experimental horses were used in this study, 8 being from a research and teaching facility owned by Michigan State University and used for theriogenology purposes, and 2 being donations to the Veterinary Teaching Hospital from local clients. The 8 research mares were kept on grass pasture throughout the year that did not contain sand. There was adequate grass coverage to prevent over-grazing. Their grass diet was supplemented with hay for 9 months which was held in feeders that sat on the ground. The pastures contained a holding area that was dirt-packed but did not contain any sand. They were supplied with *ad libitum* water. There were no known problems related to sand enteropathy with these mares. The 8 research horses were all intact mares with ages ranging from 6-24 years old. Three were Quarterhorses, 3 were Thoroughbreds, 1 was a Standardbred and 1 was a grade. One of the 2 donation horses included an 8 year-old Thoroughbred gelding whose feeding environment history was unknown; however there were no reports of previous sand colic. The other donation horse was a 17 year-old Arabian mare that was stabled indoors on wood-shavings-covered cement floors and fed grass hay and water *ad libitum*. There was no known previous incident of sand colic with this mare.

The horses were divided into two groups of five for the study and were randomly allocated into either group. The horses were numbered 1-5 in Group 1 and 6-10 in Group 2. The groups were divided based on the dose of sand that was to be administered so as to detect sensitivity of the radiographic sand accumulation objective scoring system devised in the retrospective study. Horses in Group 1 were administered sand at a dose of 4 g/Kg bodyweight once a day for 2 consecutive days. Group 2 consisted of 5 horses that were administered sand at a dose of 4 g/Kg bodyweight twice a day for 2 consecutive days.

The experiment was performed working with 2 horses at a time, 1 from each group. Each horse had a physical examination performed at the beginning of the study to obtain baseline vital statistics (rectal temperature, heart rate, respiratory rate, packed cell volume, total plasma solids, bodyweight, digital pulses and borborygmi) and to observe for any underlying disease that may confound, place bias, or preclude the study from being performed. Following the physical examination, each horse had a complete series of abdominal radiographs taken (standing lateral views, horizontal beam) from both right and left sides of the horse to ascertain if any intra-abdominal sand was present and radiographically visible.

Radiographs were taken using high intensification digital fluoroscopy (Maxiray 125, GE Advantx LFX Digital Fluoroscopy System, General Electric Medical Systems, Milwaukee, Wisconsin). A 3-phase, 12-pulse, 60-hertz generator was used that supplied power to an x-ray tube with a 1.2 mm focal spot and 10° target angle, using exposure settings of 100-110 kilovoltage peak (KVp) and 400-500 milliamperes (mA) with the time of exposure (seconds) controlled by an automatic exposure controlled phototimer. All radiographs were taken in an overlapping series consisting of 12 radiographs per side. For purposes of radiography, the abdomen was divided into 4 rows beginning ventrally and extending in a cranial-caudal direction, with 3 radiographs needed per row to view the entire abdomen (Figure 2-1). Therefore, radiograph 1 was a view of the cranioventral abdomen including the sternal and diaphragmatic flexures, radiographs 2, 3 and 6 were the ventral colons and pelvic flexure, radiographs 4 and 7 were views of the stomach region, radiographs 5, 6, and 8 were of the central abdomen including the small intestines and dorsal colon, radiographs 10, 11 were of the dorsal abdomen, and radiographs 9 and 12 were views of the small colon at the cranial brim of the pelvis. A similar format was utilized for the opposite side so that a total of 24 radiographs were obtained each time a horse had abdominal radiographs performed.

Horses had to occasionally be sedated for the radiographic procedures. Sedation was given using detomidine hydrochloride (Dormosedan®, 10 mg/ml, Orion Corporation, Espoo Finland) at 0.007 – 0.015 mg/Kg intravenously, or xylazine hydrochloride (AnaSed®, 100 mg/ml, Ben Venue Laboratories, Inc., Bedford, Ohio) at 0.5 mg/Kg intravenously.

Provided that the horses did not show any radiographic evidence of intra-abdominal sand and were healthy, the horses were kept in individual stalls, bedded with wood shavings overnight and for the duration of the study. The horses were fed timothy grass hay in a controlled fashion during the first 2 days while the sand administration was being performed. Six hours prior to any sand intubation, the horses were denied access to food so as to empty the stomach. Following the first 2 days, the horses were then fed *ad libitum* for the remainder of the study. They were allowed continual access to fresh water for the entire study.

Sand was obtained in bulk quantity for the study from a local landscaping facility. The sand used in this study was "fill" sand that was unprocessed and not consistent in its quality. It is a type of sand that compacts well and may contain some stone and clay. Sand particle size used in this study ranged from 0.05 mm - 6 mm. Fourteen and a half percent was greater than 2 mm, and the majority (57.7 %) was less than 2 mm (0.18 - 0.5 mm). Silt and clay combined for 2.3 % of the composition.

For sand administration, both horses were sedated according to the above description and restrained in stocks with lead ropes. A plastic nasogastric tube, ½" inner diameter and ¾" outer diameter, was introduced following an appropriate level of sedation had been reached. Sand (4 g/Kg bodyweight) was mixed with water that totaled a volume of no more than 15 liters and passed through the nasogastric tube into the stomach with the aid of a funnel and gravity-assisted passive-flow. The horses were then radiographed according to the described procedure. The sand administration was repeated 12 hours later for the Group 2 horse, and then both horses were radiographed again. Day 2 of the study followed the protocol from Day 1. Day 3 through 6 involved only twice daily abdominal radiographs that were separated by a 12-hour time span. The study concluded for each set of horses on Day 7.

Throughout the study, the entire fecal output was collected at intervals that coincided with the time of radiography. Feces were removed from the stall and placed in a large plastic tub filled with water. Any sand present settled to the bottom of the tub and was collected, dried, and weighed. This was performed so that intra-abdominal sand content could be calculated, as the amount administered was known.

The sensitivity of sand retrieval from the tub-system was assessed by adding 500 g of sand to a tub full of sand-free feces that was then filled with water. The sand was collected following sedimentation as was done in the experimental cases. The sand was dried and weighed and compared to the original weight. This was repeated 2 more times.

The horses were monitored throughout the study for signs of colic. Daily complete physical examinations were performed as well as abbreviated examinations for specific colic parameters every 6 hours, and hourly walk-bys for monitoring of food and water intake and clinical signs of pain. Hydration status and body weight were monitored daily. Following every intubation procedure, and if there were any signs of colic, the horses were administered flunixin meglumine (Flunixamine™, 50 mg/ml, Phoenix Scientific, Inc., St. Joseph, Missouri) at a dose of 1.1 mg/Kg intravenously.

All radiographs (digital fluoroscopic spot-film images) were adjusted for grayscale window level and window width for maximal diagnostic interpretation and digitally printed on a 4 x 1 film format (Kodak Dry View Laser Imager, model 8700, Oakdale, Minnesota). Each horse had 26 sets of radiographs, consisting of right and left lateral views taken from Day 0 (pre-sand administration check), and twice daily radiographs (am and pm) from Day 1 – Day 6, with Day 1 and 2 representing the days of consecutive sand administration. Each set of radiographs consisted of 12 images depicting the entire abdomen and these were analyzed for sand accumulation both subjectively and objectively using the scoring systems devised in the retrospective study. Unless otherwise specified, all radiographic figures are orientated with the top of the image is the dorsal aspect of the horse, the bottom is ventral, the left side is cranial and the right side is the caudal aspect of the horse. The objective scoring system has been established as being a relatively sensitive and accurate method of quantitatively assessing the amount or severity of intra-abdominal sand by establishing a "sand accumulation score" on a scale from 0-12 (0 = no sand present, 12 = a very large quantity of sand present) with a score of 7/12 indicating a high likelihood of clinical disease. The score is obtained by assessing different radiographic parameters of the sand accumulation such as homogeneity, location, number of accumulations, opacity, height and width. During the analysis, the interpreter was blinded to the group assignment of each case horse, and to the time in the study the radiographs were taken.

Statistical analysis:

All statistical tests were performed using dedicated statistical software (SAS version 9.1). All data was tested with univariate normal plots to see if they were normally distributed. Level of significance (α) for all tests was set at a p value of 0.05.

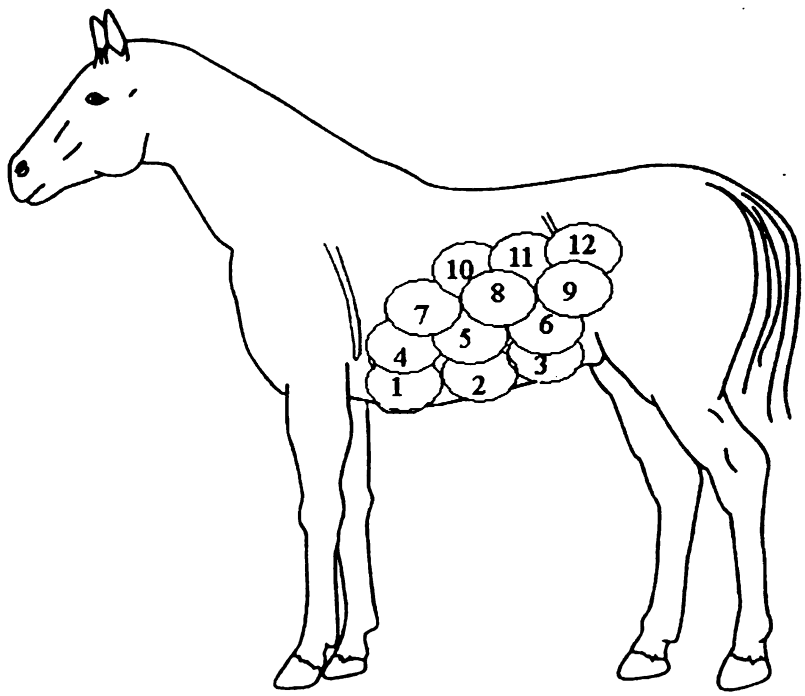
Statistical comparisons were made between the scores obtained from right and left lateral views, for both subjective and objective scoring methods, to see if obtaining both views was necessary, or provided new information, to obtain a diagnosis that one view may not accurately provide. A mix-model analysis of variance (ANOVA) for repeated measurements was performed and modified by a Tukey-Kramer adjustment for the multiple comparisons.

Statistical comparisons were also performed between Group 1 and Group 2 to determine if differences existed in the subjective and objective scores. A mix-model ANOVA for repeated measurements was performed and modified by a Tukey-Kramer adjustment for the multiple comparisons. The power of the test was also determined to assess the ability of the test to tell if a difference does exist.

Other comparisons made using similar testing methods include: 1) comparing the objective scores of Group 1 versus Group 2 over time; 2) comparing the fecal-sand output of Group 1 versus Group 2 over time; 3) comparing the objective scores for Group 1 versus Group 2 based on the amount of intra-abdominal sand; 4) comparing the 6 individual parameter scores from the objective scoring system of Group 1 versus Group 2 over time; 5) comparing the 6 individual parameter scores for Group 1 versus Group 2 based on the amount of intra-abdominal sand; and 6) comparing each individual parameter to each other by combining all data. Graphs were constructed to visualize the above comparisons and linear regression plots were calculated to ascertain if a relationship exists between the compared parameters. Graphs and regression plots were performed using a dedicated spreadsheet software package (Microsoft Excel for Windows, version 9.0).

Figure 2-1

Schematic representation of the 12 areas radiographed from each side of the horse's abdomen using high-intensification digital fluoroscopy.



Results

All 10 horses had a normal physical examination that was pertinent for the procedure. Horse #10 had a lame left front leg due to a carpal chip fracture. Hydration status, appetite, fecal output, intestinal motility and vital parameters (temperature, pulse and respiration rate) were all within normal limits. No fecal sand was collected from any horse prior to the experimental administration of sand. All pre-sand administration abdominal radiographs were negative for intra-abdominal sand except for Horse #3 who had a small quantity (subjective score = 1/5, objective score = 3/12) of sand visualized in the sternal flexure. This was considered to be due to a small quantity of sand that was nasogastrically administered 2 months previous for a trial run for this protocol. At that time, pre-sand radiographs of this horse were negative for intra-abdominal sand.

The procedure of nasogastrically administering sand took approximately 25 minutes per horse. The sand settled quickly within the nasogastric tube and often had to be re-siphoned or flushed with additional water. Only small quantities of sand could be added to the funnel-tube system so as not to plug the tube. All sand particles greater than 5 mm were removed so they would not lodge within the nasogastric tube, and to mimic a more natural setting of sand ingestion where it is considered unlikely that horses would consume these larger particles.

Throughout the study, 8/10 horses maintained their hydration and body weight (+/- 10 Kg) and did not show any signs of colic (save for mild diarrhea in 6/10 horses between Day 2-3) and were considered healthy at time of study completion. Two horses from Group 2 were the exception. Horse # 6 showed mild signs of colic on Day 2 after the fourth and final dose of sand was administered. Colic resolved with an intravenous dose (see Methods and Materials) of flunixin meglumine. Horse #10 (the 8-year old Thoroughbred gelding donation) showed mild signs of colic on Day 2 after the fourth and final dose of sand was administered. Intravenous flunixin meglumine was required twice daily for 3 days to control the colic. Colic progressed to moderate on Day 6 following 2 days of no fecal output and poor appetite. Heart rate and intestinal motility remained normal. The horse became mildly dehydrated which improved following oral fluid therapy. The horse was euthanized on Day 6 due to progressive colic, thus radiographs and fecal sand were not collected at this final time. Abdominal radiographs of both Horse #6 and 10 during their episodes of colic reflected a sand accumulation appearance compatible with sand colic, as determined by the objective scoring system. Horse #6 had an objective score of 8/12 on Day 2 and a score of 7/12 on Day 2.5. Horse #10 had an objective score of 8/12 on Day 4.5 and 5. Three medium-large sand accumulations were visualized within the ventral and mid-abdomen of Horse #10 at this time (Figure 2-2).

The radiographic procedure was performed with little difficulty. Due to the number of exposures required for the study and due to the high exposure techniques required for the abdominal radiographs, the automatic exposure control often reached its maximum exposure limits. This required intermittent tube cooling causing a slight delay in the acquisition of the images. Occasionally the horses became agitated when they had to re-enter the radiographic stocks for the opposite lateral views. Thirty-two out of 129 (25%) occasions resulted in only 1 side of the abdomen being radiographed, this being due to lack of cooperation by the horse or x-ray tube malfunction / overheating.

Initial observations of the radiographs revealed that clear differences could be seen between a sand-filled stomach, diaphragmatic flexure, sternal flexure, ventral colon, and small colon. Sand could not be reliably identified within the small intestines or the cecum. Intra-abdominal sand could be identified in its location and its progress through the intestines could be subjectively monitored. Sand was consistently seen within the stomach (radiograph number 4) following nasogastric administration. Its appearance was a well-circumscribed homogeneous mineral-opaque mass with a convex ventral margin and a straight horizontal dorsal margin in the mid-cranial abdomen (Figure 2-3). Only on 2 occasions was sand still visualized at the end of Day 1 in the stomach (Horse #6 and 10). Its progress or accumulation within the small intestines or dorsal colon could not be monitored due to the underexposure of the majority of the radiographs in the mid-central abdominal region (radiographs number 5 and 8) that corresponds to the location of the small intestines and the dorsal colons. All other areas of the abdomen were adequately penetrated and could be visualized on film. Sand was also never visualized in any horse throughout the study in the craniodorsal (radiograph number 7, Figure 2-4a, and radiograph number 10, Figure 2-4b) or mid-dorsal abdomen (radiograph number 11, Figure 2-4c). These areas represented the dorsal aspect of the stomach and dorsal colons and were consistently gas filled and contained little ingesta or feces. Ribs and vertebrae were consistently visualized in these regions. Sand was most commonly visualized within the ventral colons (radiograph number 2, Figure 2-5, radiograph number 3, Figure 2-6, and radiograph number 6) and within the sternal and diaphragmatic flexures (radiograph number 1, Figure 2-7) in the cranioventral abdomen. Sand became apparent within the ventral colon by Day 2-3 and remained within this region for the majority of the duration of the study. Its appearance varied from homogeneous to heterogeneous, well-circumscribed to poorly defined accumulations of sand that varied from mineral to soft tissue opacity. Dorsoventrally directed radiolucent bands, interpreted as haustra, were occasionally visualized. It was more typical for the sand to appear as interspersed accumulations within the other colonic digesta, becoming more defined, homogeneous and opaque in Group 2 horses (Figure 2-8) and then becoming ill-defined again towards the end of the study, while Group 1 horses did not show any signs of subjectively significant sand accumulation (Figure 2-9 a, b). Sand was inconsistently visualized within the small colon by the end of the study (radiograph number 9, and radiograph number 12, Figure 2-10) as interspersed heterogeneous accumulations mixed with feces or as very opaque fecal balls.

The fecal sand collection procedure was performed without difficulty. Of the 3 trials to assess the accuracy of the procedure, 457 g, 483 g, and 477 g of sand were recovered following the addition of 500 g of sand to sand-free feces. This represents an average of a 94% recovery rate. Initial observation of the collected sand from the trial horses revealed that the majority of the sand was fine particles throughout the duration of the study. Four out of ten horses (3 Group 2 horses and 1 Group 1 horse) had coarser sand particles (2-5 mm) being passed at Day 6 of the study as the predominant type of passed sand. The amount of passed sand over time was compared among the 10 horses (Figure 2-11). There is a significant difference ($p = 0.028$) between the 2 groups for sand output over time. Group 1 shows an increase in sand output that begins by Day 2.5, peaks at Day 3.5 and diminishes again by Day 4.5. Group 2 shows a brief and mild initial increase in sand output at Day 2, and then diminishes until Day 4.5 where it begins to increase and does not decline by the end of the study.

Subjective and objective assessments were performed on every set of radiographs as described in the retrospective study. The data was determined to be normally distributed. When comparing the subjective scores for each radiograph between right and left lateral views, no significant differences were found ($p = 0.48$). Similar results were obtained in the comparison of the objective scores for each radiograph between the right and left lateral views ($p = 0.73$). Due to these findings, only 1 radiographic view was deemed necessary to be used for the remainder of the statistical analyses. Right to left lateral views (termed as left lateral views) were selected to be utilized because the majority of the instances (31/32) when only 1 view was obtained, it was a left lateral view.

Significant differences were not observed between the 2 groups of horses using subjective scoring ($p = 0.19$, power 25.6%). Significant differences were present between the 2 groups of horses using objective scoring methods ($p = 0.0003$, power 96.0%). Since the subjective scores did not show any statistical difference for the sand accumulations between the 2 groups, only objective scores were used for the remainder of the analyses.

Trends of the objective scores over time between the 2 groups are demonstrated (Figure 2-12). Both groups show an early high score (6/12) on Day 0.5 which represents the average score due to the sand within the stomach following nasogastric intubation. Neither group reaches this score again (on average), however it is clear that Group 2 has higher objective scores on average than does Group 1 ($p = 0.0003$). Both groups are seen to have their highest average objective score assigned on Day 3 - 3.5 followed by a gradual decrease in the score. In Group 1, the highest score obtained was a 6/12 (Horse #

3 and 4). In Group 2, the highest score obtained was an 8/12 (Horse # 6 and 10) and was seen in both horses that showed signs of colic. The 3 other horses in Group 2 (Horse # 7, 8, and 9) did receive scores of 7/12 (Horse # 7 and 8 on Day 3 only, Horse # 9 on Day 4 and 4.5) but never showed any signs of colic.

When comparing the individual objective parameter score over time between the 2 groups, interesting observations are made. When looking at the location of the sand over time (Figure 2-13), both groups show a similar distribution until Day 3 when Group 2 apparently retains sand in the cranioventral abdomen longer than Group 1. Both groups do not attain an average score of 1 (meaning a cranioventral distribution of sand) until Day 2. There is no statistical difference between the 2 groups, however, based only on the location of the sand accumulation ($p = 0.64$).

The number of sand accumulations does show a significant difference between the 2 groups ($p = 0.033$). Group 2 tends to show a consistently higher score than Group 1, especially noticed at Day 4.5 (Figure 2-14) where Group 1 on average decreases in score and Group 2 on average increases in score.

The opacity of the sand accumulations does show a significant difference between the 2 groups ($p = 0.048$). Both groups obtain the highest score possible on Day 0.5, coinciding with the sand in the stomach (Figure 2-15). Following this, neither group attains this score again, with Group 2 having a persistently higher opacity score than Group 1.

Sand homogeneity also shows a significant difference between the 2 groups ($p = 0.046$). Both groups obtain the highest score possible on Day 0.5, coinciding with the sand in the stomach (Figure 2-16). Following this, neither group attains this score again, with Group 2 having a persistently higher sand homogeneity score than Group 1 especially following Day 4.

Although the graph of length of sand accumulation over time appears different for Groups 1 and 2 with Group 2 having a higher mean score than Group 1 (Figure 2-17), there is no statistical difference ($p = 0.081$).

The thickness of the sand accumulations also does not show any significant differences between the 2 groups ($p = 0.098$), although graphically there does appear to be a trend with Group 2 having a higher mean score for sand thickness than Group 1 (Figure 2-18). One note of interest is that Group 2 on average surpasses their Day 0.5 score on Day 3 and 3.5. The highest average score for Group 1 is on Day 0.5 corresponding to the sand present in the stomach.

When examining the objective score based on the amount of intra-abdominal sand, (Figure 2-19), a significant difference is not observed ($p = 0.25$) when comparing the 2 groups. There is also a poor correlation between the objective score based on the amount of intra-abdominal sand only when combining the 2 groups together ($R^2 = 0.19$), or comparing as separate groups (Group 1 $R^2 = 0.01$, Group 2 $R^2 = 0.13$). Due to the poor correlation seen at the group level, it was questioned whether a relationship may exists at the individual level. This was still not the case as the highest R^2 value calculated was 0.44 in Horse #3.

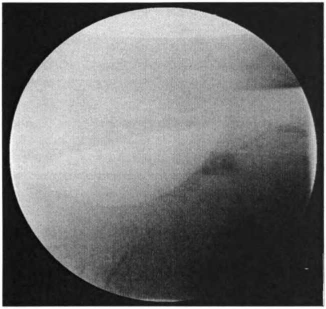
A trend between objective score and intra-abdominal sand weight was then questioned by examining this relationship across the different days to see if a particular day during the experiment had a better correlation. There are weak correlations on Day 2, 5.5, and 6 with R^2 values of 0.57, 0.67, and 0.66 respectively. When examining each group separately at each day, some trends are noticed between the objective score and the amount of intra-abdominal sand. A correlation is never seen in Group 1. Group 2 does show weak-moderate correlation between objective score and intra-abdominal sand weight on Day 2.5, 3, 3.5 and 4 with R^2 values being 0.55, 0.50, 0.51 and 0.75 respectively. Group 2 has higher R^2 values (stronger relationships) than Group 1 for 9/12 (75%) of the sessions radiographed.

Division of the objective scores into their individual parameters was performed to see if any relationship exists between these 6 parameters that form the objective score and the amount of intra-abdominal sand. Again, there was no correlation observed at this level as the highest R^2 values were obtained for the location of the sand within the abdomen (0.26) and the length of the sand accumulation (0.24).

Comparing the individual score parameters to each other, combining both group data, also did not show any significant correlation except a weak correlation between sand opacity and sand homogeneity ($R^2 = 0.5$).

Figure 2-2

One of three mid-abdominal accumulations of sand in Horse #10 (radiograph view #6) on Day 5. It is more well-defined, opaque and homogeneous than other Group 2 accumulations. It is also located in a different area within the colon than most other accumulations. Digesta within the ventral colon is seen below (ventral to) the sand. The sand is presumed to be in the dorsal colon. Labeled image is below.



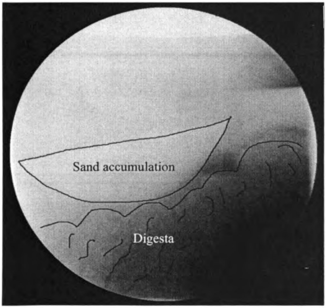
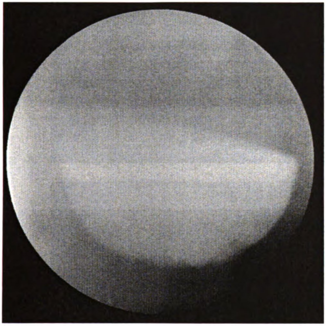


Figure 2-3

Radiograph (view #4) of sand within the stomach (Day 0.5) following nasogastric intubation and administration of 4 g/Kg sand (top). It appears well defined, opaque and homogeneous. Labeled image is below.



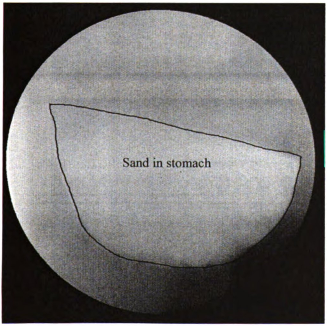
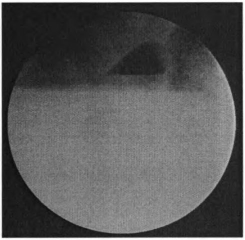
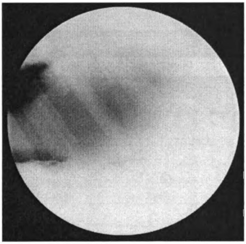


Figure 2-4 a-c

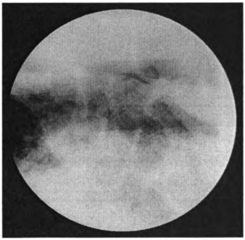
Typical appearance of the craniodorsal and mid-dorsal abdomen (radiographs # 7, 10, and 11) demonstrating gas-filled intestines, ribs, and vertebrae.



a.



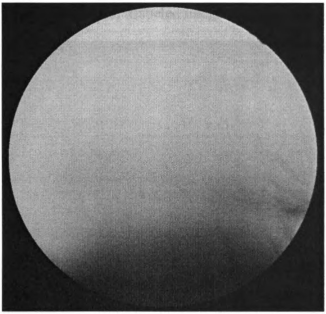
b.



c.

Figure 2-5

Sand accumulation within the ventral colon (radiograph view #2) illustrating haustra as vertical radiolucent bands. Labeled image is below.



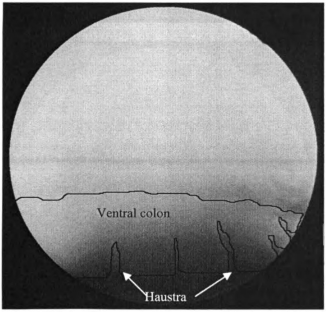
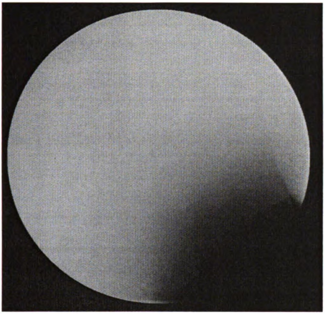


Figure 2-6

Typical appearance of the mid ventral colon (radiograph number 3). Scattered small sand accumulations are visible within the colon, presumably the pelvic flexure, alongside the flank of the horse. Labeled image is below.



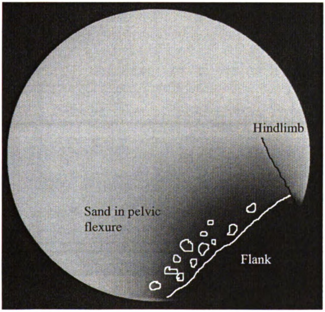
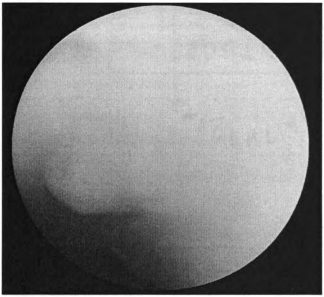


Figure 2-7

Radiograph of sand within the cranioventral abdomen (radiograph view #1) illustrating the diaphragmatic flexure and the sternal flexure with the latter being slightly more caudal and ventral than the former. Labeled image is below.



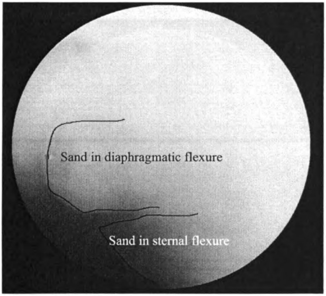
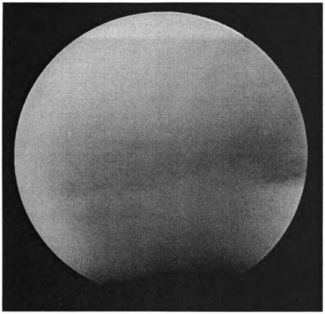


Figure 2-8

Typical appearance for sand accumulation within the mid-ventral colon (radiograph view #2) of Group 2 horses illustrating a large, well-defined opaque and homogeneous accumulation. Labeled image is below.



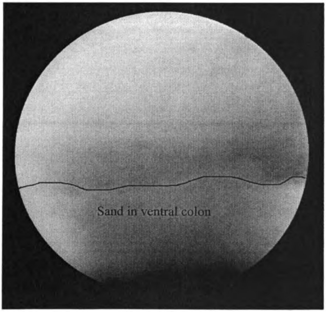
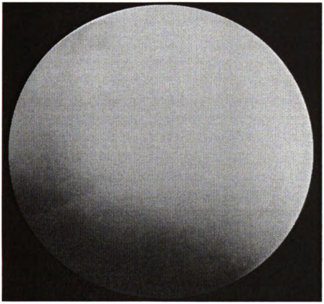


Figure 2-9a

Typical appearance for sand accumulation within the cranioventral colon (radiograph view #1) of Group 1 horses illustrating dispersed opaque sand particles within digesta and no focal accumulation. Costal cartilages are seen in the bottom left aspect of the image, cranial and ventral to the colon. Labeled image is below.



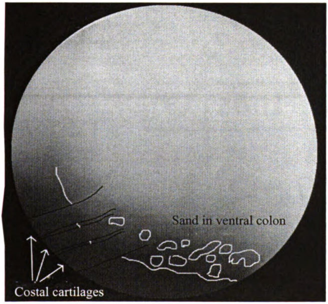
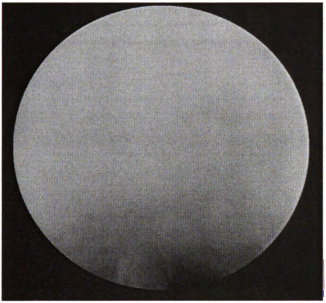


Figure 2-9b

Typical appearance for sand accumulation within the mid-ventral colon (radiograph view #2) of Group 1 horses illustrating small, poorly-defined heterogeneous accumulations. Labeled image is below.



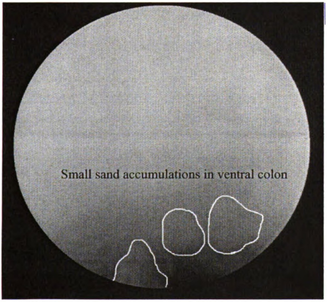
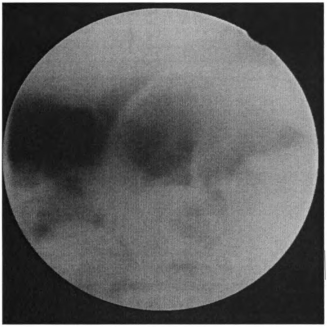


Figure 2-10

Radiograph of sand seen within the small colon (radiograph view #12) illustrating opaque feces just cranial to the pelvis. Labeled image is below.



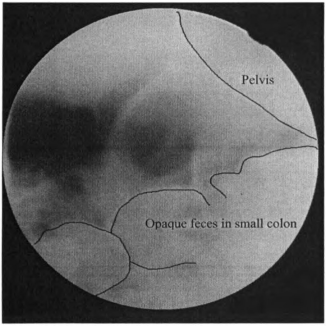


Figure 2-11

Group 1 and Group 2 average fecal sand output over time. Y-error bars denote the standard error of the means.

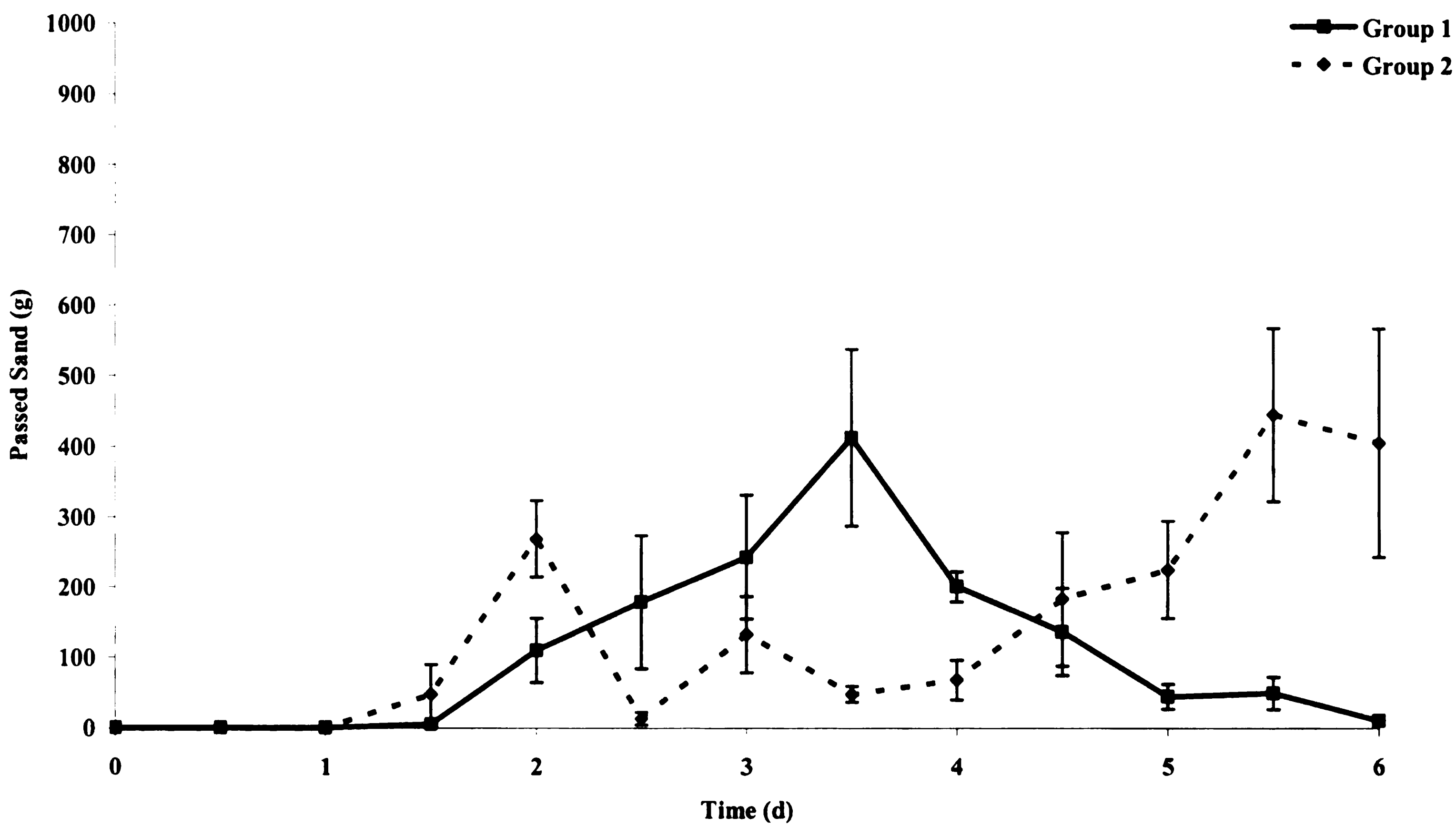


Figure 2-12

Group 1 and Group 2 average objective score trends over time. Y-error bars denote the standard error of the means.

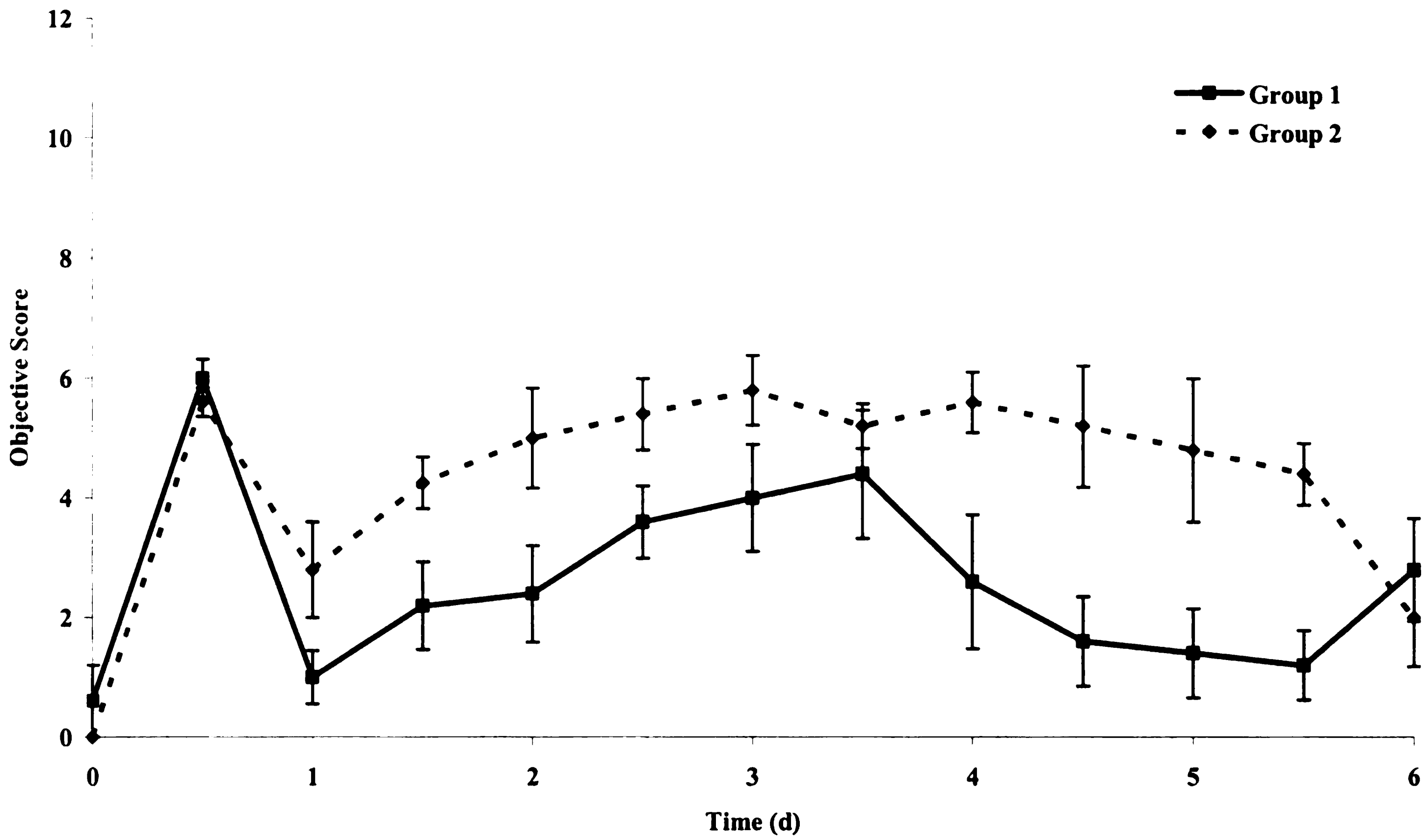


Figure 2-13

Group 1 and Group 2 average sand location score over time. Y-error bars denote the standard error of the means.

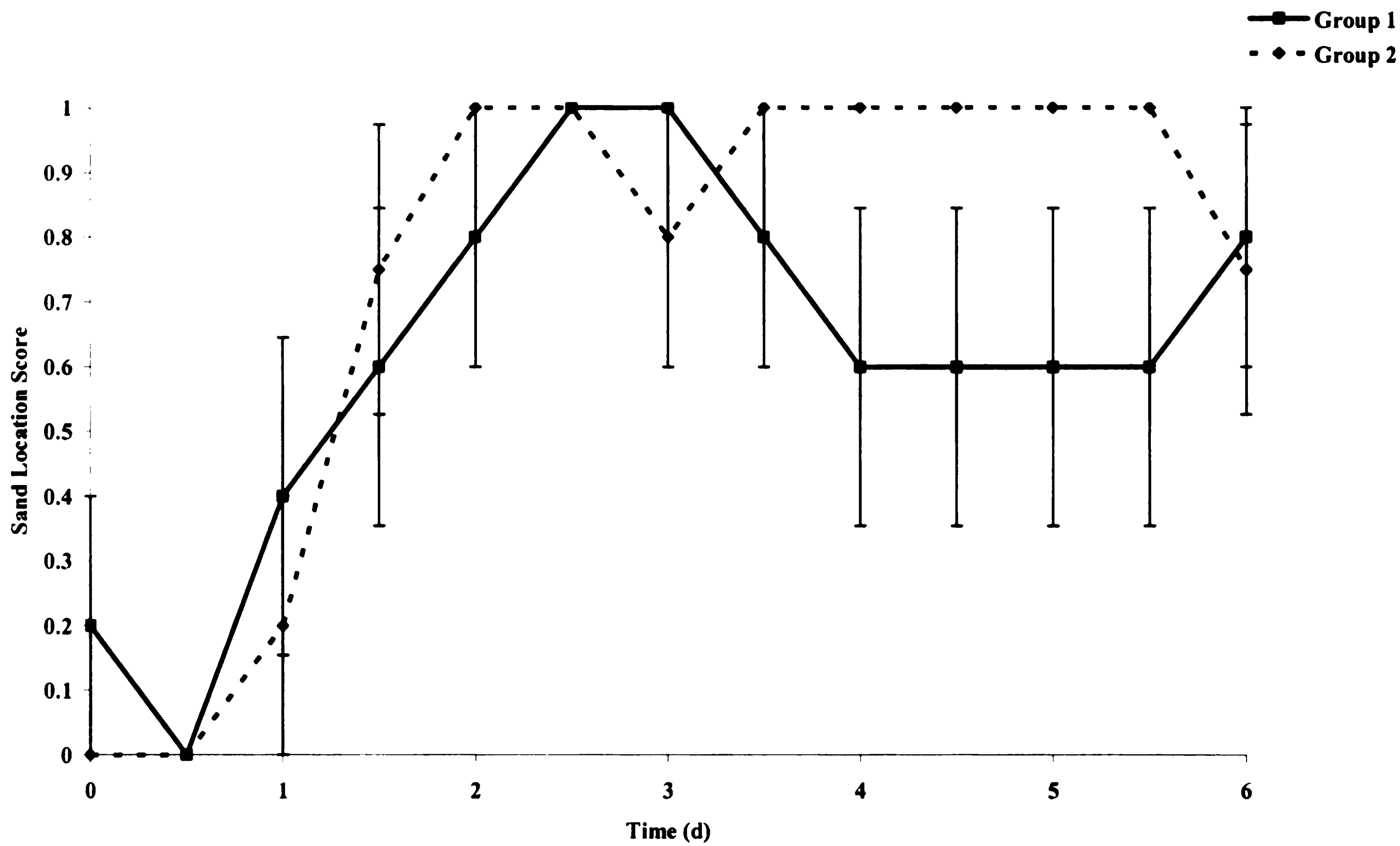


Figure 2-14

Group 1 and Group 2 average score for number of sand accumulations over time. Y-error bars denote the standard error of the means.

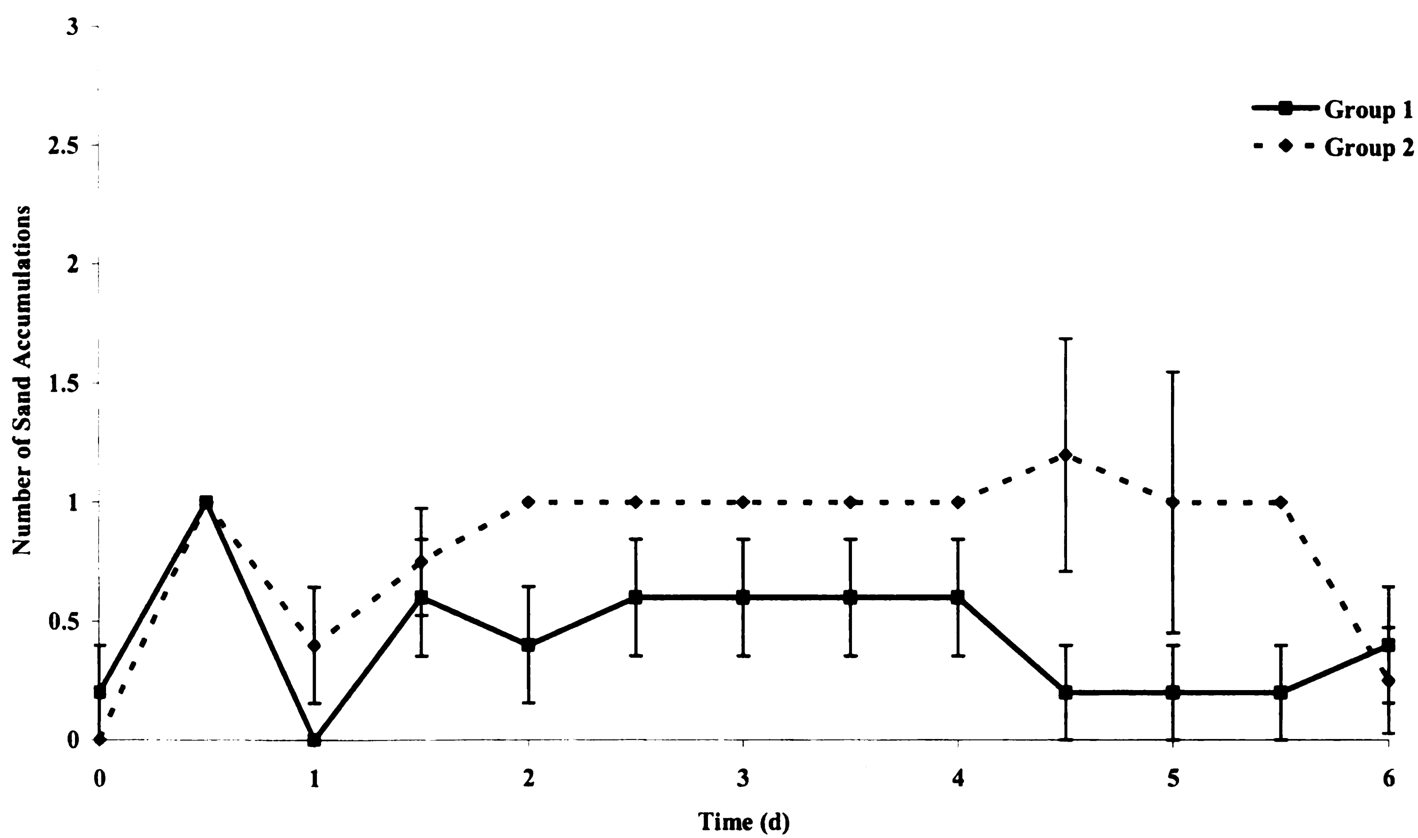


Figure 2-15

Group 1 and Group 2 average sand opacity score over time. Y-error bars denote the standard error of the means.

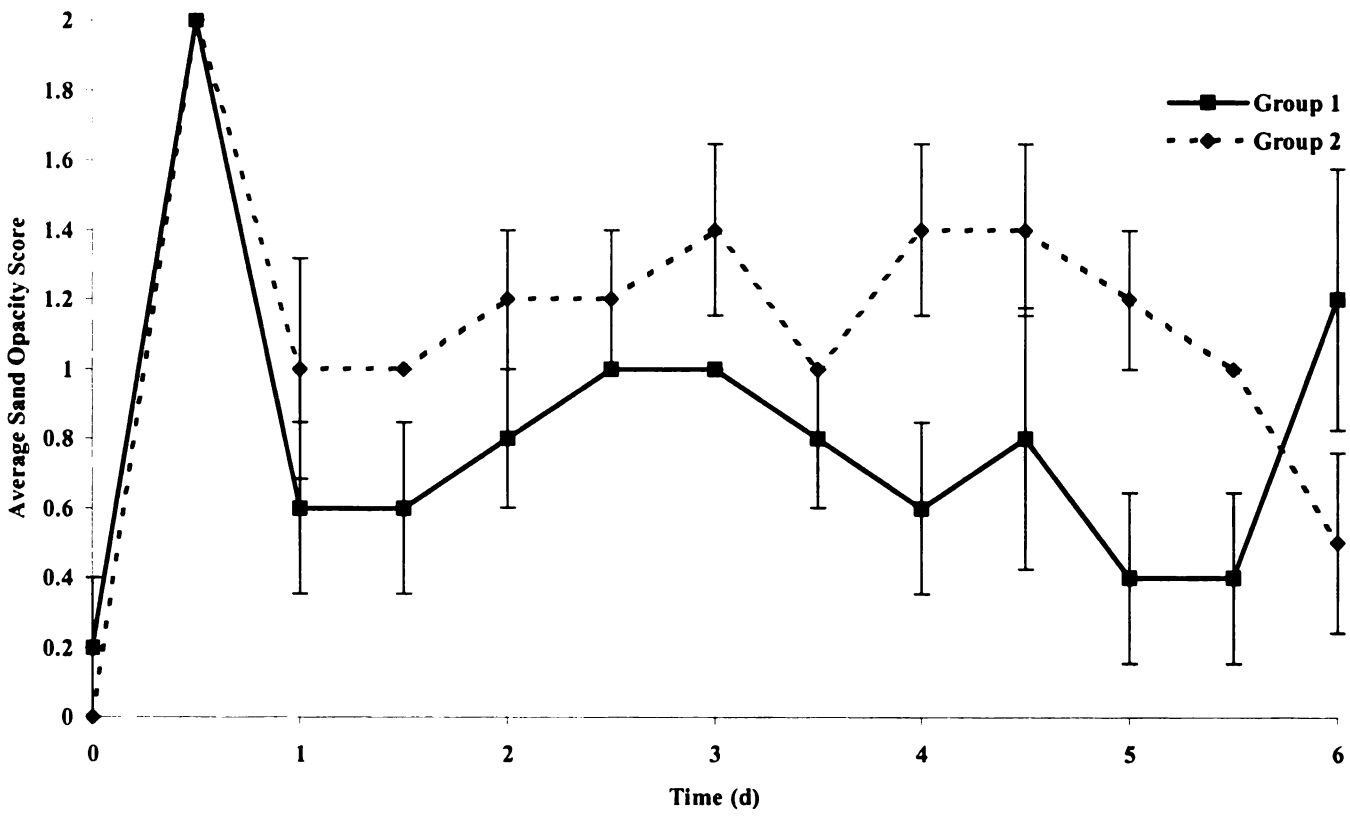


Figure 2-16

Group 1 and Group 2 average sand homogeneity score over time. Y-error bars denote the standard error of the means.

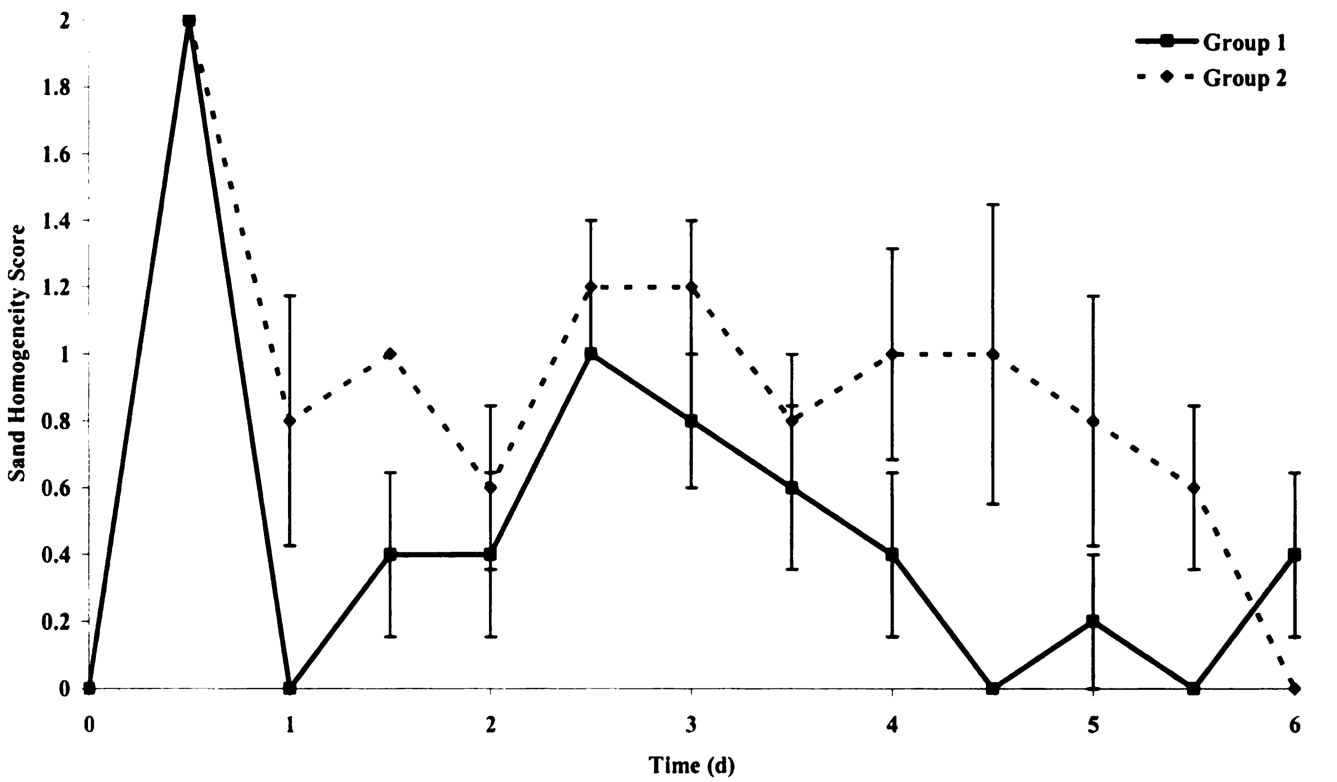


Figure 2-17

Group 1 and Group 2 average sand length score over time. Y-error bars denote the standard error of the means.

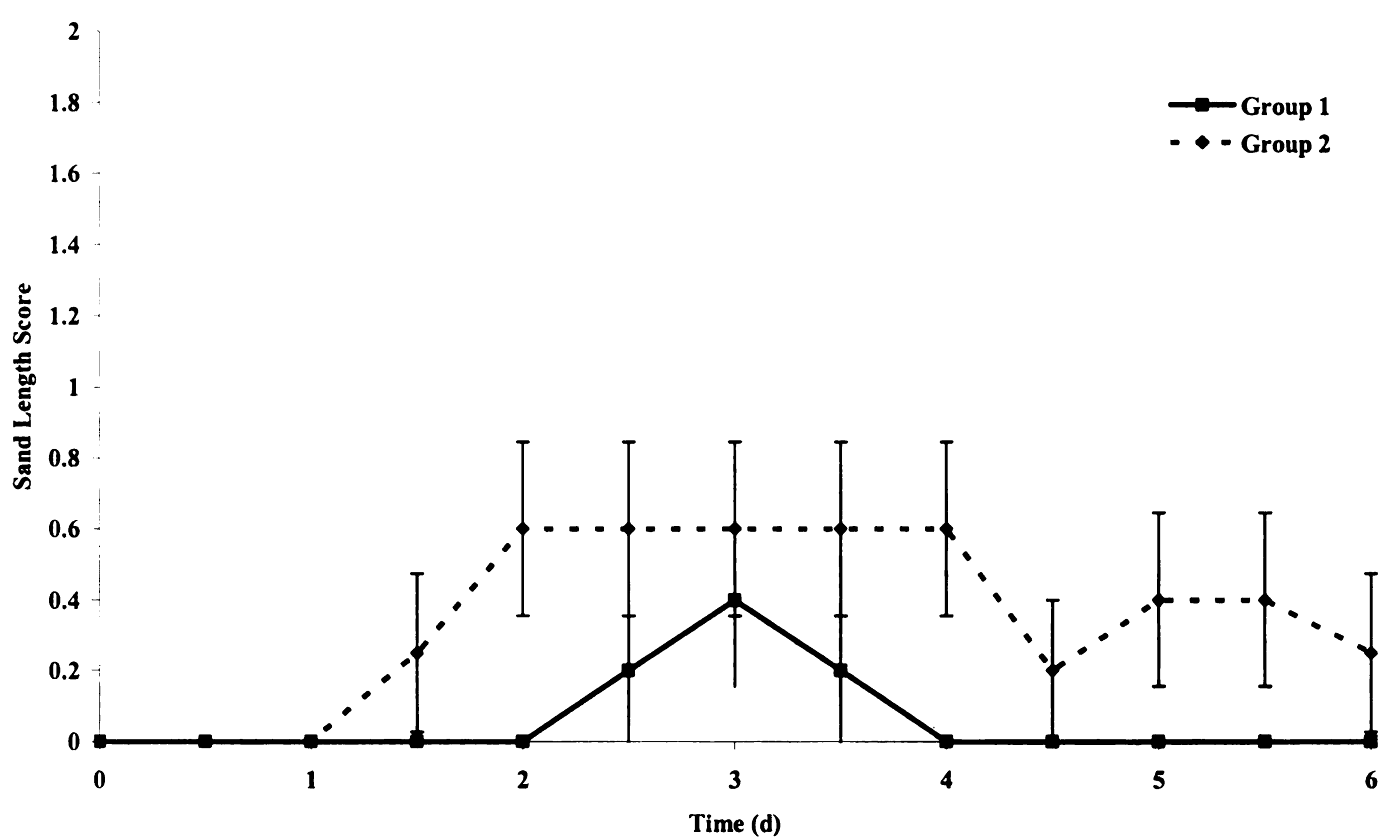


Figure 2-18

Group 1 and Group 2 average sand thickness score over time. Y-error bars denote the standard error of the means.

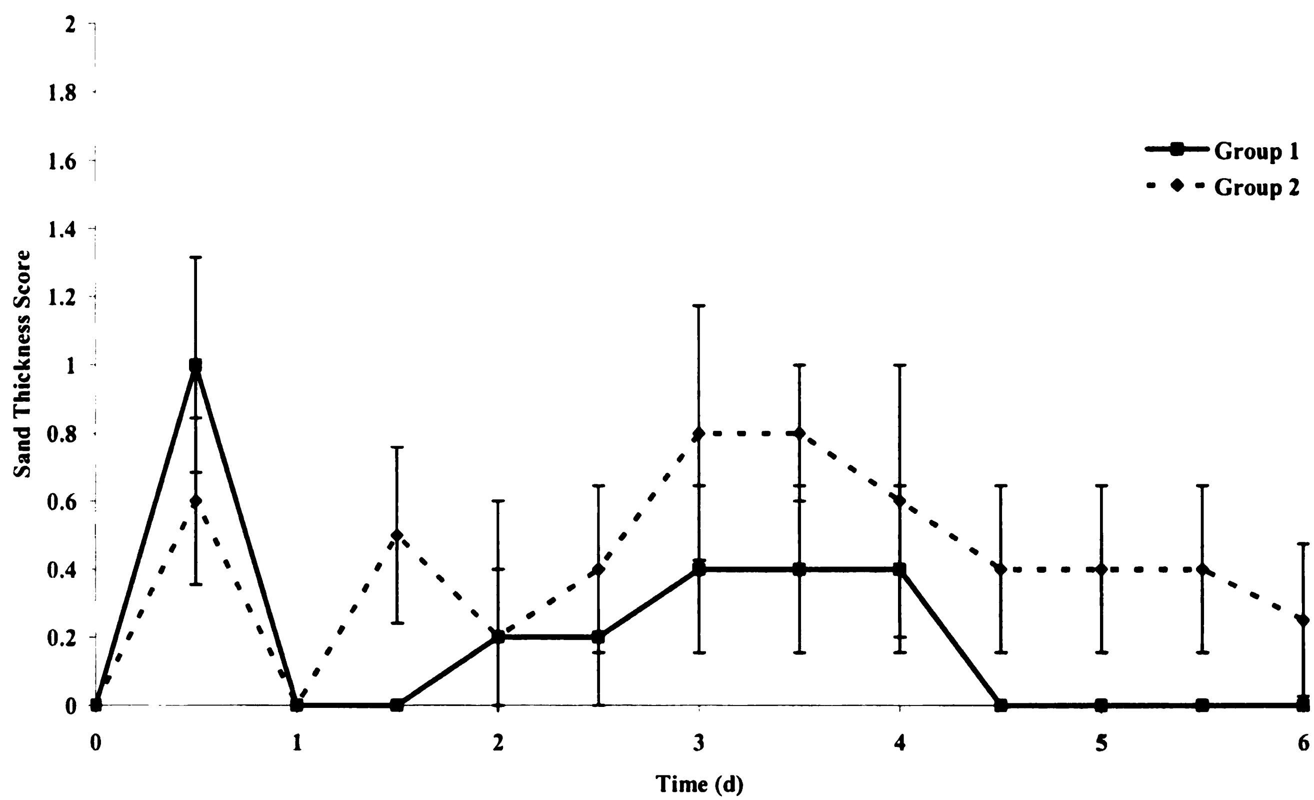
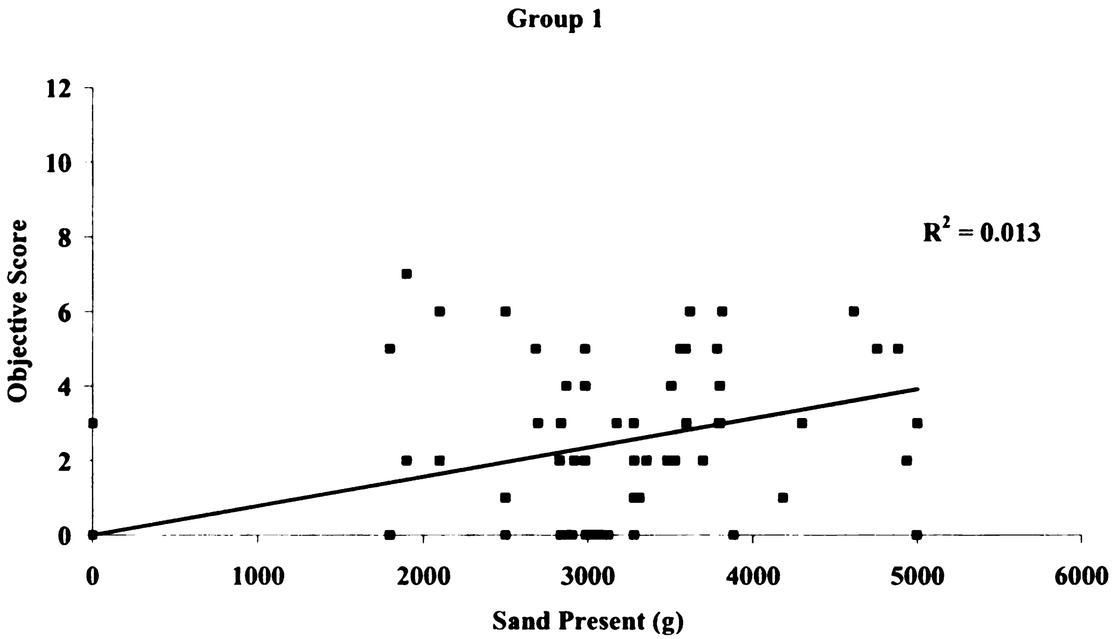


Figure 2-19

Group 1 and Group 2 objective scores based on weight of intra-abdominal sand.



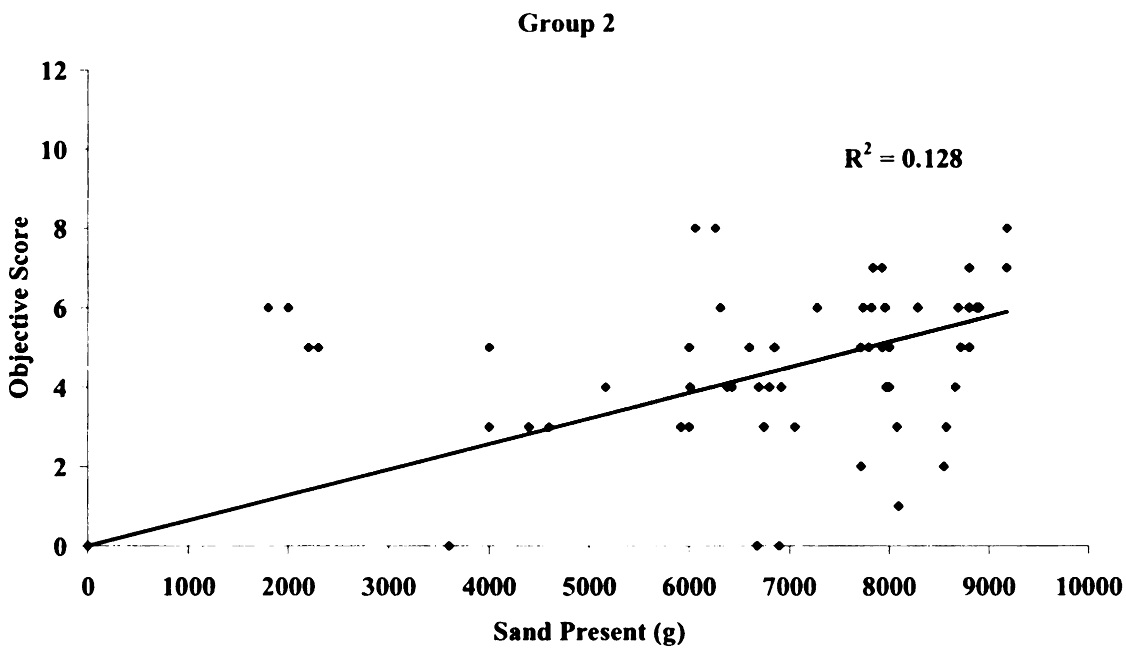
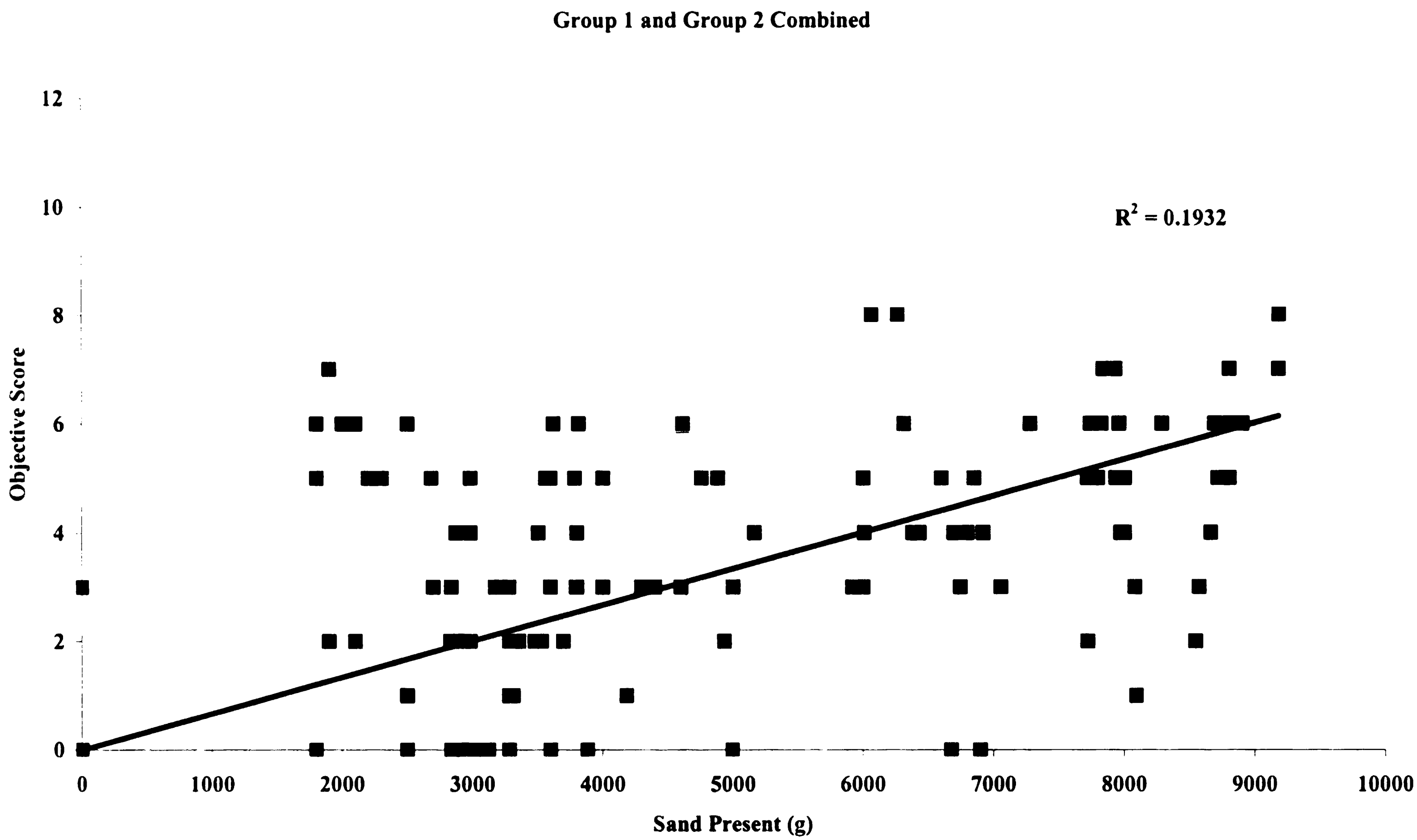


Figure 19 (con't).



Discussion

All horses in this study began the experiment healthy and 9/10 were discharged healthy. One of these 9 (Horse #6) experienced mild colic during the study (Day 2) but was responsive to medical therapy. It is presumed that the colic was the result of the 4 intubations with sand administration with secondary mild enteritis / colitis. Horse #10 was euthanized on Day 6 due to progressive deterioration from colic that was non-responsive to medical therapy. A necropsy was not performed however it is presumed that the colic was the result of sand accumulations within the right dorsal colon causing partial obstruction in this horse. Two medium-large accumulations of sand were seen in areas that were not seen to contain sand in any of the other horses (Figure 2-2). There are several confounding factors regarding this particular horse that may have predisposed it to colic and are discussed below.

From these results, it can be assumed that administering sand at this dose and through this method is generally safe, however, close attention should be given to the horses receiving the twice-daily doses especially at Day 2 - 3 of the study. Close monitoring is paramount for the health of the animals to make sure they are eating, passing feces and maintaining adequate intestinal motility and hydration. If such adverse clinical signs occur, prompt medical therapy is advised and further sand administration is discouraged. The serial radiographs in this study aided in the interpretation of the colic in Horse #10 and can provide useful information as to the etiology of the underlying problem.

The technical aspects of this study were not difficult, but time consuming. The nasogastric intubation and sand administration procedure required careful attention so as not to administer too much water with the sand, which would over-distend the stomach. The sand required a lot of water (up to 15 liters) to be mixed with to prevent settling in the tube. Other options for sand administration were considered however the method used was felt to be the best. Sand could have been administered through repeated gastrotomy but this was considered too invasive. Sand could also have been administered by nasogastric intubation and mixing with carboxymethylcellulose, which forms a gel-like substance that would help in preventing the sand from precipitating from the water. Carboxymethylcellulose was not used as it may affect gastro-intestinal motility and would then not provide an accurate representation of natural clinical conditions of sand enteropathy.

The radiographic procedure was relatively easy to perform, especially once the horses became used to the radiographic stocks. Because some horses exhibited fear and anxiety of the stocks and subsequently became stressed and agitated, only one lateral radiographic view could be obtained on occasion (25%). This was found to not be a problem in interpretation and assessing the intra-abdominal sand as the results indicate that there is no value in obtaining radiographs from both sides of the abdomen for sand accumulation. One view appears to provide the same assessment as the other view does. There are slight variations in the shape and size of the accumulations, however these are not significant.

One of the problems encountered during the radiographic procedures was the rapid heating of the x-ray tube and the required time delay for cooling. The thick body parts, large amount of ingesta and secondary scatter radiation required that very high exposure factors be used so that sufficient x-rays reached the automatic exposure control.

Since this was done at a rapid frequency (12 views per side took about 1-2 minutes), anode heating developed and maximum tube heat units were often reached. The limit on the load (KV, mA, sec) that can be safely accepted by an x-ray tube is a function of the heat energy produced during exposure, because above a certain temperature, the tungsten anode may vaporize. The total heat produced during an exposure is a product of voltage, current and exposure time expressed in the unit of joules (Curry *et al.*, 1990). The ability of an x-ray tube to make a single exposure of certain duration is termed its kilowatt rating and is specific for each x-ray tube and should be consulted before using high exposures such as in the current study. Using the exposure settings, tube heat units for one exposure in this study ranged from 11,000 J – 27,500 J depending on the area of the abdomen being radiographed. The automatic exposure control adjusted the time needed to expose the phototimer according to the specific area of the abdomen. The maximum allowable tube current decreased as the tube accumulated heat. This was generally noticed by the end of the first set (left lateral view) of 12 radiographs and through the middle of the second set (right lateral view) of 12 radiographs. Maximum exposure limits were usually met from radiograph number 4 (central abdomen) and onwards indicating that the x-ray tube was using the maximum allowed mA, given the selected KVp, to expose the phototimer. This indicated that the kilowatt rating was being approached for this particular x-ray tube. It was for this reason and due to the homogeneity of the subject that the central abdominal radiographs were poorly exposed. Radiographs after this point, despite containing a moderate amount of gas within the field of view, which would not require high exposures, still resulted in maximum tube exposure limits being reached because of the heated anode that automatically lowered the maximum allowable mA.

Another related factor is the anode heat storage capacity that is the ability of an x-ray tube to withstand heat loading over an extended period of time. This is commonly experienced during fluoroscopy used in the present study between every radiograph to localize the desired image. The anode heat storage capacity indicates the duration of time an anode can be functioning at a particular energy (J) level before causing excessive anode heating. Once an anode has reached its maximum heat capacity, it must cool before further use to prevent damage to the anode. The time required for anode cooling is also dependent on the anode heat storage capacity and the temperature the anode reached as heat loss is proportional to the fourth power of the temperature (Curry *et al.*, 1990).

The anode used in this study has a heat storage capacity of 333,000 J and the x-ray tube has a kilowatt rating of 105 – 120 using a 1.2 mm focal spot at 0.2 - 0.5 seconds of exposure (General Electric, 1990). Since the radiographs were producing approximately 11,000 – 27,500 J of heat to the anode per exposure, 12 exposures approximate a total of 132,000 – 330,000 J to the anode per radiographic series. Added on top of this heat is the heat produced by the fluoroscopy that created approximately 28,800 J (using fluoroscopic techniques of 120 KVp and 4 mA and assuming an average fluoroscopy time of 1 minute) to obtain a complete radiographic series. Total heat produced per lateral radiographic series per horse ranged from 160,800 – 358,800 J. This exemplifies how the anode storage capacity was reached fairly routinely for each radiographic series and required subsequent cooling (10 minutes) before it was ready for re-use (General Electric, 1990).

Although the above problem and details are specific for this particular study and protocol, it is a common problem in large animal abdominal radiography to have underexposure and poor quality radiographs if the x-ray tube and generator are not powerful enough (see Introduction, Chapter 1). Limitations in radiographic equipment result in further limitations in the ability to correctly interpret the radiographs which is vital in the current sand accumulation study. Underexposure may provide false negative diagnoses, or objective scores that are also falsely low. The limitations of the x-ray tube in the mid-central abdomen in these horses may be confounding the obtained results. The added time required to allow for anode cooling increases the stress on the horse which may make it more difficult to manage for additional views. These are acknowledged concerns of the study, however the retrospective part of the study demonstrated that the parameters used in this objective scoring system do have high correlations with the final diagnoses and the devised test is a more sensitive and accurate method for radiographic assessment of sand accumulation than current subjective methods (see Discussion, Chapter 1).

Two of the main concerns of this study from the onset were the ability to experimentally mimic naturally occurring gastro-intestinal sand accumulation, and to avoid clinically evident colic given the experimental protocol. Several factors that were involved in these issues were the dose and frequency of sand to be given, the method of sand administration, the type of food and frequency / quantity of food to be consumed, the amount and frequency of water consumption, and the housing and type of bedding for the horses. It was not possible to mimic natural conditions of long-term grazing exposure to sand, however, all of the above factors were considered.

It would have been desirable for the sand to be consumed orally with the feed; however the amount ingested could not be adequately controlled. One report explains the administration of sand into the cecum through a surgical typhlotomy (Hammock *et al.*, 1998); however this method would result in the sand bypassing the stomach and small intestines. This method may have provided answers as to whether the objective scoring system is sensitive to varying known amounts of colonic sand; however this current model was designed to be useful for the typical radiographic presentations that are commonly seen by the large animal practitioners where the sand is not necessarily all accumulated in the colon. It was decided therefore that the sand be given through nasogastric intubation which was also previously described with good results (Ragle, Meagher, Schrader and Honnas, 1989). This was done knowing that nasogastric intubation and sedation might have an effect on gastric emptying times and motility where it has been known to delay such factors (Mahaffey and Barber, 2002; Doherty *et al.*, 1999).

Alpha-2 adrenergic agonists such as xylazine and detomidine exert their analgesic effects on the central nervous system and the enteric nervous system (Merritt *et al.*, 1998). These drugs inhibit intestinal motility because they act by binding to the presynaptic receptors, inhibiting the release of acetylcholine thereby inhibiting intestinal contraction (Lester *et al.*, 1998). The effects of xylazine or detomidine on the proximal gastro-intestinal tract (stomach and small intestine), in particular, remains controversial where some propose an inhibitory effect on motility (Doherty *et al.*, 1999; Adams *et al.*, 1984) while others indicate a neutral or stimulatory effect (Merritt *et al.*,1989). This suggests that there may be a dose-dependant-response within different segments of the proximal gastro-intestinal tract (Lester *et al.,* 1998). It has been proposed that xylazine may induce increased amplitude of mixing contractions of isolated segments of small intestines (Stick *et al.*, 1987). Xylazine and detomidine are also known to inhibit cecal and colonic (especially the ventral colon) motility and cecal blood flow (Roger and Ruckebusch, 1987; Adams *et al.*, 1987; Rutkowski *et al.*, 1989; Clark *et al.*, 1988; Lester *et al.*, 1998) with detomidine being accepted to be a more potent and longer lasting alpha-2 adrenergic agonist than xylazine. Xylazine typically exerts its effects for 20-40 minutes while detomidine may last up to 1-2 hours. Mean amplitude, duration and number of contractions of the circular and longitudinal smooth muscle are affected, and the effects appear to be proportional to the dose (Clark *et al.,* 1988). For these reasons, alpha-2 adrenergic agonsist are discouraged from use in cases of cecal or colonic dysfunction such as impactions. Alpha-2 adrenergic agonists also exert systemic effects such as bradycardia, reduced cardiac output and transient hypertension followed by hypotension which are mediated through vascular smooth muscle receptors causing vasoconstriction (Clark *et al.,* 1988).

The horses in this experimental study were sedated with either xylazine or detomidine at similar doses as reported by Merritt *et al., (*1998), (0.5 mg/Kg and 0.01 mg/Kg respectively). The ramifications of these previous studies would likely not have a significant effect on the results of this study as sedation was given only for the nasogastric intubation and sand administration procedure. An increase in gastric emptying times and small and large intestinal transit times over the period of 1 hour would most likely not have any effect on either the radiographic appearance of the sand at 12-hour intervals, or in the amount of sand passed in 12-hour periods. All horses subjectively appeared to eat well following sedation and there was no clinical evidence of altered gastro-intestinal motility or fecal production following sedation.

The method of sand administration in this study also differs from the natural setting because this method resulted in approximately 2 Kg of sand being administered at once into the stomach as opposed to slow ingestion of small amounts over a long period of time. It can be presumed that this large volume of sand would have an effect on the gastric emptying rate and on the intestinal transit and subsequent distribution and accumulation of sand throughout the intestines, and may be different than that from small, chronic doses of sand. This method was chosen, with recognition of this fact, based on the ability to know accurate quantities of sand within the gastro-intestinal system, and to decrease multiple procedures involving sedation and nasogastric intubation which may complicate the study and be detrimental to the horses. Subjectively, this large volume of sand appeared to empty rapidly and consistently from the stomach in most horses as it was observed to have moved from the stomach by Day 1 (12 hours post sand administration). As well, the sand distribution within the colon appeared similar to distributions observed in previous clinical cases and in those previously reported (Ruohoniemi *et al., 2001*). The exception to the former statement were Horses # 6 and 10 that were seen to have small amounts of sand still present in their stomachs by Day 1. Interestingly, it was these 2 horses that experienced colic. Perhaps this initial poor (delayed) gastric emptying may have lead to subsequent poor intestinal motility and accumulation of sand resulting in colic. Whether this poor gastric emptying was the result of alpha adrenergic agonist inhibition, or individual variation is not known

Horses that present for sand enteropathy typically have been eating normally up until 1-2 days prior to presentation where they then become anorexic or show signs of colic. In this experimental study, it was desired to keep the horses eating at their normal frequency with their normal type of food since a change in diet may predispose to colic (Cohen, 2002). For 9/10 of the horses, this was *ad libitum* grass hay feedings. We elected to then feed all of these horses *ad libitum* grass hay starting after their last dose of sand. This was done despite the fact that this large quantity of ingesta may obscure small accumulations of sand from being radiographically detectable. It was desired to test the objective scoring system on typical radiographs that may have a gastro-intestinal tract full of ingesta. It is interesting to note that the 1 horse whose previous feeding history was unknown was Horse #10 who experienced moderate colic. This particular horse did not consume as much of the grass hay as the other 9 horses did during the first 2 days of the study and seemed particularly selective in the food it did eat. Its appetite declined during the rest of the study and whether or not the poor appetite prior to and during the sand administration predisposed it to colic is speculative at this point. Perhaps had the horse been fed its usual diet it would of have had a good appetite during the sand administration and not exhibited colic afterwards.

Food was withheld for 6 hours prior to sand administration in order to be sure that the stomach was empty because the sand required a lot (up to 15 liters) of water to be mixed with for passage through the nasogastric tube without settling. Since such a large volume was needed, an empty stomach was required since the equine stomach has a capacity of only 8-20 liters (Pfeiffer and MacPherson, 1990). A previous study demonstrated that the motility in the proximal gastro-intestinal tract is unaltered between fed or 24-hour fasted horses (Merritt et al., 1989). Water was also not restricted in this study as it was desirable for the horses to have continual access to water as they would in a clinical setting.

The dose of sand used in this study was determined through previous references (Hammock *et al.,* 1998; Ragle, Meagher, Schrader and Honnas, 1989) that were deemed to be safe with no signs of colic. It was desired that there be enough sand to be visualized on radiographs, a volume that has not yet been determined, but yet not enough to cause clinical disease. The protocol used in this study was modified slightly from the previously described protocols. Ragle, Meagher, Schrader and Honnas, (1989), administered 4.2 g/Kg sand through nasogastric intubation mixed with 9 ml/Kg of carboxymethylcellulose once a day for 5 straight days with no ill effects. Hammock *et al.*, 1998 administered 10 g/Kg sand into the cecum once and followed the horses through for 11 days with no ill effects. This study's protocol was to administer by nasogastric intubation 4 g/Kg sand mixed with water once (Group 1) or twice (Group 2) a day for 2 consecutive days. From this protocol, 2 horses showed signs of colic. This study's dose is similar to Ragle, Meagher, Schrader and Honnas, (1989), however carboxymethylcellulose was not used. Carboxymethylcellulose may reduce the irritation normally caused by sand by reducing the abrasion between the sand and the intestinal mucosa (Ragle, Meagher, Schrader and Honnas, 1989). This may account for the fact that 2 of the horses in the present study showed signs of colic, however carboxymethylcellulose is not a natural food consumed by horses and its presence may affect the appetite, motility or radiographic appearance of the sand so it was not used. Also, in the present study, the Group 2 horses received the same dose of sand but at double the frequency as Ragle, Meagher, Schrader and Honnas, (1989) study. Although the total volume of administered sand was the same (<10 Kg), this was accomplished in half the time as the previous study. This again may account for the colic experienced by 2 of the Group 2 horses while none of the Group 1 horses showed any adverse effects. In comparison to Hammock *et al.*, (1998) study, the total volume of sand administered to Group 2 horses was similar (10 Kg) however it was spread out over a 2 day period and was administered to the stomach and not all at once into the cecum. This study's protocol for sand administration was felt to be safe since it was not going above and beyond the total dose that the horses in Hammock *et al., (1998)* received which all had no ill effects. The fact that 2 of the Group 2 horses experienced colic demonstrates that it is not necessarily the total volume of sand that is responsible for causing colic, but is also due to its effects on passage through the entire gastro-intestinal tract and over repeated ingestions that eventually results in disease.

The selected type of housing and bedding could have an effect on the obtained results. It would have been preferred to keep the horses outdoors on their normal grass pastures, which is the typical setting for sand colic cases, however since this was a controlled experiment, it was desired that the horses be kept indoors on cement floors so that there was no access to any sand that may occur in the outdoor soil. Commercially packaged wood shavings were used as bedding so as not to risk any sand being present in the bedding that may occur with cut hay. Since changing the housing environment may induce behavioral changes or alterations in the feeding patterns of animals that may lead to colic (White, 1990), especially a change from a pasture to a stall (Cohen, 2002), this was a consideration during the study. Only Horse #5 (17-year old Arabian mare donation) did not experience a change in housing, bedding, or feeding styles as she had been housed for some time prior to the study at the Large Animal Clinic Veterinary Teaching Hospital. Most of the horses were noticed to occasionally paw the shavings, which was interpreted as boredom from the change of housing being removed from an outdoor pasture setting. Aside from this, no conclusions can be made as to any adverse effect of the change of housing or bedding on the animals' behavior or appetite as there was no apparent clinical change in any of these factors. Again, only Horse #10 might be the exception as he did experience colic that may be attributable to the change in housing with resultant anorexia.

Of the risk factors for colic, signalment has been positively associated with certain types of colic (Cohen, 2002); however sand colic in particular does not appear to have any predisposing signalment factors. In general, older horses are at an increased risk of colic as are Arabians (Cohen, 2002; Kaneene *et al.*, 1997). The horses used in this study were of varying age with the oldest being 26 years old who did not show any ill effects throughout the study. The Arabian mare (Horse #5) also did not show any adverse effects. The age and breed dispersion of the 10 horses was not a controllable factor in this study, however they were all adults with breeds (Quarterhorse, Thoroughbred, Arabian, and Standardbred) typical of presenting with sand colic at the Large Animal Clinic Veterinary Teaching Hospital and also observed in a previous study (White and Lessard, 1986). The one variant in the signalment of the experimental horses was Horse #10 who was the only gelding in the study, as the rest were mares. This is most likely not the reason for the moderate colic observed in this horse, however, it is another confounding factor with this horse in particular. Kaneene *et al.*, (1997), concluded that geldings were at a reduced risk for colic; however this appears to be controversial. Equid activity may also be a predisposing risk factor for colic (Kaneene *et al.,* 1997), as racing horses may be at a reduced risk. Horse #10 was a previous racing Thoroughbred prior to the study however due to the carpal chip induced lameness it was retired from such activity. All other mares were pasture horses.

It was desired that the sand used in this study be representative of the sand that is found in Michigan soil. Michigan sand is typically a silicate type that is either glacier deposit and is sharp and not weathered, or is a dune deposit that is rounded and weathered. Sand particle size in Michigan ranges from 2 µm – 2 mm (Warncke, personal communication). The sand used in this study was "fill-type", unprocessed sand whose range in particle size and distribution appears to be representative of typical Michigan soil sand. This was an important factor in the design of this project as the design was to try and mimic, as close as possible, natural conditions of sand colic. Although not referenced, it is assumed that different sized sand particles may be more or less likely to result in colonic accumulation and/or irritation resulting in colic. Larger particles may settle-out from the ingesta faster than smaller particles, which may also become incorporated within the digesta and passed out through the feces. It has been reported that coarse sand frequently accumulates in the right dorsal colon, transverse colon and pelvic flexure, and fine sand typically accumulates in the left and right ventral colon, and associated sternal and diaphragmatic flexures (Snyder and Spier, 1992). Although sand particle size could not be evaluated on the abdominal radiographs of the current project, 3/5 of the group 2 horses and 1/5 of the Group 1 horses showed evidence of passing predominantly large-size sand particles at the end of the study (Day 6). This does appear to give credence to the fact that larger particles may be more likely to accumulate and cause colic or to have a longer intestinal transit time as compared to smaller sized sand particles that tend to be incorporated within the colonic digesta and have normal intestinal transit time, providing there is good motility.

Intestinal transit time is intimately linked to intestinal motility. Transit time is defined as the time it takes for material to travel from one portion of the gastro-intestinal tract to another. An increase in propulsive motility decreases transit time, whereas an increase in retentive motility increases the transit time. There are many factors that affect gastro-intestinal emptying rates and intestinal transit times such as the composition of the material (liquid moves through faster than solid food), moisture content (high moisture food empties faster than dry food), fat or fiber content (low fat - high fiber meals have a more rapid gastric emptying rate than high fat - low fiber meals), volume of ingesta, the chemical and physical properties of the ingesta (pH, temperature), various reflex mechanisms (cephalogastric, gastrogastric, gastrocolic, enterogastric), medications (alpha-2 adrenergic agonists such as xylazine), and the presence of pain (enteritis / colitis, colic), emotional stress, noise, anxiety, physical restraint and gastric intubation which can all slow emptying rates and transit times.

Gastro-intestinal motility is carried out through both an extrinsic and intrinsic (enteric) nervous system. The extrinsic nervous system synapses with the intrinsic system to modify its control through parasympathetic and sympathetic pathways. The intrinsic (enteric) nervous system consists of a myenteric plexus that regulates the circular and longitudinal smooth muscle layers, and a submucosal plexus which has hormonal secretory control. The regulation of gastro-intestinal motility is through slow waves of basic electrical rhythm (BER) that produce muscular tone to the intestinal tract through the pacemaker cells of the enteric nervous system. The electric pacemaker for the small intestine is in the longitudinal muscle of the proximal duodenum (Clark, 1990). Slow waves initiated in the longitudinal muscle are conducted to the circular muscle layer at a rate dependent on the electrical coupling between the 2 layers. Spike potentials from the extrinsic nervous system added onto this BER causes contraction of the smooth muscle, resulting in motility (Adams *et al.*, 1984). During fasting, there is a migrating motor complex that sweeps through the gastro-intestinal tract at regular intervals and acts as a housekeeping function to remove indigestible solids and sweep bacteria towards and through the colon. This migrating motor complex is characterized by cyclical alterations between no spike potentials followed by intermittent spike potentials followed by regular spike potentials with propulsion occurring during the intermittent and regular spike potential phases (Adams *et al.*, 1984).

Types of intestinal contractions include peristalsis that are propulsive movements through circular muscular contraction progressing aborally (example: migrating motor complex), segmental contractions that are localized and slow the aboral movement of digesta to increase exposure for absorption and is more prominent in the small intestine, and tonic contractions that are prolonged and function to isolate one segment of intestine from another and also slows aboral movement of digesta (Clark, 1990). Mean transit time (includes gastric emptying and small intestinal transit) to the cecum in fed horses is approximately 3 hours (Clark, 1990). This is the result of gastric slow waves that occur at a frequency of 2.5/min and small intestinal slow waves at 14.5/min (Merritt *et al.*, 1989).

The functions of the stomach, small intestine and large intestine are quite different. The stomach stores and processes food so that it can be presented to the duodenum at a controlled rate. The small intestine allows digestion and absorption of the nutrients. The colon functions to absorb water and electrolytes, store feces and ferment organic matter that escape digestion and absorption of the small intestine. It must absorb a fluid volume approximately the same as the total extracellular fluid during 24 hours (Snyder and Spier, 1996). Both liquid and particulate matter move through the cecum at a relatively fast rate (Snyder and Spier, 1996) as the mean transit time through the cecum for liquid is 5 hours (Clark, 1990).

Progressive motility abruptly decreases in the large colon as transit time through this segment is about 50 hours (Clark, 1990). The ventral and dorsal colons are the major retention sites of ingesta. Much of the soluble and most of the insoluble carbohydrates pass through the small intestine, and therefore are digested in the large intestine. So microbial digestion with volatile fatty acid production in the large intestine supplies a large percentage of the horse's energy requirements. This function of the cecum and colon requires that the digesta be constantly mixed and retained long enough to complete digestion of cellulose. Consequently, resistance to flow of ingesta is apparent at the pelvic flexure and proximal transverse colon with no retrograde movement between dorsal and ventral colon. As particle size increases (2 cm), a selective retention occurs in the dorsal colon.

Motor activity in the large intestine originates in the cecum, proximal right ventral colon, and pelvic flexure (Clark, 1990). The right and left ventral colon and pelvic flexure both have oral and aboral directed motor activity, resulting in retention of ingesta enabling microbial digestion (Snyder and Spier, 1996). The proximal colon has intermittent periods of anti-peristalsis (originating from colonic pacemaker cells) where food can stall for long periods of time allowing mixing and preventing passage until absorption is complete. In addition, segmental and peristaltic contractions of the large intestine also move ingesta aborally through the small colon to be voided. Motility and contractions of the distal colon is similar to that of the small intestine.

Within the cecum, there is a coordinated series of spike bursts beginning at the base and progressing towards the apex that is responsible for retropulsion and retention of ingesta for bacterial fermentation and is initiated by a pacemaker in the cecal base (Rutkowski *et al.*, 1989). These spike bursts occur at velocity of 3.8 cm/sec at a frequency of 0.46 spikes bursts/min. A second type of progressive series of spike bursts begin at the cecal apex and are conducted through the cecocolic orifice and into the right ventral colon and are responsible for progressive motility propelling food from the cecum into right ventral colon and are initiated by a pacemaker in the cecal apex (Rutkowski *et al.*, 1989). These occur with a velocity of 6 cm/sec at a frequency of 0.4 spike bursts/min.

Intramural ganglia (intrinsic plexus) within the large colon modulate the contractile activity of the smooth muscle so appropriate contractions occur. Impulses are sent through mechanoreceptors and chemoreceptors in the smooth muscle. Thus luminal distension from ingesta initiates a stimulus. The number of propulsive and retropulsive contractions is directly related to the fullness of the cecum and large intestine (Clark, 1990). Normal colonic motor profile during interdigestive periods consist of spike bursts of 5-12 seconds duration grouped in complexes that migrate from right ventral colon to beyond the pelvic flexure and of localized spike bursts of under 5 seconds duration (Roger and Ruckebusch, 1987). These short spike burst are associated with mixing contractions while the long spike bursts are associated with retropulsion and propulsion. It is alterations in these short and long spike bursts that result in either constipation or hypermotility (Adams *et al.*, 1984).

The above physiological description of normal gastro-intestinal function and motility illustrates how complex it is and how many factors may be involved in the process. Since the large colon functions in water absorption and microbial digestion, conditions interfering with these processes may result in severe dehydration and luminal distension, with subsequent pain, shock and colic (Snyder and Spier, 1992). Large colon intra-luminal obstructions in general tend to occur at locations of normal anatomical luminal narrowing or at pacemaker centers including the cecocolic orifice, the pelvic flexure and the transverse colon (Allen and Tyler, 1990). Sand accumulations resulting in colic have been commonly observed to be found in the right dorsal colon, transverse colon, left and right ventral colons, the sternal and pelvic flexures and the small colon (Ragle, Meagher, Lacroix and Honnas, 1989) with there being some debate as to whether sand of different particle size accumulates in different segments (Snyder and Spier, 1992; Hammock *et al., 1998*).

With sand enteropathy, the sand may irritate the colonic mucosa and induce diarrhea resulting in decreased transit times, and subsequent dehydration and weight loss. Sand may also settle out from the digesta and accumulate in the colonic lumen. Sand may collect in the large colon because it is a fermentative area of reduced flow and the sand would consequently settle out in this segment of the gastro-intestinal tract as opposed to the stomach or small intestine where motility is strong, fast and aborally propulsive (Colahan, 1987). It is for this reason, that abdominal radiographs typically demonstrate sand accumulations in the cranioventral abdomen where the left ventral colon turns at the sternal flexure into the right dorsal colon (Bertone *et al.*, 1988; Ford and Lokai, 1979; Ruohoniemi *et al.*, 2001). A study by Ragle, Meagher, Schrader and Honnas, (1989), demonstrated that horses that are non-obstructive typically show sand in the cranioventral abdomen on radiographs (in the left ventral colon and sternal flexure). A retrospective study of surgical cases of sand colic by Ragle, Meagher, Lacroix and Honnas (1989), showed that the majority of sand was found be accumulating and obstructing the right dorsal colon. There then appears to be a disparity in locations where sand accumulates, depending if the horse is obstructive (requiring surgery) or not, although they may be clinical for sand colic in both instances.

The results of this present study support this concept as almost all of the accumulations of sand were visualized on abdominal radiographs in the cranioventral and mid-ventral abdomen, corresponding to the left ventral colon and sternal flexure. These horses either did not show any adverse clinical signs or showed mild and brief signs of colic. The only horse that showed significant clinical signs of colic and was not passing feces and not responding to medical therapy was Horse #10. This horse was euthanized as it was deemed a surgical case of sand colic. Interestingly enough, 2 of the 3 accumulations of sand in Horse #10 were seen in locations not typically seen throughout the rest of the study, presumed to be the right dorsal colon, which adds credence to the study of Ragle, Meagher, Lacroix and Honnas (1989).

Altered motility is another key feature of gastro-intestinal inflammation. Both increased and decreased motility can occur from inflammatory diseases with diarrhea being the result of increased motility. Motility was not a specific focus in the present study; however some discussion can be made in light of the interpretations of the obtained data. From the above stated literature, it appears that total normal gastro-intestinal transit time approximates 50-60 hours (2-2.5 days). Gastro-intestinal motility was assessed in each patient at regular intervals as part of a health check. All patients had normal auscultable motility as determined by characteristic sounds (borborygmi) heard at regular intervals. Mild diarrhea was noticed in 6/10 of the horses (all 5 Group 2 horses, 1 Group 1 horse) between Day 2 and Day 3. This was assumed to be due to the large volume of water that was administered with the sand through nasogastric intubation. This time frame appears to coincide with the estimated gastro-intestinal transit time mentioned above. Fecal sand was present in significant amounts starting on Day 2 in both groups, usually coinciding with the diarrhea. This again demonstrates normal gastro-intestinal motility. From this point in time on, there is a divergence in the sand output between the 2 groups. Although both groups started producing sand in their feces by Day 2, Group 2 then declined their fecal-sand output while Group 1 showed evidence of high fecal-sand output through Day 4 when they then started to decline to baseline (0) levels. This timepoint (Day 4) also corresponds to 2 days following their last dose of sand, which indicates that the gastro-intestinal motility and transit times were unaltered in the Group 1 horses. This demonstrates that a dose of sand of 4 g/Kg once a day for 2 days (total amount not above 5 Kg) does not appear to affect their normal gastro-intestinal tract physiology. Group 2 horses showed a decline in fecal-sand output following Day 2 and did not show a significant difference in their output pattern from Group 1 until Day 5 when their output increased to a level equal that of Group 1 on Day 3 (their day of maximum sand output) and continued to plateau at the end of the study. From this can be inferred that this higher dose of sand (4 g/Kg twice a day for 2 days, total amount not above 10 Kg) can induce altered gastro-intestinal physiology resulting in prolonged transit times from the accumulation and irritation processes of the sand. It is interesting that the auscultable motility of the intestinal tract was always within normal limits. Auscultation cannot differentiate between the different types of intestinal contraction (propulsive or retentive) so it can be assumed from this study that the sand caused a decrease in the peristaltic propulsive contractions and increased in the retentive ("mixing") type of contractions in an attempt to dislodge the sand accumulations.

The significant difference of fecal-sand output between the 2 groups is shown graphically in Figure 2-11 as the 2 lines diverge from one another after Day 2 and from that point on there are only 2 instances (Day 3 and 4.5) where the standard error of the means overlaps between the two. Although the total amount of sand output was higher in Group 2 horses, this was not evident until approximately Day 4.5 of the study. Before this day, Group 1 horses had a significantly different (higher) fecal sand output than Group 2.

There are some limitations to these interpretations of gastro-intestinal motility and transit times based on fecal-sand output. Through the method described for fecal-sand collection in the Chapter 2 Materials and Methods section, there is obviously some loss of sand in the procedure as some may not settle out from the feces and would be washed away in the process. A 94% recovery rate was determined for this procedure; however this percentage may vary according to sand particle size and fecal consistency. At most, this limitation would increase the actual amount of fecal-sand output as seen in Figure 2-11. If a 94% recovery rate can be assumed, there is a significant difference between the 2 groups for percent of sand passed compared to that administered over the 6-day period ($p = 0.020$, Table 2-1). The average percentage of fecal-sand output for Group 1 is 33.9% while Group 2 only passed on average 18.9% of the total sand administered. This means that the horses were discharged with roughly 66% (Group 1) and 81% (Group 2) of the sand still within their gastro-intestinal tract. This corresponds to, on average, 1.4 Kg for Group 1, and 3.6 Kg for Group 2. Interestingly enough, 3/5 of the Group 1 horses and 2/5 of the Group 2 horses did not show any radiographical evidence of intra-abdominal sand accumulation at the time of discharge. This means that at least 3.6 Kg sand may be within the gastro-intestinal tract and not evident on radiographs. This depends of course on the distribution of the sand as less than 2 Kg of sand could be clearly seen accumulated within the stomach, but the same quantity may not be visible if dispersed throughout the colon with no significant accumulations present. According to Figure 2-19, sand quantities ranging from 1800-6670 g were not radiographically visible as an objective score of 0 was assigned. This particular score however was assigned on a day (Day 0.5) after this same quantity was seen as an accumulation in the stomach after administration. It is most likely due to the fact that the sand was now dispersed within the small intestines which were not visualized adequately during the study. These results also demonstrate that radiographs may not depict gastro-intestinal sand up to a weight of 6670 g. This may be significant especially given the fact that the amount required to cause clinical signs is not known (Bertone *et al.*, 1988). There were no quantities of sand given that were smaller than 1800 g to assess how much sand there needs to be before it is radiographically visible.

The difference in percentage of sand output between the 2 groups is again most likely reflective of the delayed transit time and decrease in the propulsive intestinal contractions that was experienced by the Group 2 horses. It would be interesting to note how long it would have taken these horses to reach the same level of sand output (33%) as the Group 1 horses had. Also of note, is that despite there being still significant sand within the gastro-intestinal tract of all horses at 6 days, their 12-hour output was not indicative of this at the end of the study. Even Group 1 horses that had approximately 66% of the sand still inside their intestinal tract had a mean 12-hour output of 35 g over the last 3 sampling periods (Day 5-6). This is in comparison to a mean 12-hour output of 412.4 g at the time of their maximum fecal-sand output (Day 3.5). This would tend to indicate that at least in these Group 1 horses, who were interpreted to have a normal gastro-intestinal physiology despite the sand, passed a larger percentage of the sand out that was carried through the intestinal tract within the normal transit time (2 days) and the remainder was slowly passing out with subsequent gastro-intestinal movements. Although a plateau level of sand output was reached at the 6 day period for the Group 2 horses, and a decline was not observed, it can be assumed that these horses would eventually follow the pattern of the Group 1 horses and decline in their output of sand with still a large percentage within their intestines that would slowly pass in small amounts over time. This variation in fecal sand output demonstrates that there is indeed poor correlation between such diagnostic methods and establishing a diagnosis of sand colic and that even small amounts of sand may be passing in the feces in clinically normal horses. This is in agreement with other current literature (Snyder and Spier, 1992); however previous reports stated that a positive diagnosis can always be made by rectal sand recovery (Ferraro, 1973).

Another limitation in the interpretation of this data is that it is not possible to assume that all of the sand passed with the feces could be collected. There is obviously a chance that not all the feces were collected however an attempt was made to be thorough. The major problem arose from the fact that 6/10 horses experienced diarrhea to a mild degree due to the large volume of water given to administer the sand. There is therefore some degree of insensible loss of sand through the watery feces that could not be reliably collected. The bedding was made of wood shavings, and if diarrhea was passed onto the shavings, an attempt was made to dump the pile of shavings into the water bucket to settle out the sand. Diarrhea that passed over the stall walls or onto bare floor could not be collected reliably. This again would provide in an underestimation of the sand passed by the horses. It is again interesting to note that the diarrhea occurred over the Day 2-3 period of the study, which was the point of increase in Group 1 fecal-sand output, and a point of decrease in the Group 2 horses. This may mean that in fact Group 2 horses never experienced a decline in their sand output as Figure 2-11 indicates, or that Group 1 horses may have experienced an earlier rise in their sand output which may reflect an increase in the gastro-intestinal transit and motility that may be seen with diarrhea.

Another limitation in the interpretation of the results is the lack of a complete control in Group 1. In retrospect, it would have been more scientifically correct to sedate, nasogastrically intubate and administer 15 liters of water to all of the Group 1 horses at the same frequency as Group 2 horses. This would have assured that the results obtained would have been truly due to the difference in the quantity of sand administered between the 2 groups only. Whether the colic seen in Group 2, its fecal sand passage, or its objective and subjective scores could actually be due to the additional sedation, water content or nasogastric intubations and not actually from the increased sand quantity is speculative. Theoretically, the additional water that Group 2 horses received may have resulted in more insensible sand loss through diarrhea and have falsely lowered their fecal sand output. Likewise, the additional water in Group 2 horses may have altered the radiographic appearance and structure of the sand accumulation and subsequently altered the objective and subjective scores. Perhaps if Group 1 horses had received the same dose of water as Group 2 horses, it would have intermixed with the sand, resulting in smaller and more dispersed accumulations and their scores would actually be lower than what was observed. This would theoretically strengthen the differences seen between the 2 groups. Had Group 1 horses been subject to the same amount of sedation and intubations as Group 2 horses, they may have experienced altered (slowed) gastric emptying and intestinal transit times. This in turn may have lead to signs of colic, and prolonged retention of sand in the stomach as Horses #6 and 10 experienced.

Another goal of this study was to develop a technical protocol for radiographic imaging for suspect cases of sand colic. It was determined from the retrospective part of the study that poor radiographic quality had a negative effect on the establishment of a diagnosis of sand colic, which was also concluded in another similar study with enteroliths (Yarbrough *et al.*, 1994). It was also observed that many studies did not have a complete view of the entire abdomen to evaluate and interpret. All radiographs evaluated in the retrospective study were in a right to left lateral view. The results of the prospective part of the study indicate that high exposure factors (KVp > 90, mA >400) are required for radiographic imaging of the equine abdomen, in agreement with current literature (Butler *et al., 2000*). In some cases however, this still is not enough for complete penetration as was seen in the central abdomen in some of the experimental horses. However, since this region corresponds to the small intestine predominantly and that sand does not typically accumulate in this region, the exposure factors used are deemed satisfactory for a diagnostic study.

A mainstay in diagnostic radiology is that 2 orthogonal views are required for full assessment of a certain structure in order to obtain a 3-dimentional image from 2-dimentional radiographs. Since this is not possible in awake adult equines, standing lateral views are obtained, and usually from only one side of the abdomen. Additional benefits of opposite lateral views are obvious as a lesion may be better visualized if it closer to the film. This shorter object-film distance results in better resolution, which may improve the interpretation and diagnosis. It was determined from this study that 2 opposite lateral views are not necessary for a diagnosis of sand accumulation in horses. The additional view did not significantly alter the final score or diagnosis in either subjective or objective methods of assessment. An orthogonal view, such as a ventrodorsal would be obviously beneficial as it may provide more information as to the true size of the accumulation, however is not feasible in routine cases for "sand-scans". Also, it was discovered from the retrospective part of the study that using 1 lateral view with an objective method of assessment, the obtained score has a high correlation with a diagnosis, especially if a score of 7 or higher is obtained.

Using the described 12-overlapping-image method to radiograph the abdomen allowed clear visualization of the individual sections of the gastro-intestinal tract. Stomach, dorsal and ventral colon, sternal flexures, diaphragmatic flexures, pelvic flexures and small colon could all be differentiated. Small intestine and cecum could not be reliably seen, nor could differentiation between left or right colon be made. Sand was seen in all locations except in the dorsal abdomen and in the mid-central abdomen. The dorsal abdomen consisted primarily of the gas caps of the stomach and intestines, and the mid-central abdomen consisted primarily of the small intestines and dorsal colons. Overlapping of the images provided assurance that parts of the intestine and/or sand accumulation were not missed on interpretation, and that the entire accumulation could be measured.

Based on the above findings, the following recommendations are made for radiographic imaging of an adult equine for sand colic. Using exposure factors of 100 KVp and >400 mA, good quality overlapping radiographs of the entire ventral 2/3 of the abdomen are recommended from 1 side only. Despite that few radiographs had any discernible findings in the mid-central abdomen, this region is still recommended to be radiographed. This region may show sand accumulations in the dorsal colon that also resides in this area as this area is more commonly obstructed in surgical cases, as was seen in Horse #10. Radiographing the majority of the abdomen for assessing sand accumulation is against many popular beliefs that state that the cranioventral abdomen is alone sufficient (Ruohoneimi *et al.*, 2001). This study clearly demonstrates that this is insufficient and would result in a potentially non-diagnostic study. Whereas in certain cases, such as enterolithiasis, the entire abdomen need not be radiographed if an enterolith can be visualized as a diagnosis can be made (Yarbrough *et al.*, 1994). If cases of sand enteropathy, just visualizing 1 accumulation is not sufficient, and the entire abdomen need be searched or imaged so as to provide a complete assessment of the gastro-intestinal sand. As sand was never seen in the dorsal 1/3 of the abdomen, and that there was no correlation of gas-distended intestines with sand colic (see Retrospective part), the dorsal 1/3 of the abdomen is not recommended to be imaged if sand colic is the primary or sole differential. If other causes of colic are sought, the entire abdomen need be radiographed as this dorsal section may provide vital information on certain cases, such as gas distention from colonic entrapment or torsion.

The radiographic appearance of the sand seen in the experimental horses closely resembles the description of other sand accumulations from reported cases (Ruohoneimi *et al.*, 2001). This is despite the fact that in the current study, sand was administered as a bolus to the stomach and still appears radiographically similar to sand that is chronically ingested. Sand accumulations varied from distinct to dispersed within digesta, usually tapering cranial-caudal margins, convex ventral margins, and horizontal or irregular dorsal margins. The ventral margins were usually smooth and concave because they were resting along the floor of the colon and usually had haustra defining their location. The dorsal margin was often more indistinct than the ventral margins because of the overlying or intermixed ingesta. Accumulations within the sternal flexure had rounded cranial margins and were located more caudoventral than accumulations in the diaphragmatic flexures, which also had rounded cranial margins. The stomach could be differentiated based on its location being more dorsal and caudal than the colonic flexures. The dorsal margins of the stomach sand accumulations always had straight, horizontal borders due to the sand-water interface from the intubation procedure. The size and consistency of the various accumulations could easily be appreciated; however overlapping radiographs were essential for full assessment. Ruohoneimi *et al.*, (2001) also states that although large accumulations may not be entirely visible on the cranioventral abdominal radiograph, the average size and appearance can be assumed from the portion that is seen. The above results demonstrate that if this approach was used, false interpretations may occur as it is clear that the accumulations may drastically change shape, size, and consistency from 1 end to the other of the accumulation. Whereas the cranial extent of an accumulation may appear large and homogeneous and opaque, it may become more narrow and small and heterogeneous and less opaque further caudally. It is also important that the entire accumulation to be visible for full assessment for the objective scoring assessment to work properly. This again states the importance of a complete abdominal radiographic series.

Results from the Retrospective part of the study demonstrated that current subjective methods of assessing sand accumulation and correlating these assessments with a diagnosis is unreliable and inaccurate. This theory needed to be tested on a prospective model that attempted to mimic a clinical setting. With differences in known amounts of intra-abdominal sand content between the 2 groups (Group 1 having roughly half the quantity that Group 2 had), subjective methods of assessment could not reliably differentiate the 2 groups as being separate. Using the devised objective scoring assessment, Group 1 and Group 2 could be differentiated. The obtained results support the proposed hypothesis. This indicates that current subjective methods are indeed highly inaccurate as they may not allow radiographic differentiation between a volume of sand that is half of another. Because of this, it is advised that subjective assessment not be used when attempting to establish a diagnosis of sand colic or to interpret relative quantity of sand seen on the radiographs and correlate these assessments with a clinical diagnosis. Colic from sand accumulation may result from many factors such as its overall length and height, its opacity and homogeneity and its location within the abdomen. Only when all of these parameters are assessed can an adequate evaluation be made as to the degree (severity, quantity) of the sand accumulation. Using the devised objective scoring method allows each of these parameters to be individually assessed and included into a total score for an individual horse. Further studies are recommended to assess how sensitive this testing method is to be able to differentiate amounts of sand. By using smaller divisions between the 2 groups, the limits of this scoring method can then be assumed.

The objective scoring method was also touted to be relatively accurate in its assessment for a positive diagnosis of sand colic. The Retrospective part of the study demonstrated that an objective score of at least 7/12 was 83% precise in diagnosing colic due to the sand accumulation. Precision values for scores above and below this value are not known, but can simply be inferred. For example a score of 12/12 does not guarantee 100% that there is sand colic, it simply means that it is highly likely. The scores obtained in this experimental study confirm these findings from the Retrospective part of the study. The highest score that any of the Group 1 horses received was a 7 for Horse #3 on Day 0.5. This day always provided the highest score for this group as 4 horses had a score of 6 and the other horse had a score of 5. This high score reflects the entire dose of sand within the stomach immediately following nasogastric intubation and administration of the sand and therefore is misleading. The sand seen in the stomach following administration is very opaque, homogeneous and has significant height (due to the shape of the stomach). If such an accumulation were to be seen further distally along the gastro-intestinal tract, this would result in a score in a similar range (5-7) but would be more meaningful since it would represent a colonic (not gastric) accumulation. The highest score obtained by the Group 1 horses following Day 0.5 was a 6/12 which means that despite more sand present than on Day 0.5, it is distributed throughout the intestinal tract and not significantly accumulating. To add credence to this, none of the Group 1 horses showed any signs of colic, and none had a post Day 0.5 score of 7 or higher. Whether Horse #3 or 4 were close to experiencing colic on Day 3-4 of the study (objective scores of 6), is speculative and again states that despite a fairly significant accumulation of sand, clinical signs may not be present, and that other causes should be sought if clinical signs are present.

Group 2 horses also showed a repeatedly high score at Day 0.5 following gastric sand administration as all 5 horses had a score of 5-6. At times following this, Horse #6 had a high-score of 8 on Day 8 and showed signs of colic on that day, Horse #7 had a high-score of 7 once on Day 3 and did not colic, Horse #8 had a high-score of 7 on Day 3 and did not colic, Horse #9 had a high-score of 7 on Days 4 and 4.5 and did not colic, and Horse #10 had a high-score of 8 over Days 4.5-5 and did colic on those days. The average Group 2 high-score after Day 0.5 was still only 6, which was on Day 3. These results demonstrate that all of Group 2 horses had scores reaching at least 7 sometime during their study and only 2/5 (40%) of these demonstrated colic, which were those 2 horses that had scores of 8/12. Horse 9 did not colic despite having a score of 7/12 over 2 imaging periods.

These results can be explained because despite the Retrospective study claiming that a score of 7/12 is 83% precise in diagnosing sand colic, there are 17% of cases that will be misdiagnosed. These differences may also be explained because this was an experimental setting and does not truly mimic natural sand colics that were the types of cases that were evaluated in the Retrospective part of the study. Perhaps, the horses were demonstrating more subtle signs of colic that were either not noticed or misinterpreted. For example, the pawing of the bedding was interpreted as being due to boredom from the change from pasture to a stall and may have been a sign of colic. There were however, no additional supportive findings of colic (such as tachycardia, anorexia, decreased intestinal motility, or lack of fecal production). This does however demonstrate that this relative ordinal scale of 0-12 does work as all of the horses (2/2) that had scores of 8/12 did show signs of colic.

The objective scores over the duration of the 6-day study demonstrate that the average group score decreased following Day 0.5 due to distribution of the sand in the intestines following gastric emptying (Figure 2-12). Scores obtained at this time were low (1-4) because despite there being the same amount of sand in the abdomen as before, it is now within the small intestines mixed with the chyme, not accumulating, and located in an area prone to underexposure. As intestinal motility moved the sand further into the large colon, scores again increased from Day 1.5 - 3.5 due to the visualization of the sand, and its accumulation in the ventral colon. Following this time period, Group 2's scores decline much more slowly than Group 1's scores because of the prolonged accumulation and increased amount of intestinal sand present in this group. This clearly demonstrates the ability of the objective scoring method to differentiate between horses with different amounts of sand.

Because one of the objectives of this scoring system was to find out if it could tell relative quantities of sand visible on radiographs, the objective scores were plotted against the weight of the sand that was known to be present in the abdomen at that time (Figure 2-19). The results indicate that this method cannot predict quantity of sand present in the horse, as the score is based on many factors, not just the weight of the sand. The graph shows a prominent dispersion of values around the regression line indicating a poor cause and effect relationship. Even making the above comparisons within each group, and with each horse within each group, does not demonstrate any relationship. Previous work states that radiographs can clearly show the amount of sand in the cranioventral abdomen, (Ruohoneimi *et al.*, 2001); however this study does not support this statement. The chart in Figure 2-19 depicts how many scores of 0 were given to cases that had relatively high amounts of intra-abdominal sand, but this sand might not have been radiographically apparent. Since it was found that a score of 7/12 could predict sand colic with 83% precision, extrapolating from Figure 2-19 shows that a score of 7-8 requires approximately 11,000-12,000 g of sand. When extrapolating from each individual horse's chart (Appendix E), although certain individuals showed signs of colic with sand weights of only 6000 g, the average trend shows that approximately 10,000 g (10 Kg) may be required to cause signs of colic, or obtain an objective score of 7/12. This is in agreement with some research (Ragle, Meagher, Lacroix and Honnas, 1989), yet contradictory to another that states that over 20 Kg is required (Ragle, Meagher, Schrader and Honnas, 1989). This study was also designed on the premise that 10 Kg (the maximum total dose) would not cause clinical signs. The above interpretation of required weight is made in light of the acknowledged differences between this study and naturally occurring sand colic.

The fact that Group 2 horses exemplify better relationships between their objectives score and the amount of intra-abdominal sand on particular days as compared to Group 1 horses is interesting. Group 2 had relatively strong relationships between their score and the amount of sand on Day 2.5, 3, 3.5 and 4 whereas Group 1 never had a strong relationship on any given day. This may mean that a certain threshold quantity of sand is required in order for this objective scoring method to show relationships with the weight of the sand and that quantities below this value are either not radiographically visible or are not accumulating in a fashion to demonstrate a relationship. What this threshold level may be is unknown, and further studies would be recommended to see how sensitive this objective scoring method is to a certain baseline level of sand.

There are some significant differences between the 2 groups in terms of the individual parameter scores over time. Group 2 had a higher location score than Group 1 in the 2nd half of the study, indicating that they often had their accumulating sand localized to the cranioventral abdomen (Figure 2-13). This was the most standard location for the sand to accumulate in these horses however, for reasons mentioned previously, entire abdominal radiographs are advised so as not to miss any accumulations for evaluation. Sand was commonly seen in the mid-ventral abdomen, which would be missed on cranioventral abdominal views only. It is also observed that it takes approximately 2 days before the sand is seen accumulating in the cranioventral abdomen.

Before this point, sand is within the small intestine and cecum or is passing through the colon in small amounts.

Group 2 horses had a significantly higher number of accumulations score than Group 1 horses, especially towards the end of the study (Figure 2-14). During this time (Day 4 onward), it was common not to see any sand accumulating in the Group 1 horses, while Group 2 horses still had a distinct accumulation visible. Horse #10 had 3 distinct accumulations seen towards the end of the study and showed signs of colic. These results are in agreement with another report that states that the number of accumulations has a positive association with clinical signs (Kaikkonen *et al.,* 2000).

Opacity and homogeneity scores were significantly higher in Group 2 horses compared to Group 1 (Figures 2-15, 2-16). This means that Group 2 horses had more dense accumulations than Group 1 horses due to the poor motility and clearance. This increase in density of the accumulation will tend to lead to more focal pressure and weight to that part of the colon, causing an increased likelihood of clinical signs. Interestingly, it was only sand homogeneity and opacity that was found to be significantly correlated to one another when comparing the individual parameters together. This can be interpreted that as once an accumulation becomes more opaque, it also becomes more homogeneous. This is inferred as a more solid and denser accumulation of pure sand. Group 1 horses had lower score because the lower volume of sand did not cause altered gastro-intestinal motility and the sand remained interspersed with the digesta. Also of note, the highest opacity or homogeneity score never got as high as the score given to the stomach accumulation that had a maximum value of 2 in both groups for both parameters. So despite the increase in sand weight over time, it never accumulates to such density as is seen in the stomach immediately following intubation.

Although sand accumulation length and height did not show any significant difference between the 2 groups there is a visible difference (Figures 2-17, 2-18). This again demonstrates that the overall shape of the accumulation alone does not have a relationship with clinical signs. Accumulations may vary from long and skinny to short and thick, but inferring a diagnosis from such appearances will be misleading. Only Group 2 had sand height scores that were higher than the height score given for the stomach and these scores were seen on Day 3 and 3.5 when significant accumulation started to develop. This can create an impression as to how large some of these accumulations were in these horses. Group 1's sand height scores never equaled their stomach's sand height score as their sand never accumulated to such degree.

From the above, it can be seen that objective scores are based on not only the weight, but how many accumulations it is formed in, how dense it is, where it is in the gastro-intestinal tract, and if is a long and skinny accumulation or a thick accumulation. Even comparing the individual parameter scores to the known amount of sand present did not reveal any strong relationships. This demonstrates that sand colic is multifactorial in its etiology and is not only based on 1 parameter such as weight, density, size, or location of the sand but depends on the interrelationships of all of these parameters acting together to cause the resultant signs of colic.

The poor relationships and correlations demonstrated in the above results for the various comparisons may be in fact due to the small sample size used (2 groups of 5 horses) or population homogeneity and may require larger group sizes in order to demonstrate significant relationships. This sample size was found to be adequate however, for differentiating the 2 groups based on the objective scoring system (power = 96%). This method of assessment was critical and sufficiently objective enough that it could detect differences between the 2 groups using just 5 horses per group. The subjective method of assessment had a low power (25.6%) in its ability to test differences between the 2 groups. Using the subjective method of assessment, approximately 25 horses per group would have been needed to see a difference. This again demonstrates the improvement of the objective method from the subjective method of radiographic assessment of sand accumulations.

Table 2-1

Percentage of sand passed over a six-day period following nasogastric administration of 4 g/Kg for the first two days. (Based on a 94% recovery rate).

Horse #	Sand Collected (g)	Actual Amount of Sand Passed (g)	Amount of Sand Administered (g)	% of Sand Passed After 6 Days
1	1218	1296	4200	30.9
2	2163	2301	5000	46
3	1118	1189	3800	31.3
4	1659	1765	5000	35.3
5	731	778	3600	21.6
6	2523	2684	9200	29.2
7	1202	1279	8000	16
8	706	751	8800	8.5
9	2081	2214	8000	27.7
10	1191	1267	7200	17.6

Conclusion

The objective of this study was to improve the sensitivity and specificity of abdominal radiography as a diagnostic tool for intestinal sand accumulation in horses. The goal was to develop an objective method of radiographic interpretation of intestinal sand accumulation on equine abdominal radiographs. It is felt that this study demonstrates that this goal has been achieved and the hypothesis proven correct. Through the creation of an objective scoring system, sand accumulation on equine abdominal radiographs can now be assessed in a more reliable, reproducible and accurate fashion than current subjective methods of assessment. Objective assessments can reliably differentiate different amounts of sand on radiographs that subjective methods could not.

The devised objective scoring system uses the combination of a subset of scores from 6 parameters (location, number of accumulations, opacity, homogeneity, length and height) to provide a total score ranging from 0-12. It was found that a score of 7 or higher had an 83% chance of correctly predicting sand colic. This objective method of assessment was then tested on experimental horses that were nasogastrically administered sand in 2 different doses, Group 1 receiving half of the dose that Group 2 received. By examining the radiographs following sand administration, objective methods of assessment could differentiate the 2 groups whereas subjective methods could not. It was also relatively accurate method of assessment as all (2/2) horses that demonstrated clinical signs of colic had objective scores of 8/12 at the times of colic. There was no score given higher than 7 for horses that did not experience colic.

Radiographic interpretation of sand accumulation goes beyond trying to estimate the approximate amount of sand in the gastro-intestinal tract. Clinical signs of colic depend not only on the total weight of the offending sand, but also on where it is located, how many accumulations are present, its density and its combined length and height. It is all of these interrelationships that need to be considered when evaluating abdominal radiographs.

It was determined that this protocol (4 g/Kg sand bid for 2 days) for the Group 2 horses may induce altered gastro-intestinal motility and result in relatively significant sand accumulation and cause colic. In the one horse that experienced signs of colic sufficient to warrant surgery, sand was visualized in the dorsal colon as opposed to the ventral colon that was the most common sight of accumulation in asymptomatic or medically responsive colic cases.

It was determined that radiographing from 1 side of the abdomen only is satisfactory as opposite lateral views do not significantly alter the interpretation. The ventral 2/3 of the abdomen should be radiographed however and not just the cranioventral abdomen if searching for signs of sand accumulation.

Results from this study should aid in establishing a protocol for abdominal radiography for sand colic, and provide a more accurate method of assessing the sand accumulation. This in turn will provide a better assessment of the severity of the disease and subsequently a better and more accurate therapeutic regimen. Results from this research should aid in establishing better correlations between the diagnostics, clinical examination and surgical findings, which is often a problem with sand colic.

Future tangents of this research may involve establishing a minimum quantity of sand that is radiographically visible within the colon utilizing the objective scoring system. This would help in then knowing how much sand there has to be in order for a score to be assigned. As well, future work may also involve establishing a more strict sensitivity of this objective scoring system. The current project demonstrated that this method can differentiate 2 doses, one being double the other. Performing this research with more groups and with smaller differences in the doses between the 2 groups may establish the limits of the benefits of the objective scoring system. Correlating all of the above results with surgical or necropsy findings will also help in validating this proposed new objective assessment system for sand accumulation on equine abdominal radiographs.

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APPENDIX A

Table 2-2

Tabulated sand parameters. X = no data available.

Group #	Horse #	Day	Sand Administered (g)	Sand Administered Total (g)	Sand Passed (g)	Sand Passed Total (g)	Sand Present Within Abdomen (g)
1	1	0	0	0	trace	trace	0
1	1	0.5	2100	2100	trace	trace	2100
1	1	1	0	2100	trace	trace	2100
1	1	1.5	2100	4200	17	17	4183
1	1	2	0	4200	300	317	3883
1	1	2.5	0	4200	88	405	3795
1	1	3	0	4200	287	692	3505
1	1	3.5	0	4200	333	1025	3175
1	1	4	0	4200	193	1218	2982
1	1	4.5	0	4200	trace	1218	2982
1	1	5	0	4200	trace	1218	2982
1	1	5.5	0	4200	trace	1218	2982
1	1	6	0	4200	trace	1218	2982
1	2	0	0	0	trace	trace	0
1	2	0.5	2500	2500	trace	trace	2500
1	2	1	0	2500	0	trace	2500
1	2	1.5	2500	5000	4	4	4996
1	2	2	0	5000	113	117	4883
1	2	2.5	0	5000	586	703	4297
1	2	3	0	5000	600	1303	3697
1	2	3.5	0	5000	670	1973	3027
1	2	4	0	5000	150	2123	2877
1	2	4.5	0	5000	40	2163	2837
1	2	5	0	5000	trace	2163	2837
1	2	5.5	0	5000	trace	2163	2837
1	2	6	0	5000	trace	2163	2837

Table 2-2 (con't).

Group #	Horse #	Day	Sand Administered (g)	Sand Administered Total (g)	Sand Passed (g)	Sand Passed Total (g)	Sand Present Within Abdomen (g)
1	3	0	0	0	trace	trace	0
1	3	0.5	1900	1900	trace	trace	1900
1	3	1	0	1900	0	trace	1900
1	3	1.5	1900	3800	trace	trace	3800
1	3	2	0	3800	trace	trace	3800
1	3	2.5	0	3800	17	17	3783
1	3	3	0	3800	164	181	3619
1	3	3.5	0	3800	59	240	3560
1	3	4	0	3800	283	523	3277
1	3	4.5	0	3800	358	881	2919
1	3	5	0	3800	90	971	2829
1	3	5.5	0	3800	132	1103	2697
1	3	6	0	3800	15	1118	2682
1	4	0	0	0	0	0	0
1	4	0.5	2500	2500	0	0	2500
1	4	1	0	2500	trace	trace	2500
1	4	1.5	2500	5000	trace	trace	5000
1	4	2	0	5000	65	65	4935
1	4	2.5	0	5000	179	244	4756
1	4	3	0	5000	142	386	4614
1	4	3.5	0	5000	800	1186	3814
1	4	4	0	5000	220	1406	3594
1	4	4.5	0	5000	239	1645	3355
1	4	5	0	5000	44	1689	3311
1	4	5.5	0	5000	30	1659	3281
1	4	6	0	5000	trace	1659	3281

Table 2-2 (con't).

Group #	Horse #	Day	Sand Administered (g)	Sand Administered Total (g)	Sand Passed (g)	Sand Passed Total (g)	Sand Present Within Abdomen (g)
1	5	0	0	0	0	0	0
1	5	0.5	1800	1800	0	0	1800
1	5	1	0	1800	0	0	1800
1	5	1.5	1800	3600	3	3	3597
1	5	2	0	3600	69	72	3528
1	5	2.5	0	3600	26	98	3502
1	5	3	0	3600	21	119	3481
1	5	3.5	0	3600	200	319	3281
1	5	4	0	3600	158	477	3123
1	5	4.5	0	3600	45	522	3078
1	5	5	0	3600	89	611	2989
1	5	5.5	0	3600	84	695	2905
1	5	6	0	3600	36	731	2869
2	6	0	0	0	0	0	0
2	6	0.5	2300	2300	0	0	2300
2	6	1	2300	4600	0	0	4600
2	6	1.5	2300	6900	2	2	6898
2	6	2	2300	9200	19	21	9179
2	6	2.5	0	9200	2	23	9177
2	6	3	0	9200	276	299	8901
2	6	3.5	0	9200	27	326	8874
2	6	4	0	9200	186	512	8688
2	6	4.5	0	9200	607	1119	8081
2	6	5	0	9200	361	1480	7720
2	6	5.5	0	9200	665	2145	7055
2	6	6	0	9200	378	2523	6677

Table 2-2 (con't).

Group #	Horse #	Day	Sand Administered (g)	Sand Administered Total (g)	Sand Passed (g)	Sand Passed Total (g)	Sand Present Within Abdomen (g)
2	7	0	0	0	trace	trace	0
2	7	0.5	2000	2000	trace	trace	2000
2	7	1	2000	4000	0	trace	4000
2	7	1.5	2000	6000	trace	trace	6000
2	7	2	2000	8000	28	28	7972
2	7	2.5	0	8000	12	40	7960
2	7	3	0	8000	122	162	7838
2	7	3.5	0	8000	15	177	7823
2	7	4	0	8000	27	204	7796
2	7	4.5	0	8000	79	283	7717
2	7	5	0	8000	439	722	7278
2	7	5.5	0	8000	429	1151	6849
2	7	6	0	8000	51	1202	6798
2	8	0	0	0	0	0	0
2	8	0.5	2200	2200	0	0	2200
2	8	1	2200	4400	trace	trace	4400
2	8	1.5	2200	6600	trace	trace	6600
2	8	2	2200	8800	trace	trace	8800
2	8	2.5	0	8800	trace	trace	8800
2	8	3	0	8800	trace	trace	8800
2	8	3.5	0	8800	84	84	8716
2	8	4	0	8800	56	140	8660
2	8	4.5	0	8800	88	228	8572
2	8	5	0	8800	26	254	8546
2	8	5.5	0	8800	260	514	8286
2	8	6	0	8800	192	706	8094

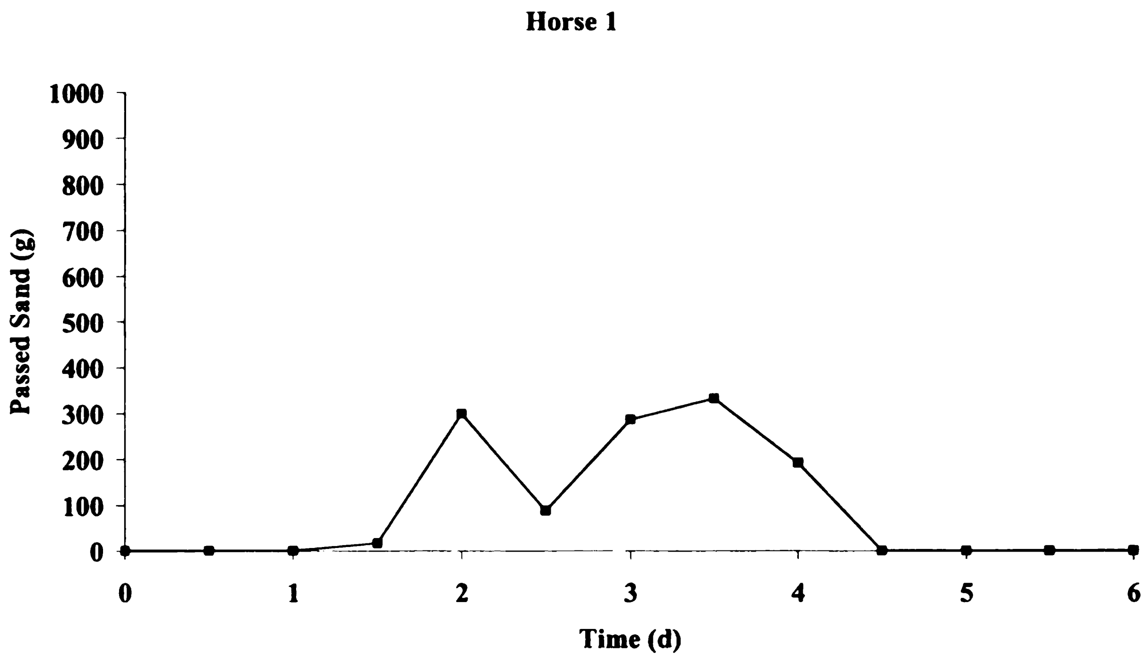
Table 2-2 (con't).

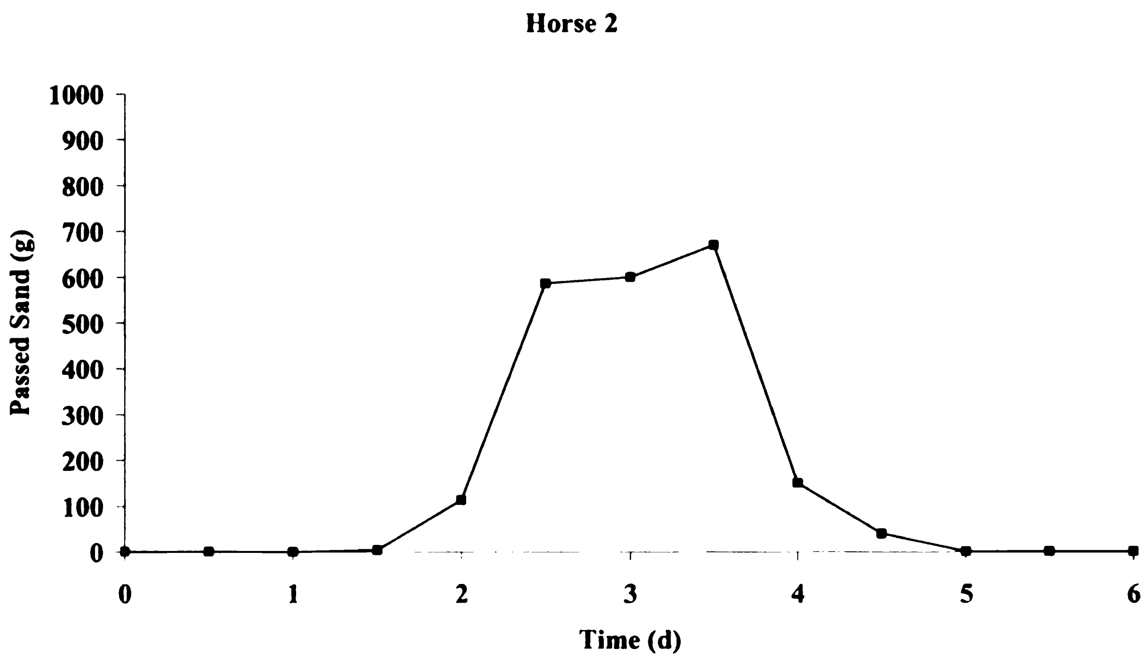
Group #	Horse #	Day	Sand Administered (g)	Sand Administered Total (g)	Sand Passed (g)	Sand Passed Total (g)	Sand Present Within Abdomen (g)
2	9	0	0	0	0	0	0
2	9	0.5	2000	2000	0	0	2000
2	9	1	2000	4000	0	0	4000
2	9	1.5	2000	6000	trace	trace	6000
2	9	2	2000	8000	trace	trace	8000
2	9	2.5	0	8000	trace	trace	8000
2	9	3	0	8000	trace	trace	8000
2	9	3.5	0	8000	65	65	7935
2	9	4	0	8000	7	72	7928
2	9	4.5	0	8000	92	164	7836
2	9	5	0	8000	97	261	7739
2	9	5.5	0	8000	820	1081	6919
2	9	6	0	8000	1000	2081	5919
2	10	0	0	0	0	0	0
2	10	0.5	1800	1800	0	0	1800
2	10	1	1800	3600	0	0	3600
2	10	1.5	1800	5400	235	235	5165
2	10	2	1800	7200	220	455	6745
2	10	2.5	0	7200	52	507	6693
2	10	3	0	7200	264	771	6429
2	10	3.5	0	7200	50	821	6379
2	10	4	0	7200	68	889	6311
2	10	4.5	0	7200	50	939	6261
2	10	5	0	7200	200	1139	6061
2	10	5.5	0	7200	52	1191	6009
2	10	6	X	X	X	X	X

APPENDIX B

Figure 2-20

Individual and group average graphical representation of sand output over time.





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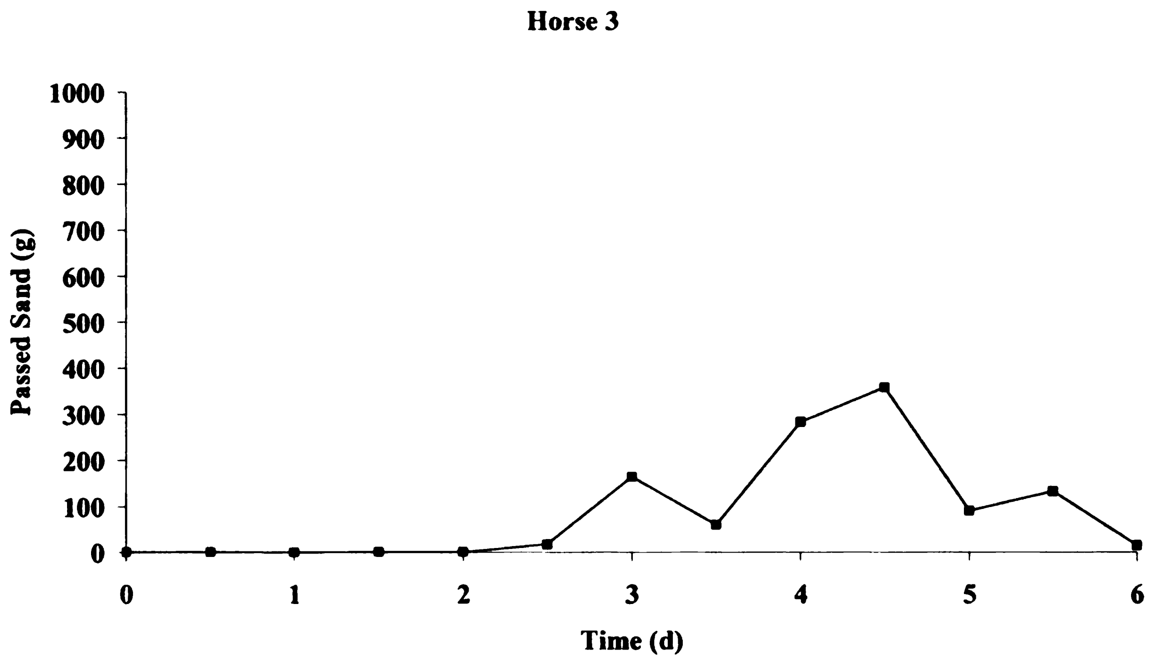

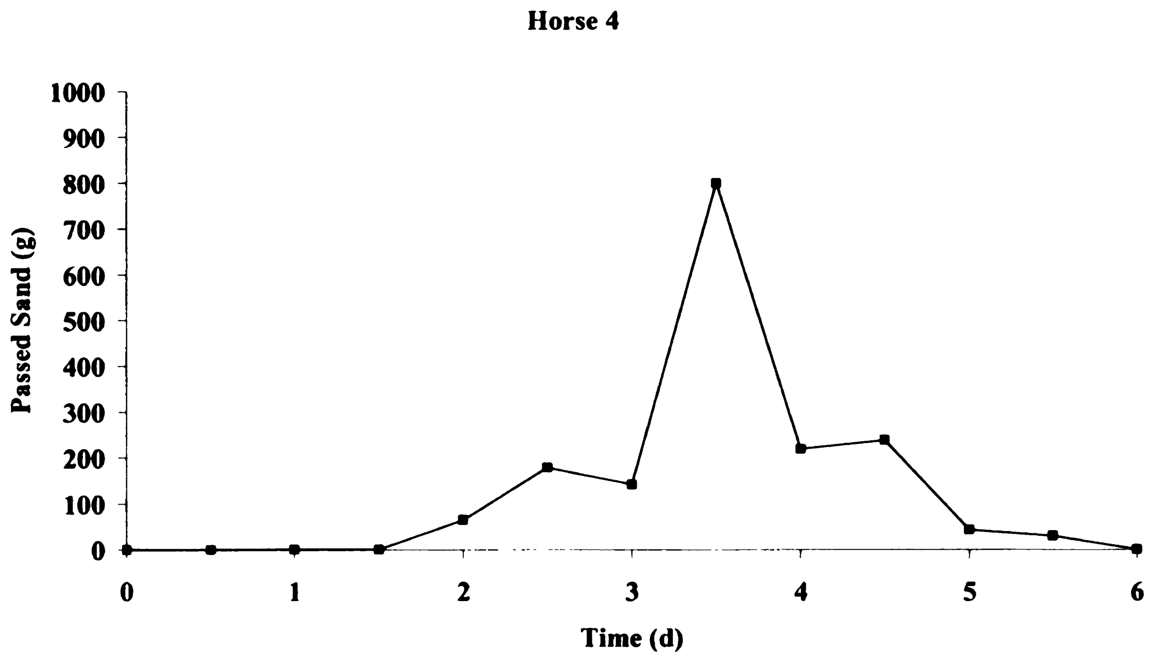

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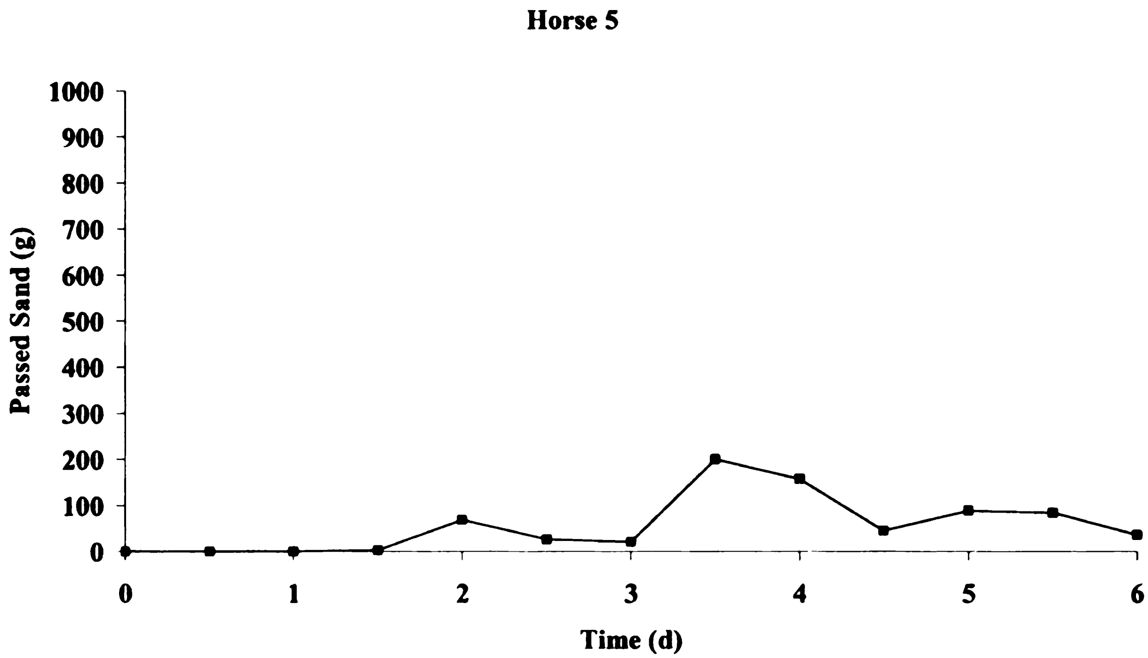

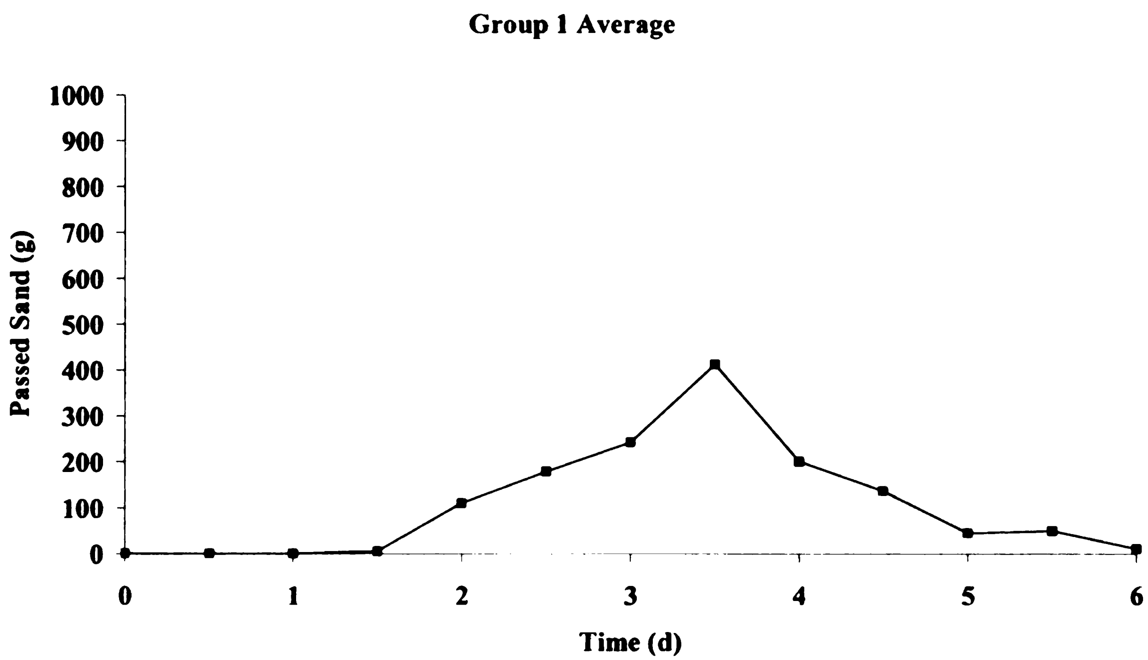

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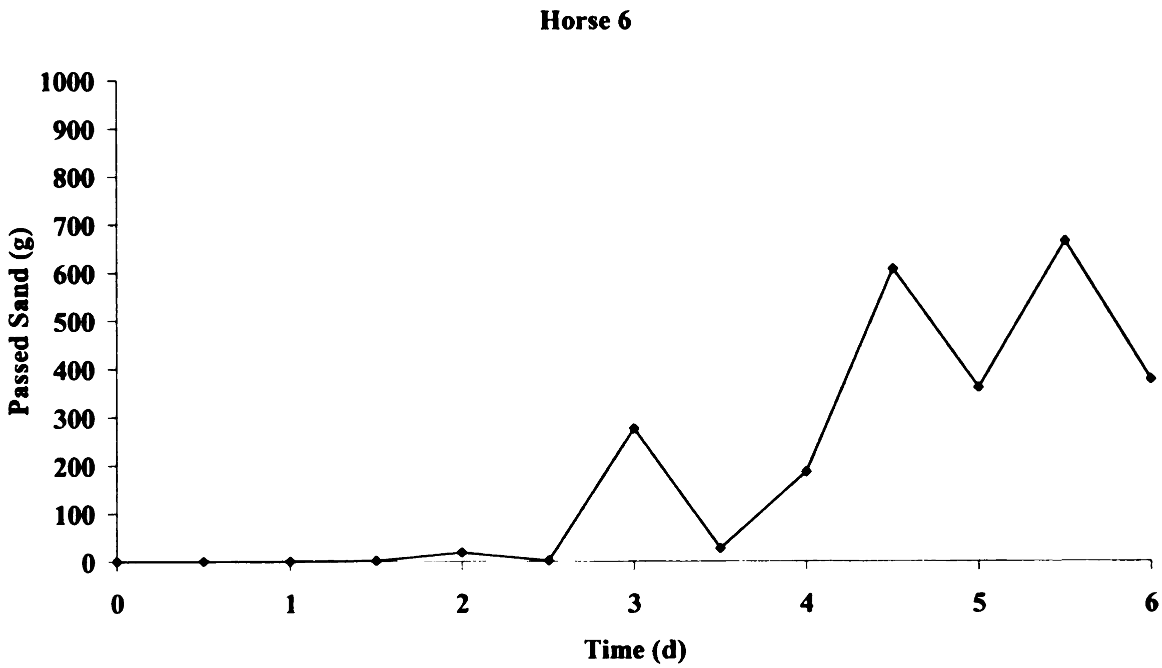

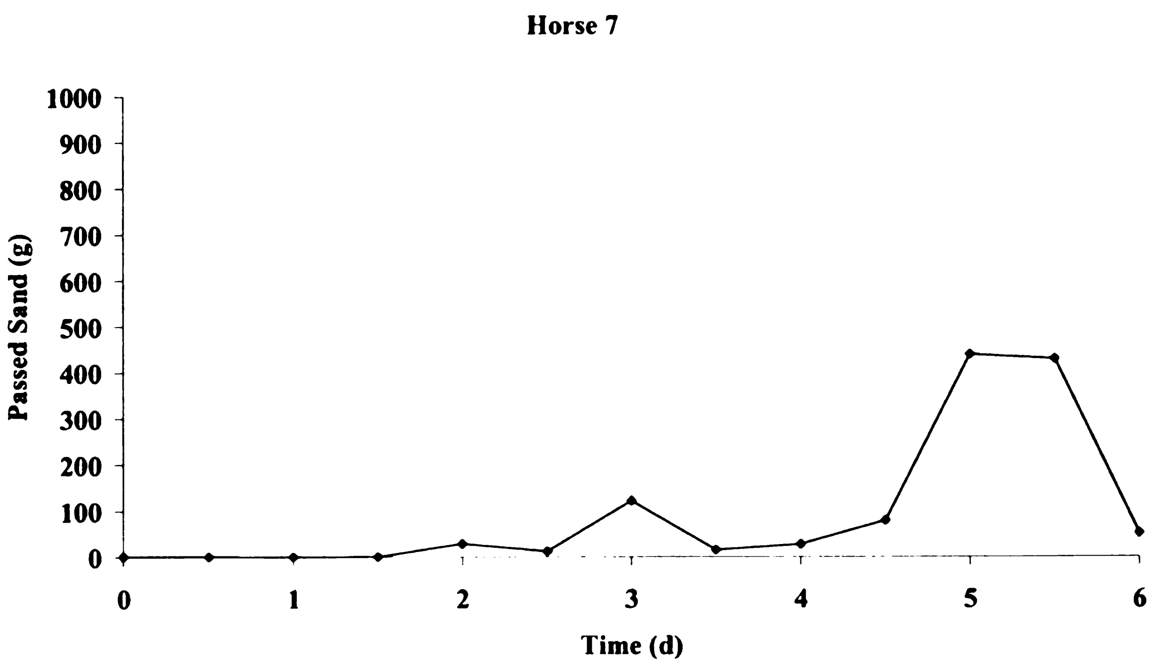

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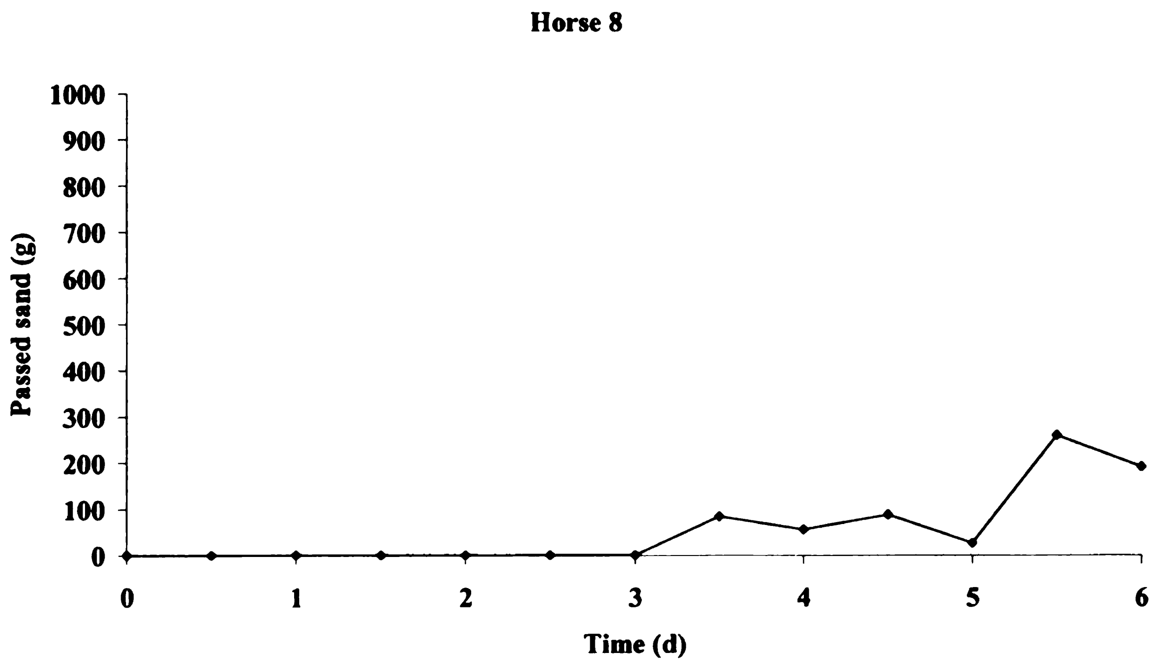

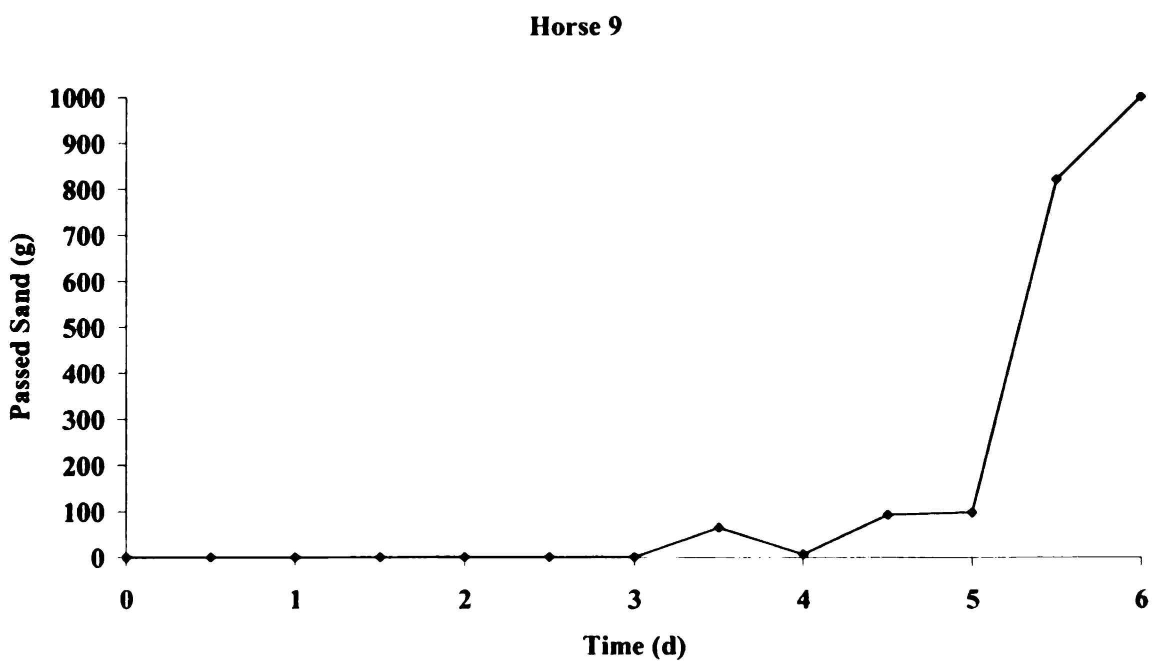

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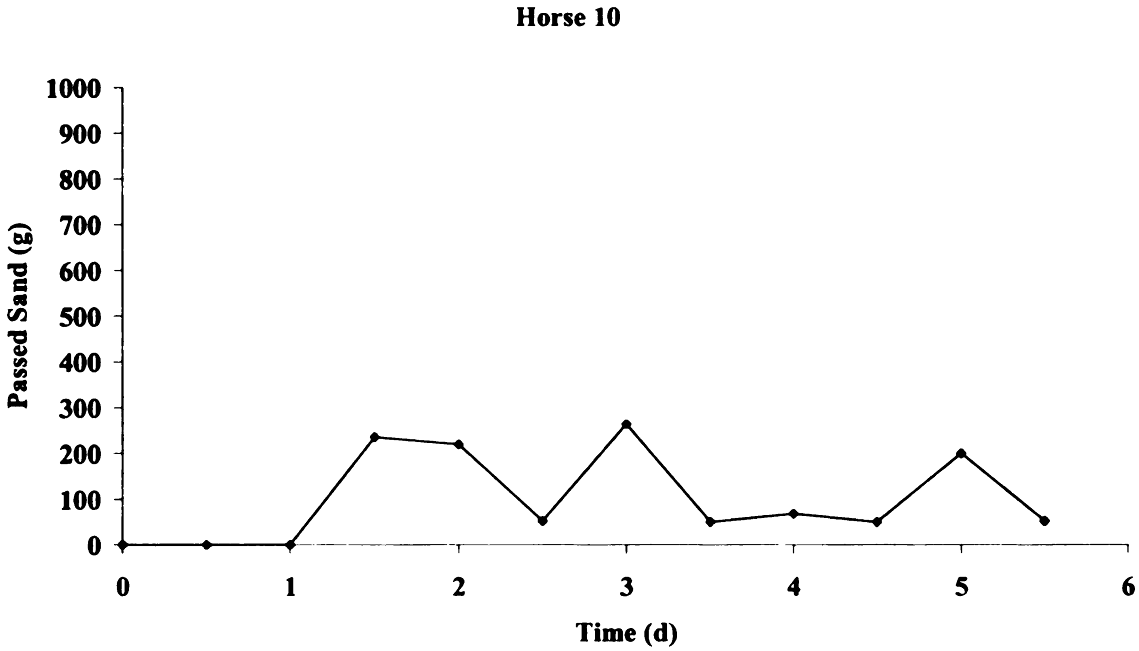
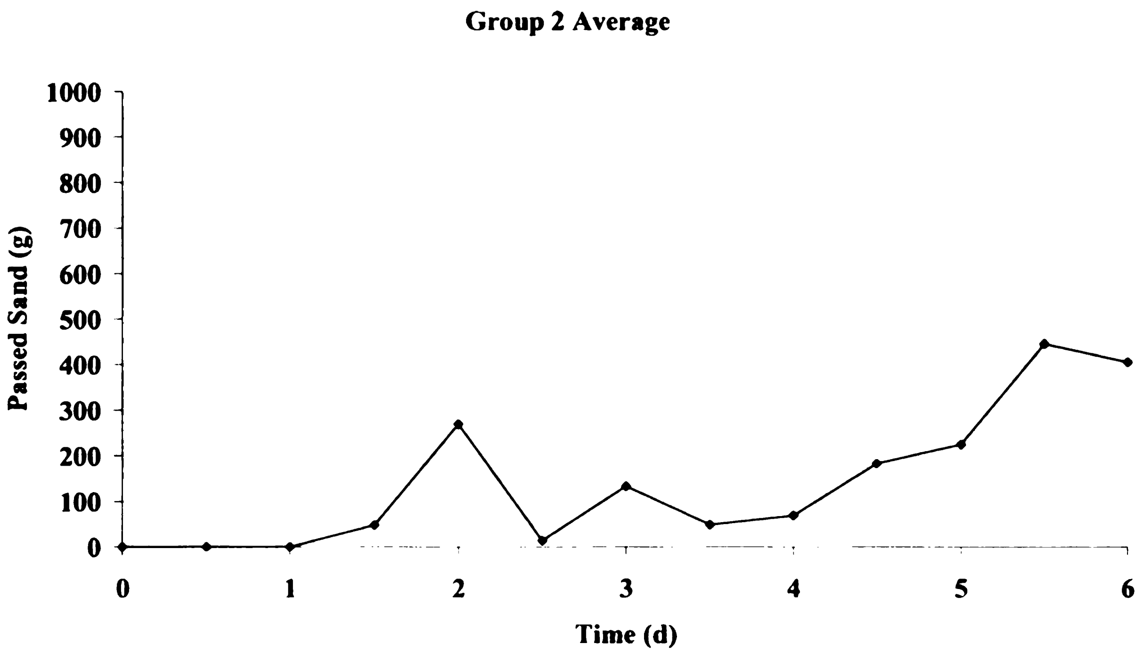




APPENDIX C

Table 2-3

Subjective and objective scores (with breakdown for each parameter) for each horse.

X = no data available.

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
1	1	0	L	0	0	0	0	0	0	0	0
1	1	0	R	0	0	0	0	0	0	0	0
1	1	0.5	L	2	6	0	1	2	2	1	0
1	1	0.5	R	2	6	0	1	2	2	1	0
1	1	1	L	1	2	1	0	1	0	0	0
1	1	1	R	1	2	1	0	1	0	0	0
1	1	1.5	L	1	1	1	0	0	0	0	0
1	1	1.5	R	1	1	1	0	0	0	0	0
1	1	2	L	0	0	0	0	0	0	0	0
1	1	2	R	2	4	1	0	1	1	0	0
1	1	2.5	L	2	3	1	1	1	1	0	0
1	1	2.5	R	X	X	X	X	X	X	X	X
1	1	3	L	1	4	1	1	1	1	0	0
1	1	3	R	1	6	1	1	1	1	2	0
1	1	3.5	L	1	3	1	1	1	0	0	0
1	1	3.5	R	X	X	X	X	X	X	X	X
1	1	4	L	1	5	1	1	1	1	1	0
1	1	4	R	1	4	1	1	1	1	0	0
1	1	4.5	L	1	4	1	1	2	0	0	0
1	1	4.5	R	1	3	1	1	1	0	0	0
1	1	5	L	1	4	1	1	1	1	0	0
1	1	5	R	1	3	1	1	1	0	0	0
1	1	5.5	L	1	2	1	0	1	0	0	0
1	1	5.5	R	1	2	1	0	1	0	0	0
1	1	6	L	1	2	1	0	1	0	0	0
1	1	6	R	1	2	1	0	1	0	0	0

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
1	2	0	L	0	0	0	0	0	0	0	0
1	2	0	R	1	4	0	1	1	2	0	0
1	2	0.5	L	4	6	0	1	2	2	1	0
1	2	0.5	R	2	5	0	1	2	2	0	0
1	2	1	L	0	0	0	0	0	0	0	0
1	2	1	R	0	0	0	0	0	0	0	0
1	2	1.5	L	0	0	0	0	0	0	0	0
1	2	1.5	R	0	0	0	0	0	0	0	0
1	2	2	L	3	5	1	1	1	1	1	0
1	2	2	R	1	5	1	1	1	0	1	0
1	2	2.5	L	1	3	1	0	1	1	0	0
1	2	2.5	R	1	3	1	0	1	1	0	0
1	2	3	L	1	2	1	0	1	0	0	0
1	2	3	R	0	0	0	0	0	0	0	0
1	2	3.5	L	0	0	0	0	0	0	0	0
1	2	3.5	R	X	X	X	X	X	X	X	X
1	2	4	L	0	0	0	0	0	0	0	0
1	2	4	R	X	X	X	X	X	X	X	X
1	2	4.5	L	0	0	0	0	0	0	0	0
1	2	4.5	R	X	X	X	X	X	X	X	X
1	2	5	L	0	0	0	0	0	0	0	0
1	2	5	R	X	X	X	X	X	X	X	X
1	2	5.5	L	0	0	0	0	0	0	0	0
1	2	5.5	R	X	X	X	X	X	X	X	X
1	2	6	L	1	3	1	0	2	0	0	0
1	2	6	R	X	X	X	X	X	X	X	X

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
1	3	0	L	1	3	1	1	1	0	0	0
1	3	0	R	1	3	1	1	1	0	0	0
1	3	0.5	L	4	7	0	1	2	2	2	0
1	3	0.5	R	3	7	0	1	2	2	2	0
1	3	1	L	1	2	1	0	1	0	0	0
1	3	1	R	1	2	1	0	1	0	0	0
1	3	1.5	L	1	4	1	1	1	1	0	0
1	3	1.5	R	1	4	1	1	1	1	0	0
1	3	2	L	1	3	1	1	1	0	0	0
1	3	2	R	1	3	1	1	1	0	0	0
1	3	2.5	L	1	5	1	1	1	1	0	1
1	3	2.5	R	1	5	1	1	1	1	0	1
1	3	3	L	3	6	1	1	1	1	1	1
1	3	3	R	2	6	1	1	1	1	1	1
1	3	3.5	L	1	5	1	1	1	1	1	0
1	3	3.5	R	2	4	1	1	1	0	1	0
1	3	4	L	1	3	1	1	1	0	0	0
1	3	4	R	1	3	1	1	1	0	0	0
1	3	4.5	L	1	2	1	0	1	0	0	0
1	3	4.5	R	1	2	1	0	1	0	0	0
1	3	5	L	1	2	1	0	1	0	0	0
1	3	5	R	1	2	1	0	1	0	0	0
1	3	5.5	L	1	3	1	1	1	0	0	0
1	3	5.5	R	1	3	1	1	1	0	0	0
1	3	6	L	2	5	1	1	2	1	0	0
1	3	6	R	2	5	1	1	2	1	0	0

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
1	4	0	L	0	0	0	0	0	0	0	0
1	4	0	R	0	0	0	0	0	0	0	0
1	4	0.5	L	2	6	0	1	2	2	1	0
1	4	0.5	R	1	4	0	1	2	1	0	0
1	4	1	L	1	1	0	0	1	0	0	0
1	4	1	R	1	1	0	0	1	0	0	0
1	4	1.5	L	1	3	1	1	1	0	0	0
1	4	1.5	R	2	3	1	1	1	0	0	0
1	4	2	L	1	2	1	0	1	0	0	0
1	4	2	R	2	5	1	1	1	1	1	0
1	4	2.5	L	2	5	1	1	1	1	1	0
1	4	2.5	R	1	5	1	1	1	1	1	0
1	4	3	L	1	6	1	1	1	1	1	1
1	4	3	R	X	X	X	X	1	X	X	X
1	4	3.5	L	3	6	1	1	1	1	1	1
1	4	3.5	R	X	X	X	X	X	X	X	X
1	4	4	L	1	5	1	1	1	1	1	0
1	4	4	R	X	X	X	X	X	X	X	X
1	4	4.5	L	2	2	1	0	1	0	0	0
1	4	4.5	R	X	X	X	X	X	X	X	X
1	4	5	L	1	1	1	0	0	0	0	0
1	4	5	R	X	X	X	X	X	X	X	X
1	4	5.5	L	1	1	1	0	0	0	0	0
1	4	5.5	R	0	1	1	0	0	0	0	0
1	4	6	L	0	0	0	0	0	0	0	0
1	4	6	R	X	X	X	X	X	X	X	X

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
1	5	0	L	0	0	0	0	0	0	0	0
1	5	0	R	0	0	0	0	0	0	0	0
1	5	0.5	L	1	5	0	1	2	2	0	0
1	5	0.5	R	1	5	0	1	2	2	0	0
1	5	1	L	0	0	0	0	0	0	0	0
1	5	1	R	0	0	0	0	0	0	0	0
1	5	1.5	L	1	3	0	1	1	1	0	0
1	5	1.5	R	1	3	0	1	1	1	0	0
1	5	2	L	0	2	1	0	1	1	0	0
1	5	2	R	0	2	1	0	1	1	0	0
1	5	2.5	L	0	2	1	0	1	1	0	0
1	5	2.5	R	0	2	1	0	1	1	0	0
1	5	3	L	0	2	1	0	1	1	0	0
1	5	3	R	0	2	1	0	1	1	0	0
1	5	3.5	L	0	2	1	0	1	1	0	0
1	5	3.5	R	0	2	1	0	1	1	0	0
1	5	4	L	0	0	0	0	0	0	0	0
1	5	4	R	0	0	0	0	0	0	0	0
1	5	4.5	L	0	0	0	0	0	0	0	0
1	5	4.5	R	0	0	0	0	0	0	0	0
1	5	5	L	0	0	0	0	0	0	0	0
1	5	5	R	0	0	0	0	0	0	0	0
1	5	5.5	L	0	0	0	0	0	0	0	0
1	5	5.5	R	0	0	0	0	0	0	0	0
1	5	6	L	1	4	1	1	1	1	0	0
1	5	6	R	1	4	1	1	1	1	0	0

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
2	6	0	L	0	0	0	0	0	0	0	0
2	6	0	R	0	0	0	0	0	0	0	0
2	6	0.5	L	1	5	0	1	2	2	0	0
2	6	0.5	R	2	6	0	0	2	2	1	0
2	6	1	L	1	3	1	1	1	0	0	0
2	6	1	R	1	3	1	1	1	0	0	0
2	6	1.5	L	X	X	X	X	X	X	X	X
2	6	1.5	R	X	X	X	X	X	X	X	X
2	6	2	L	2	8	1	1	2	1	2	1
2	6	2	R	2	8	1	1	2	1	2	1
2	6	2.5	L	3	7	1	1	1	2	1	1
2	6	2.5	R	3	7	1	1	1	2	1	1
2	6	3	L	1	6	1	1	2	1	0	1
2	6	3	R	3	6	1	1	1	1	1	1
2	6	3.5	L	2	6	1	1	1	1	1	1
2	6	3.5	R	2	6	1	1	1	1	1	1
2	6	4	L	1	6	1	1	2	1	0	1
2	6	4	R	1	5	1	1	1	1	0	1
2	6	4.5	L	1	3	1	0	1	1	0	0
2	6	4.5	R	2	3	1	0	1	1	0	0
2	6	5	L	1	2	1	0	1	0	0	0
2	6	5	R	1	2	1	0	1	0	0	0
2	6	5.5	L	1	3	1	1	1	0	0	0
2	6	5.5	R	1	3	1	1	1	0	0	0
2	6	6	L	0	0	0	0	0	0	0	0
2	6	6	R	0	0	0	0	0	0	0	0

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
2	7	0	L	0	0	0	0	0	0	0	0
2	7	0	R	0	0	0	0	0	0	0	0
2	7	0.5	L	2	6	0	1	2	2	1	0
2	7	0.5	R	4	8	0	1	2	2	2	1
2	7	1	L	2	3	0	1	1	1	0	0
2	7	1	R	1	2	0	0	1	1	0	0
2	7	1.5	L	2	5	1	1	1	1	0	1
2	7	1.5	R	1	4	1	1	1	1	0	0
2	7	2	L	2	4	1	1	1	0	0	1
2	7	2	R	2	4	1	1	1	0	0	1
2	7	2.5	L	1	6	1	1	2	1	0	1
2	7	2.5	R	2	6	1	1	1	1	1	1
2	7	3	L	2	7	1	1	1	1	2	1
2	7	3	R	3	7	1	1	1	1	2	1
2	7	3.5	L	4	6	1	1	1	1	1	1
2	7	3.5	R	X	X	X	X	X	X	X	X
2	7	4	L	3	5	1	1	1	0	1	1
2	7	4	R	X	X	X	X	X	X	X	X
2	7	4.5	L	1	5	1	1	1	0	1	1
2	7	4.5	R	X	X	X	X	X	X	X	X
2	7	5	L	3	6	1	1	1	1	1	1
2	7	5	R	X	X	X	X	X	X	X	X
2	7	5.5	L	2	5	1	1	1	0	1	1
2	7	5.5	R	X	X	X	X	X	X	X	X
2	7	6	L	1	4	1	0	1	0	1	1
2	7	6	R	X	X	X	X	X	X	X	X

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
2	8	0	L	0	0	0	0	0	0	0	0
2	8	0	R	0	0	0	0	0	0	0	0
2	8	0.5	L	2	5	0	1	2	2	0	0
2	8	0.5	R	2	5	0	1	2	2	0	0
2	8	1	L	1	3	1	0	1	1	0	0
2	8	1	R	2	3	1	0	1	1	0	0
2	8	1.5	L	3	5	1	1	1	1	1	0
2	8	1.5	R	2	4	1	1	1	1	0	0
2	8	2	L	1	5	1	1	1	1	0	1
2	8	2	R	3	6	1	1	1	1	1	1
2	8	2.5	L	2	6	1	1	1	1	1	1
2	8	2.5	R	2	6	1	1	1	1	1	1
2	8	3	L	3	7	1	1	1	2	1	1
2	8	3	R	X	X	X	X	X	X	X	X
2	8	3.5	L	2	5	1	1	1	1	1	0
2	8	3.5	R	X	X	X	X	X	X	X	X
2	8	4	L	1	4	1	1	1	1	0	0
2	8	4	R	X	X	X	X	X	X	X	X
2	8	4.5	L	2	3	1	1	1	0	0	0
2	8	4.5	R	X	X	X	X	X	X	X	X
2	8	5	L	1	2	1	0	1	0	0	0
2	8	5	R	X	X	X	X	X	X	X	X
2	8	5.5	L	2	6	1	1	1	1	1	1
2	8	5.5	R	2	6	1	1	1	1	1	1
2	8	6	L	1	1	1	0	0	0	0	0
2	8	6	R	X	X	X	X	X	X	X	X

Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
2	9	0	L	0	0	0	0	0	0	0	0
2	9	0	R	0	0	0	0	0	0	0	0
2	9	0.5	L	1	6	0	1	2	2	1	0
2	9	0.5	R	1	6	0	1	2	2	1	0
2	9	1	L	1	5	0	1	2	2	0	0
2	9	1	R	0	0	0	0	0	0	0	0
2	9	1.5	L	1	3	1	0	1	1	0	0
2	9	1.5	R	1	3	1	0	1	1	0	0
2	9	2	L	1	5	1	1	1	1	1	0
2	9	2	R	1	5	1	1	1	1	1	0
2	9	2.5	L	2	4	1	1	1	1	0	0
2	9	2.5	R	X	X	X	X	X	X	X	X
2	9	3	L	1	5	1	1	1	1	1	0
2	9	3	R	3	7	1	3	1	1	1	0
2	9	3.5	L	2	5	1	1	1	0	1	1
2	9	3.5	R	3	6	1	1	1	1	1	1
2	9	4	L	2	7	1	1	1	1	2	1
2	9	4	R	2	7	1	1	1	1	2	1
2	9	4.5	L	2	7	1	1	2	2	1	0
2	9	4.5	R	1	4	1	1	1	1	0	0
2	9	5	L	1	6	1	1	1	1	1	1
2	9	5	R	2	6	1	1	1	1	1	1
2	9	5.5	L	1	4	1	1	1	1	0	0
2	9	5.5	R	X	X	X	X	X	X	X	X
2	9	6	L	1	3	1	1	1	0	0	0
2	9	6	R	1	3	1	1	1	0	0	0

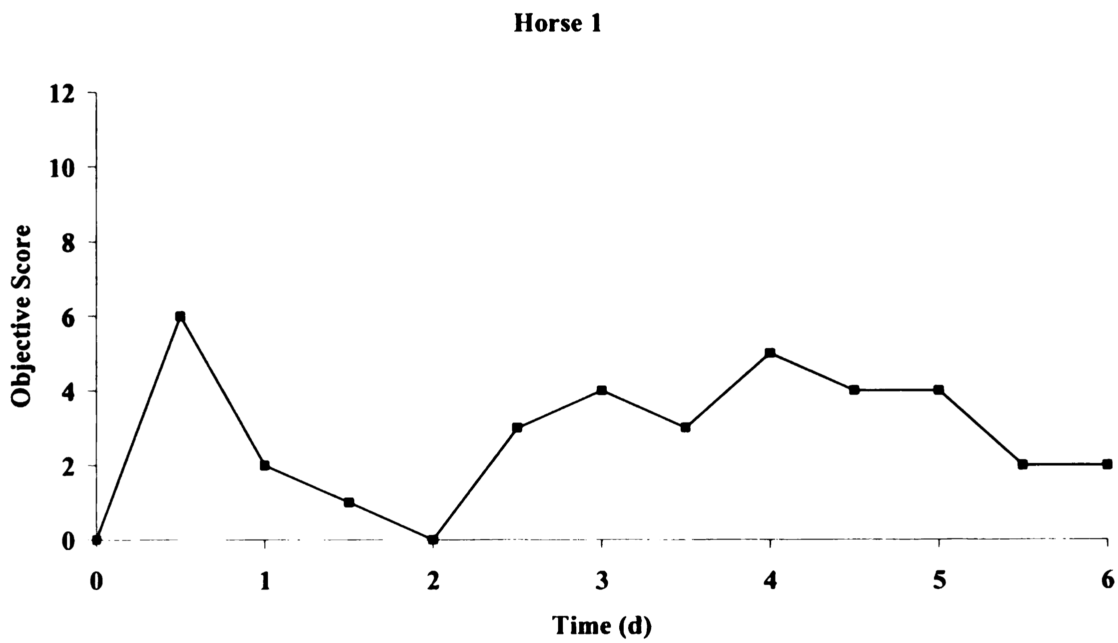
Table 2-3 (con't).

Group #	Horse #	Day	View	Subjective Score (0-5)	Objective Score (0-12)	Location Score (0-1)	Accumulation # Score (0-3)	Opacity Score (0-2)	Homogeneity Score (0-2)	Sand Thickness Score (0-2)	Sand Length Score (0-2)
2	10	0	L	0	0	0	0	0	0	0	0
2	10	0	R	0	0	0	0	0	0	0	0
2	10	0.5	L	3	6	0	1	2	2	1	0
2	10	0.5	R	3	6	0	1	2	2	1	0
2	10	1	L	0	0	0	0	0	0	0	0
2	10	1	R	X	X	X	X	X	X	X	X
2	10	1.5	L	0	4	0	1	1	1	1	0
2	10	1.5	R	1	4	0	1	1	1	1	0
2	10	2	L	0	3	1	1	1	0	0	0
2	10	2	R	0	0	0	0	0	0	0	0
2	10	2.5	L	2	4	1	1	1	1	0	0
2	10	2.5	R	1	4	1	1	1	1	0	0
2	10	3	L	0	4	0	1	2	1	0	0
2	10	3	R	1	3	0	1	1	1	0	0
2	10	3.5	L	1	4	1	1	1	1	0	0
2	10	3.5	R	1	4	1	1	1	1	0	0
2	10	4	L	1	6	1	1	2	2	0	0
2	10	4	R	2	6	1	1	2	2	0	0
2	10	4.5	L	1	8	1	3	2	2	0	0
2	10	4.5	R	1	8	1	3	2	2	0	0
2	10	5	L	1	8	1	3	2	2	0	0
2	10	5	R	2	8	1	3	2	2	0	0
2	10	5.5	L	0	4	1	1	1	1	0	0
2	10	5.5	R	1	8	1	3	2	2	0	0
2	10	6	L	X	X	X	X	X	X	X	X
2	10	6	R	X	X	X	X	X	X	X	X

APPENDIX D

Figure 2-21

Individual horse and group average objective score trends over time.



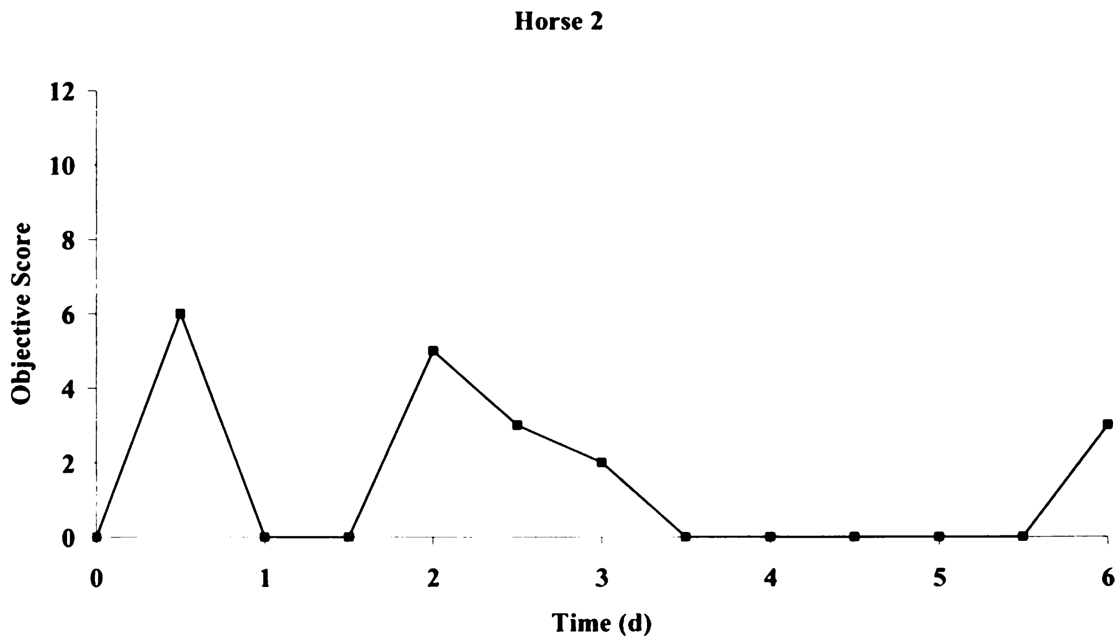
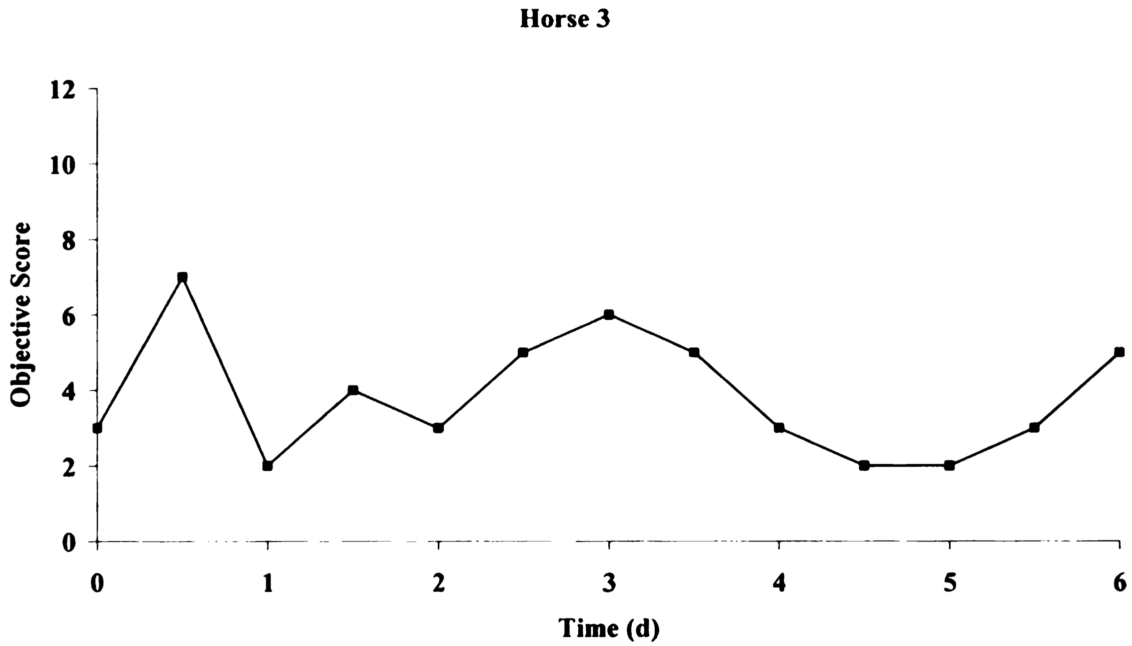


Figure 2-21 (con't).



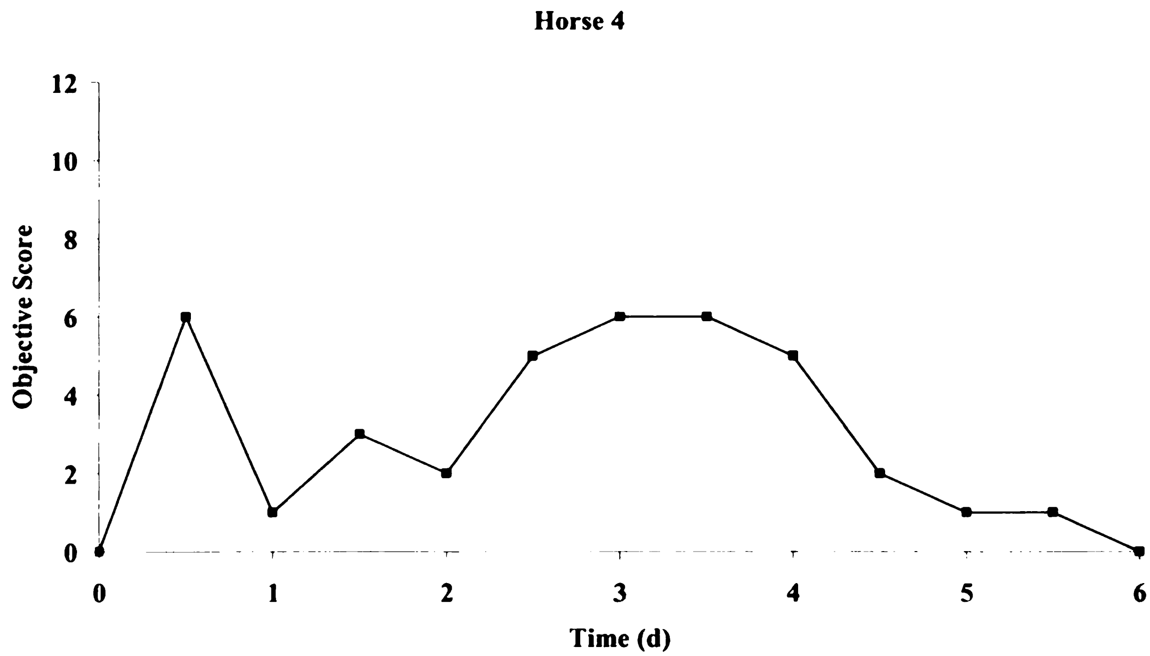
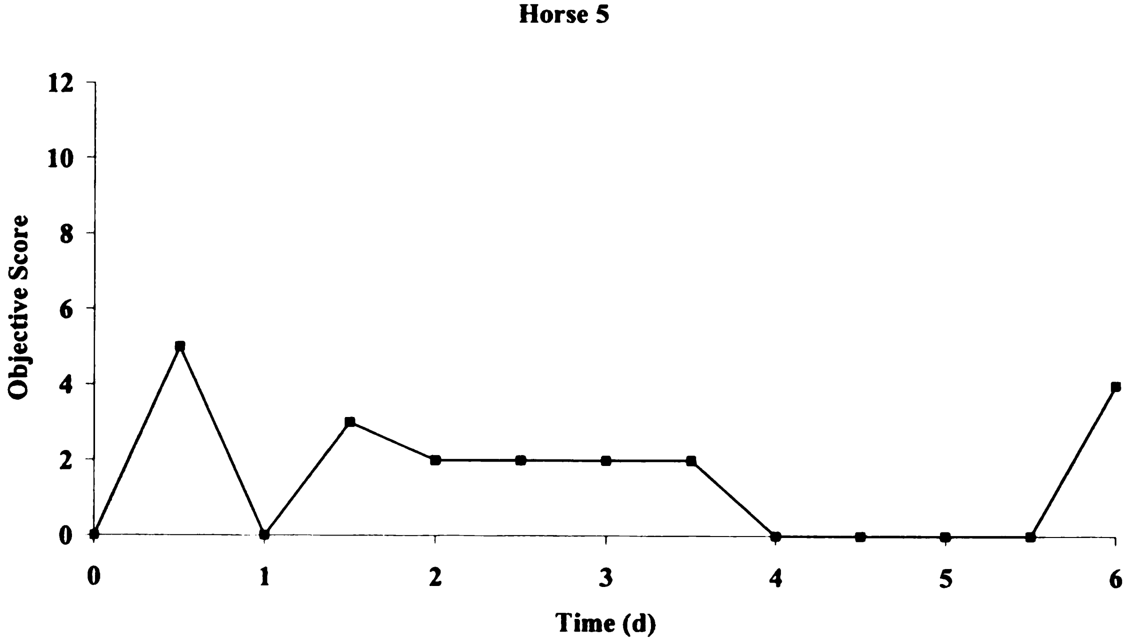


Figure 2-21 (con't).



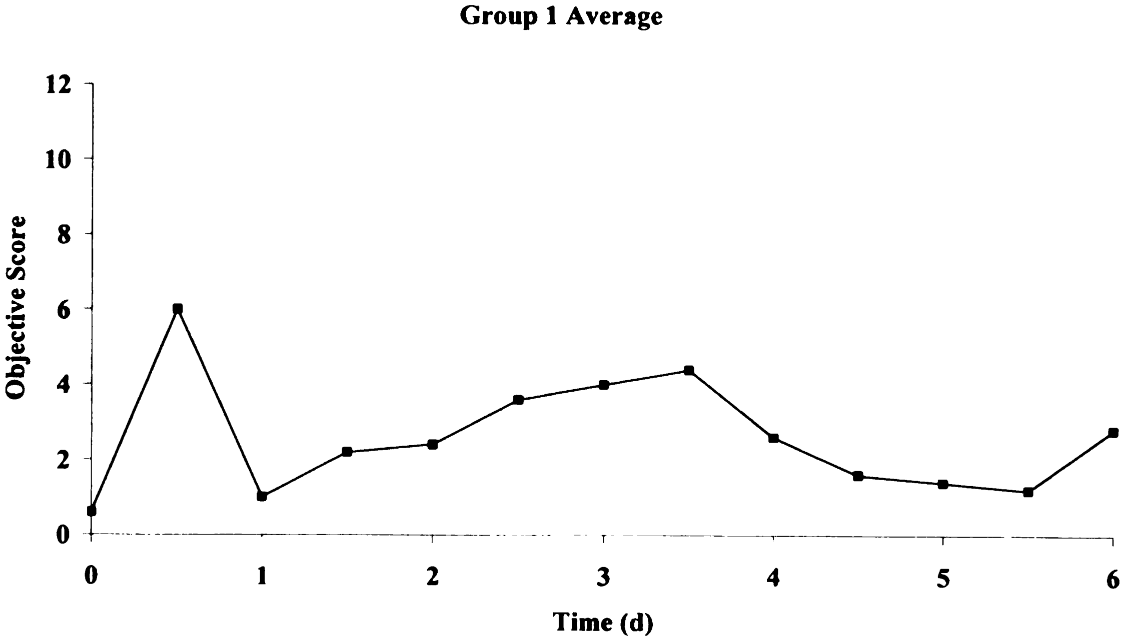
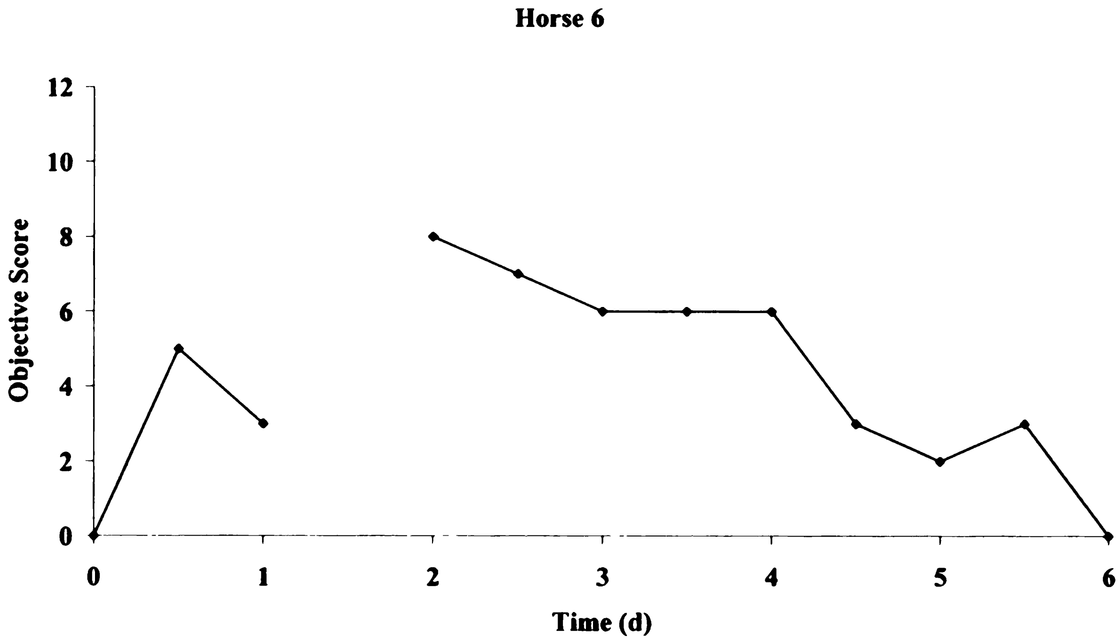


Figure 2-21 (con't).



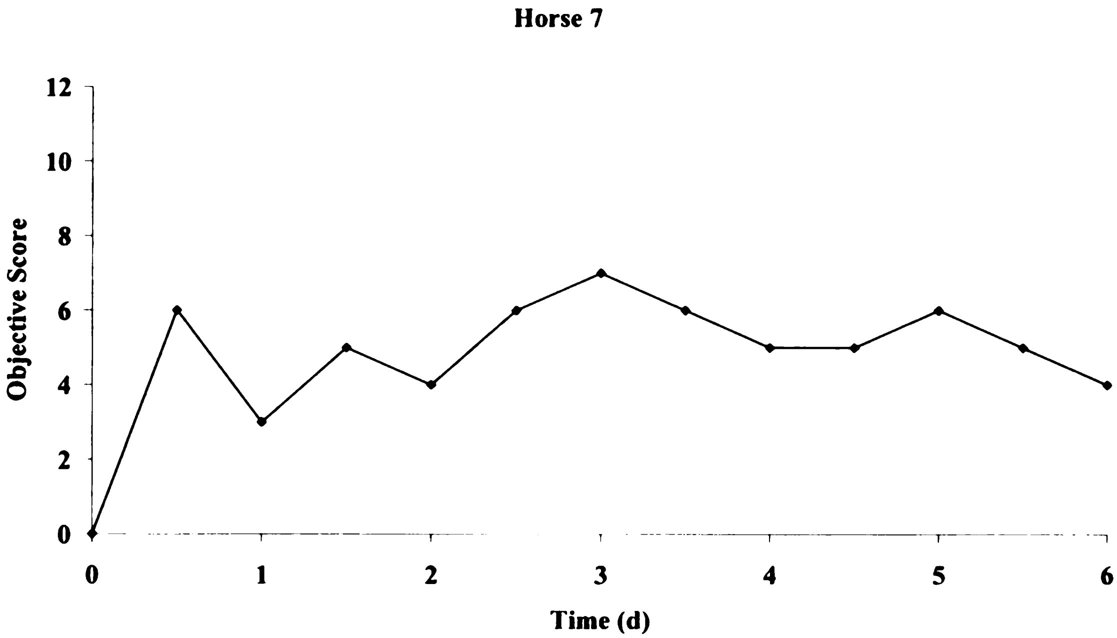
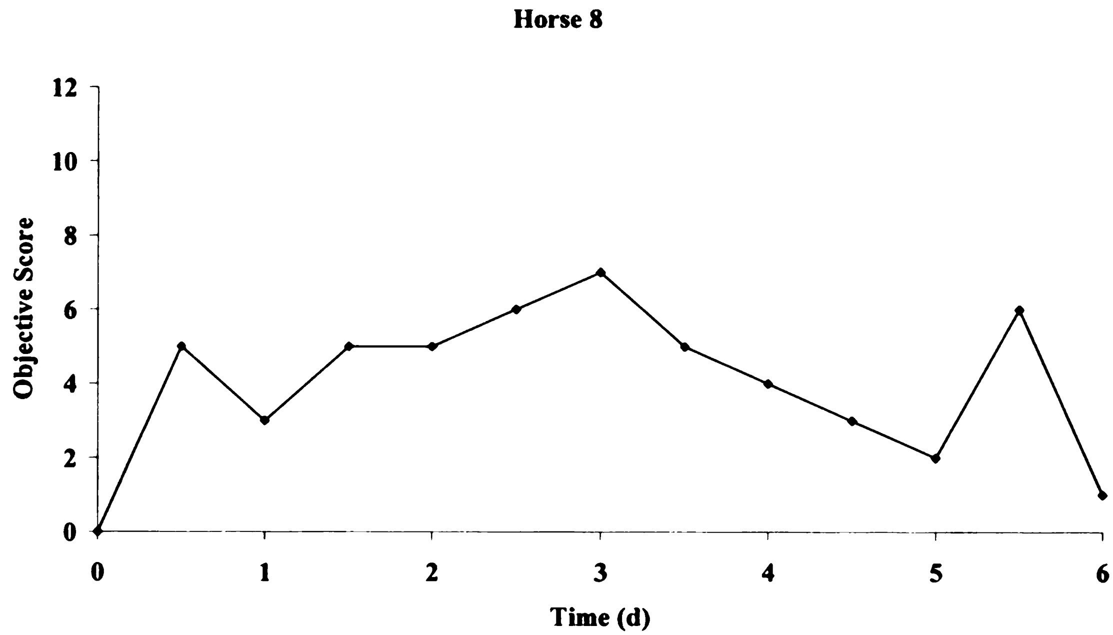


Figure 2-21 (con't).



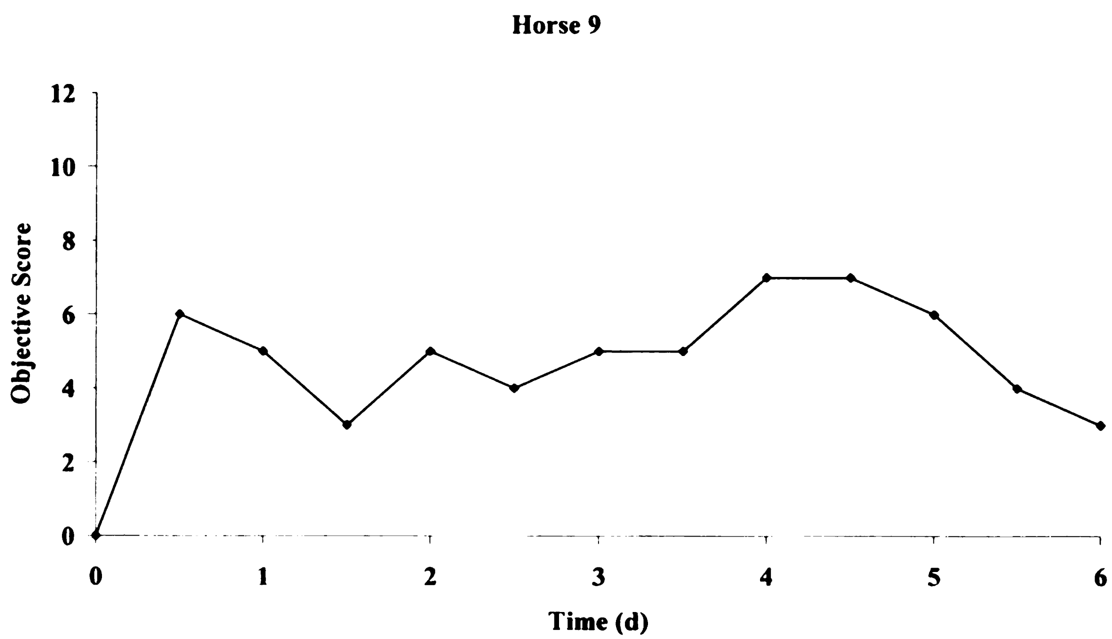
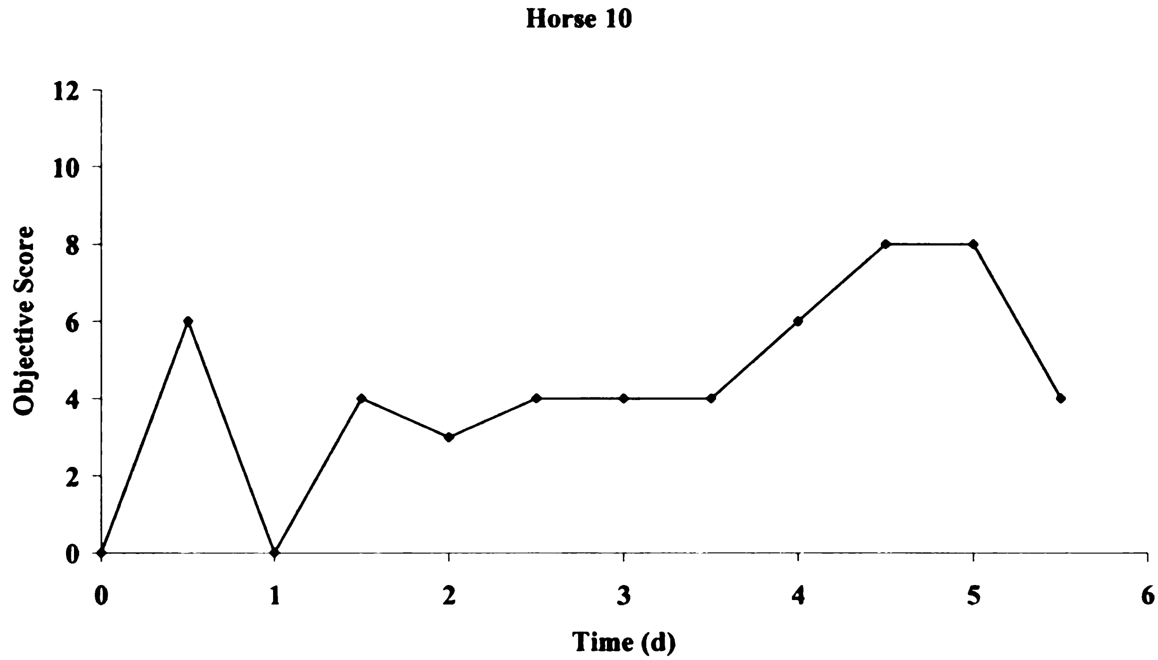
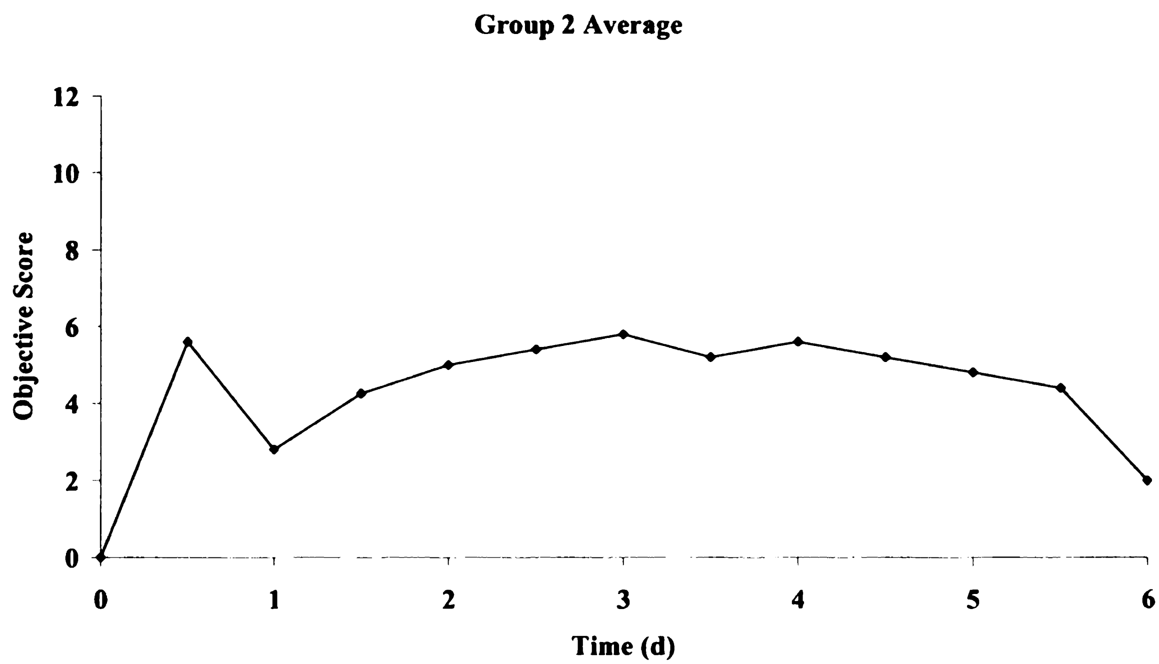


Figure 2-21 (con't).

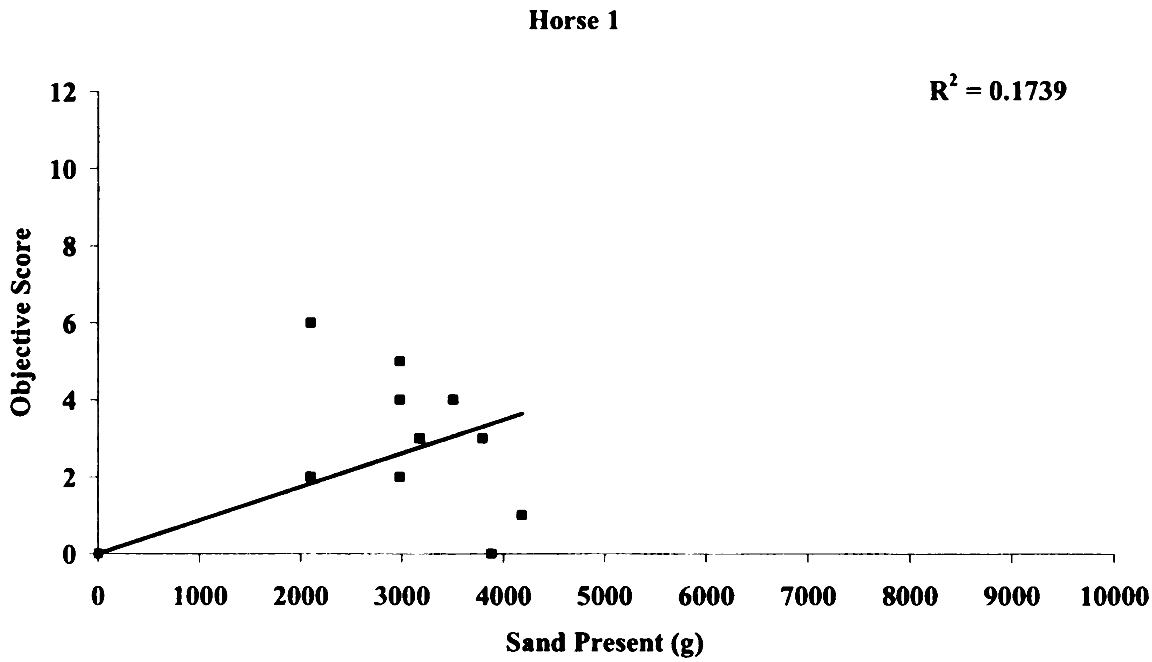




APPENDIX E

Figure 2-22

Individual horse objective scores based on weight of intra-abdominal sand.



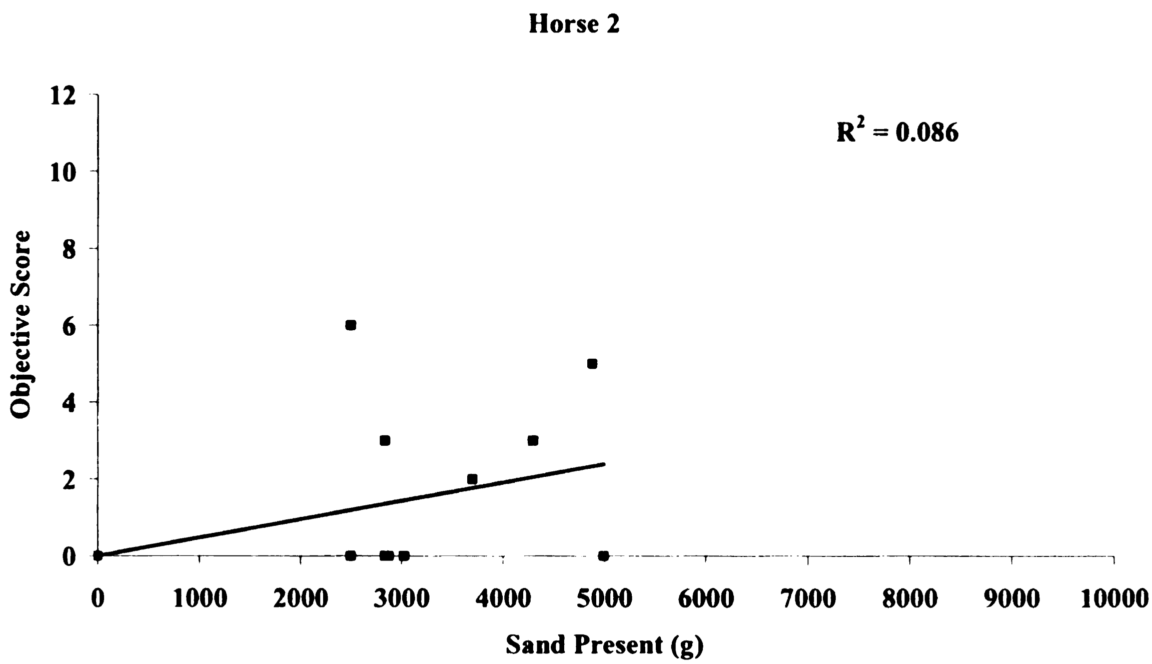
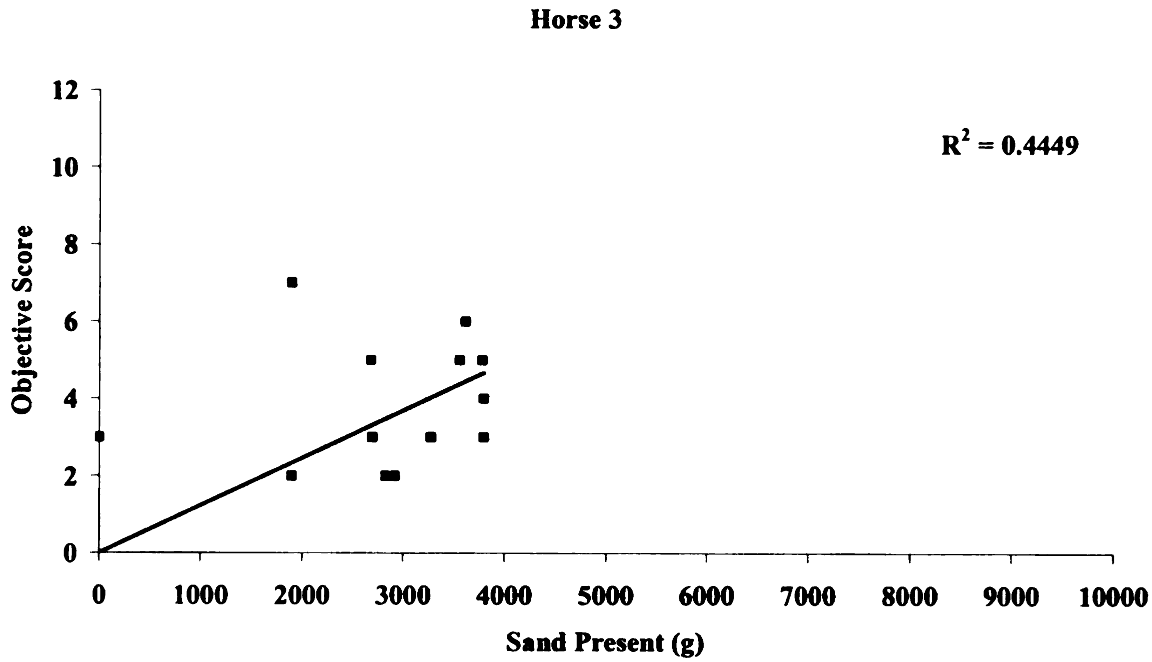
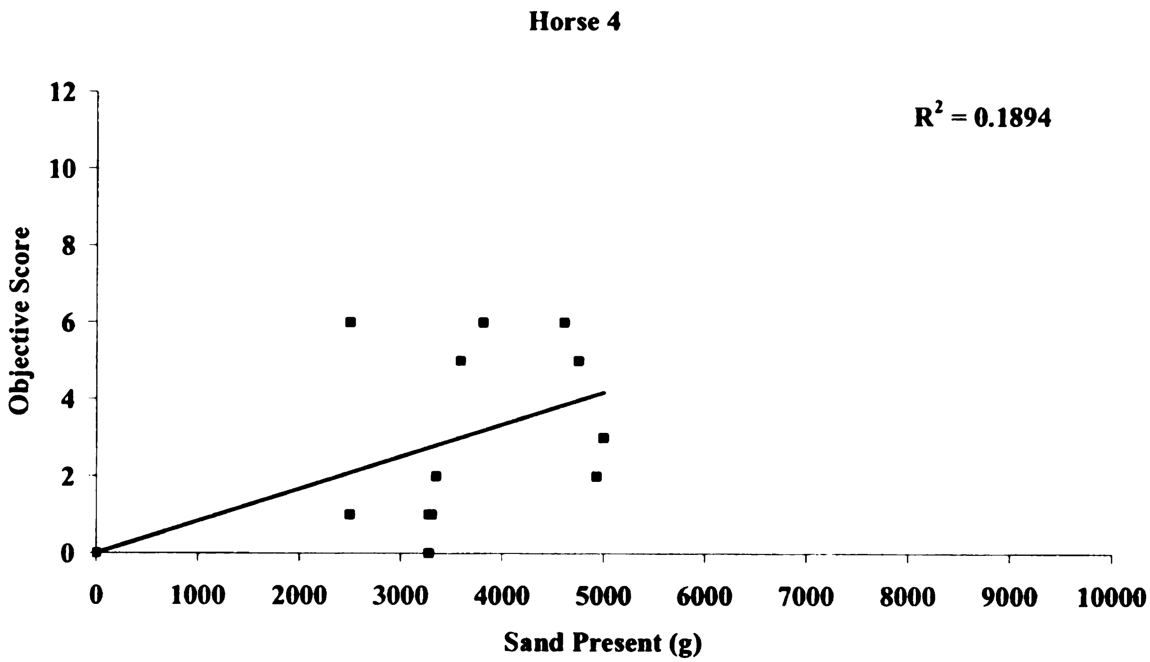


Figure 2-22 (con't).





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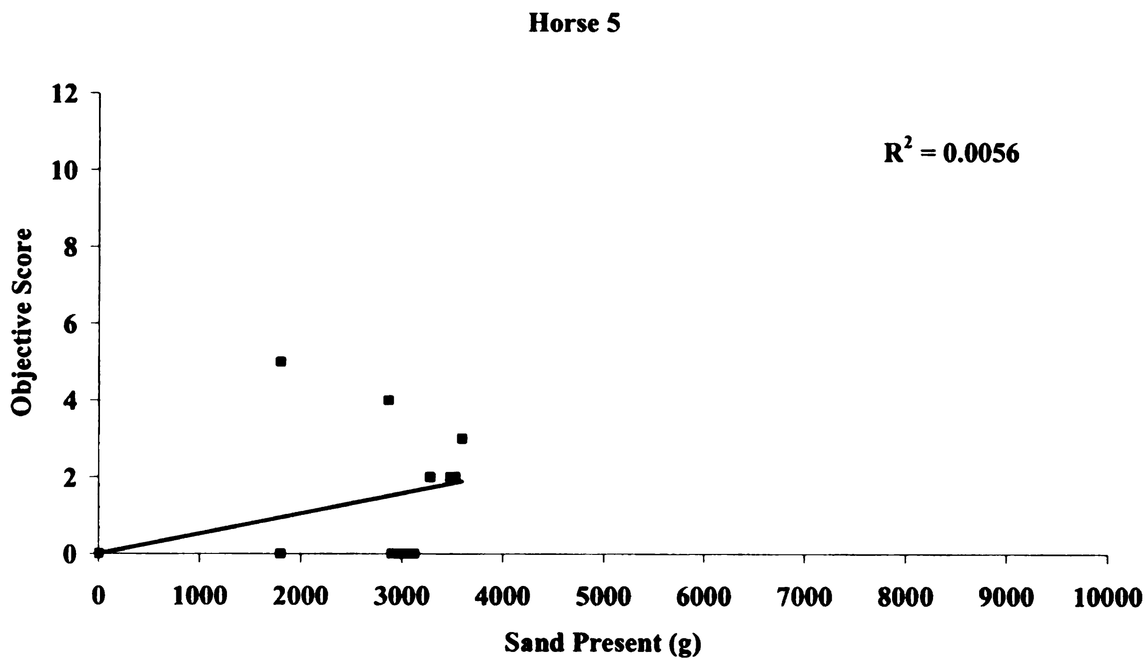

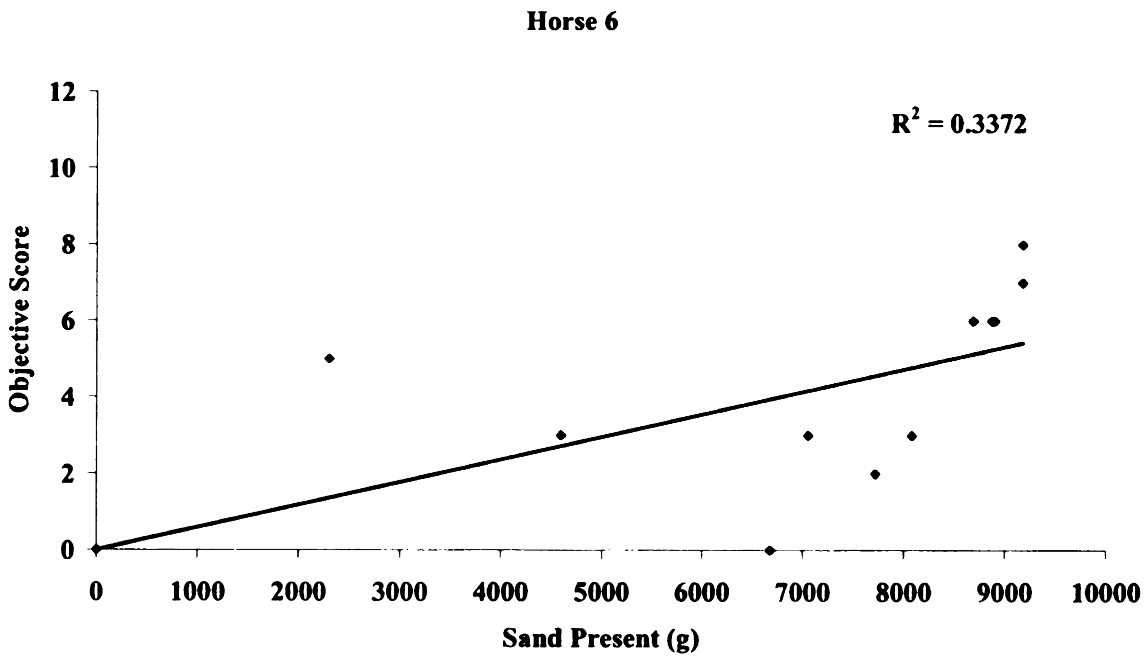

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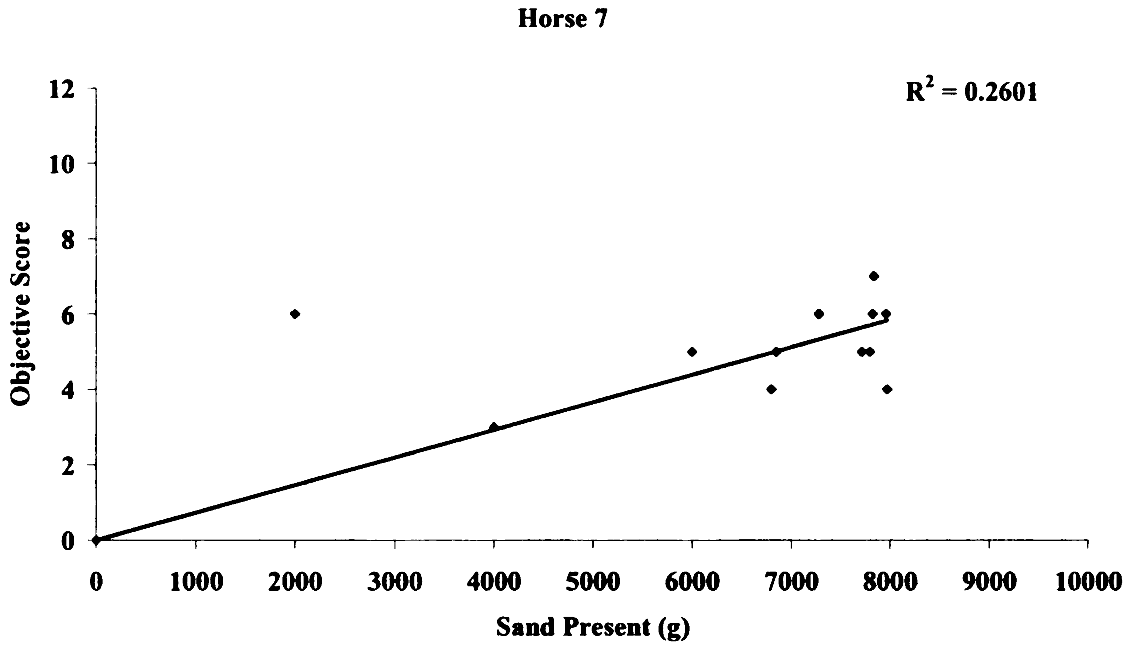


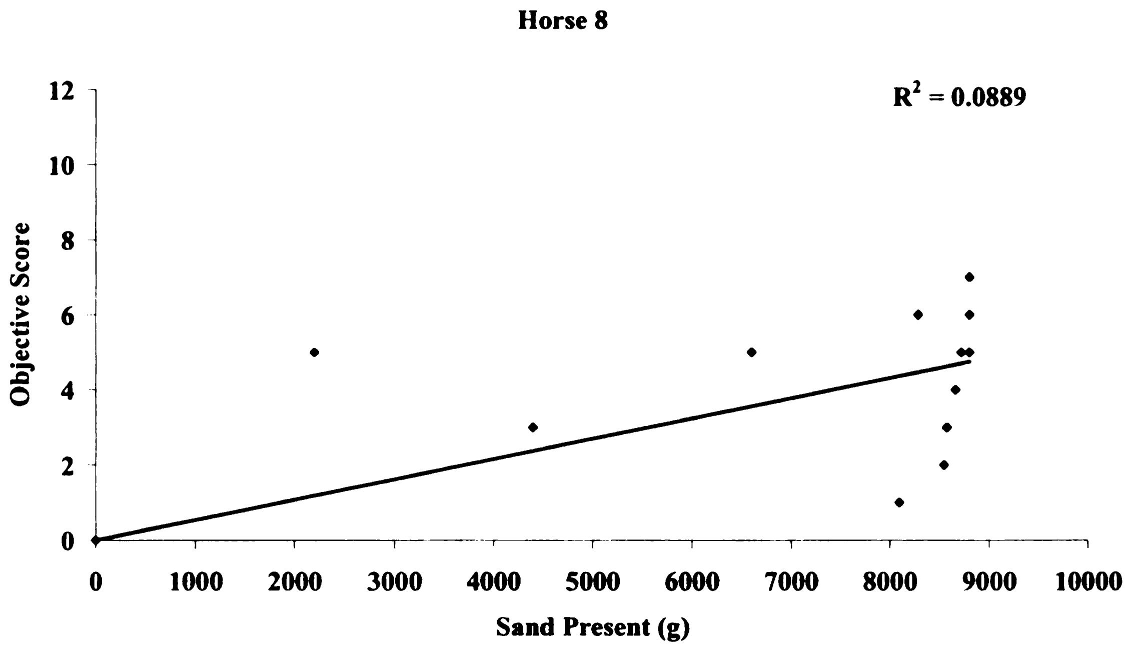
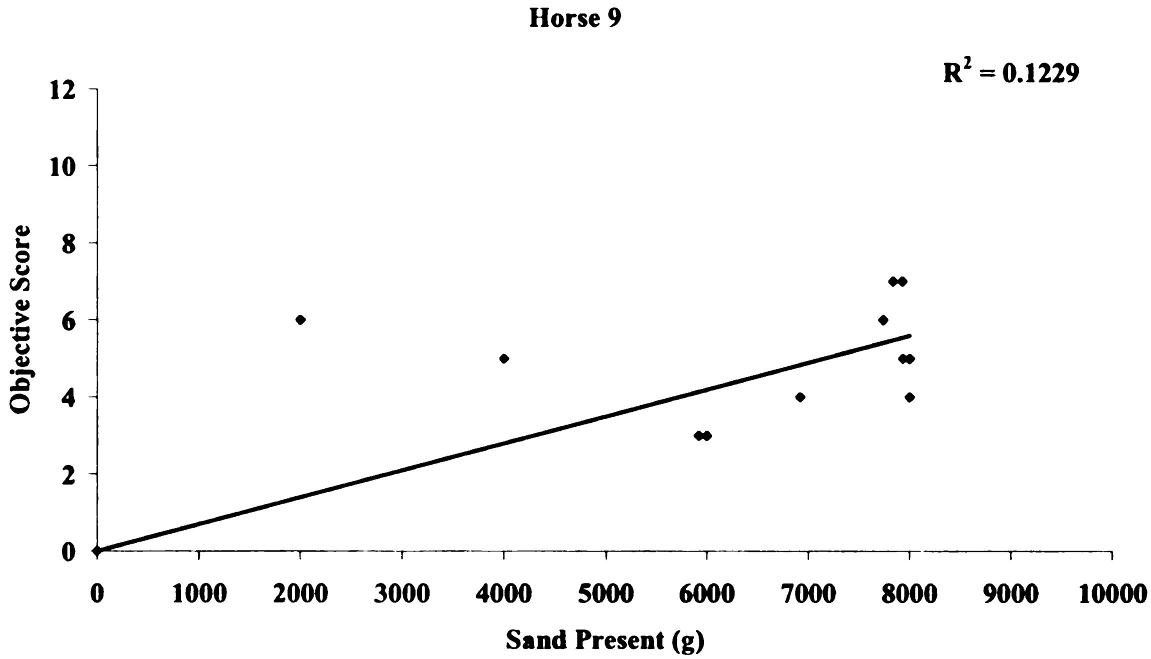
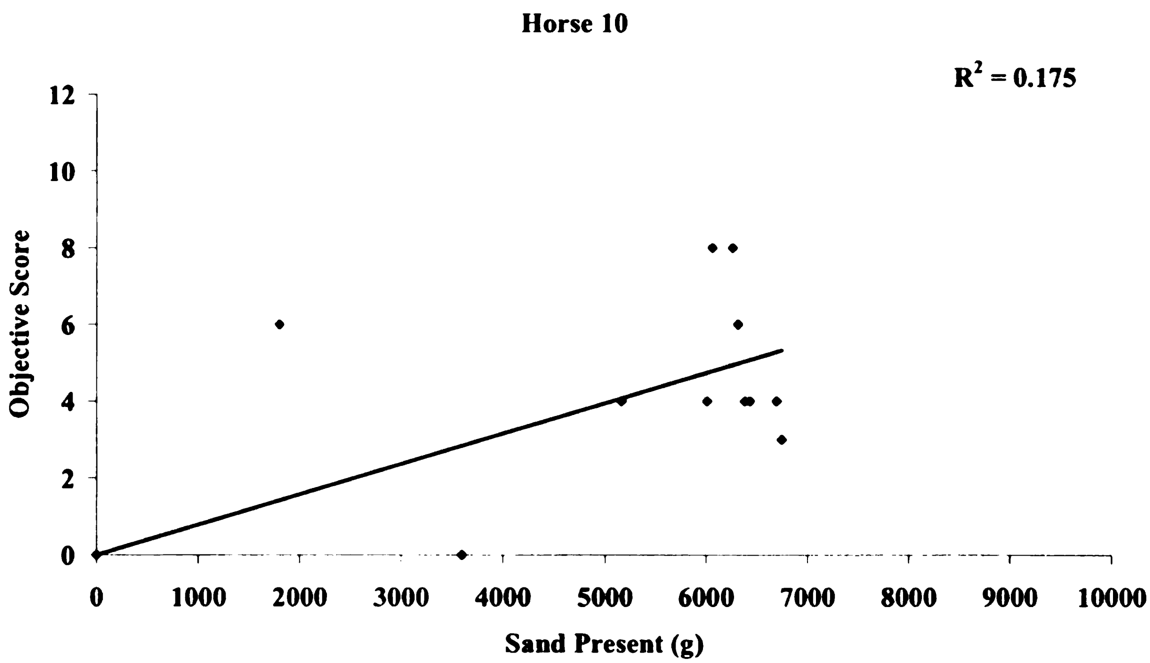


Figure 2-22 (con't).

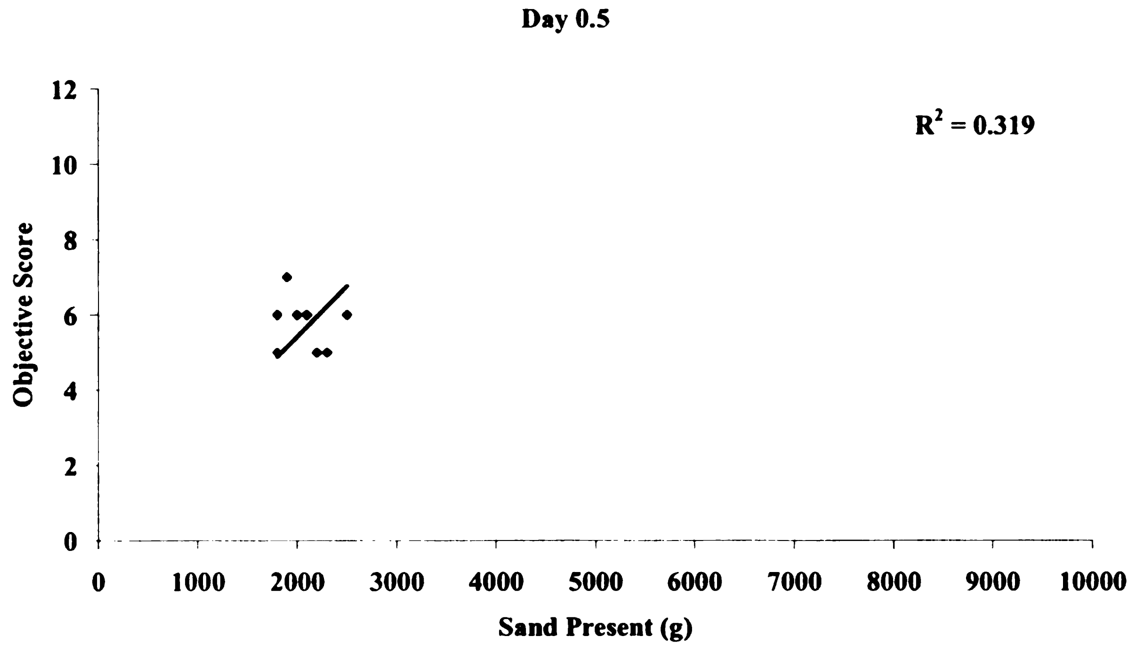




APPENDIX F

Figure 2-23

Combined Group 1 and Group 2 objective scores based on weight of intra-abdominal sand on a given day.



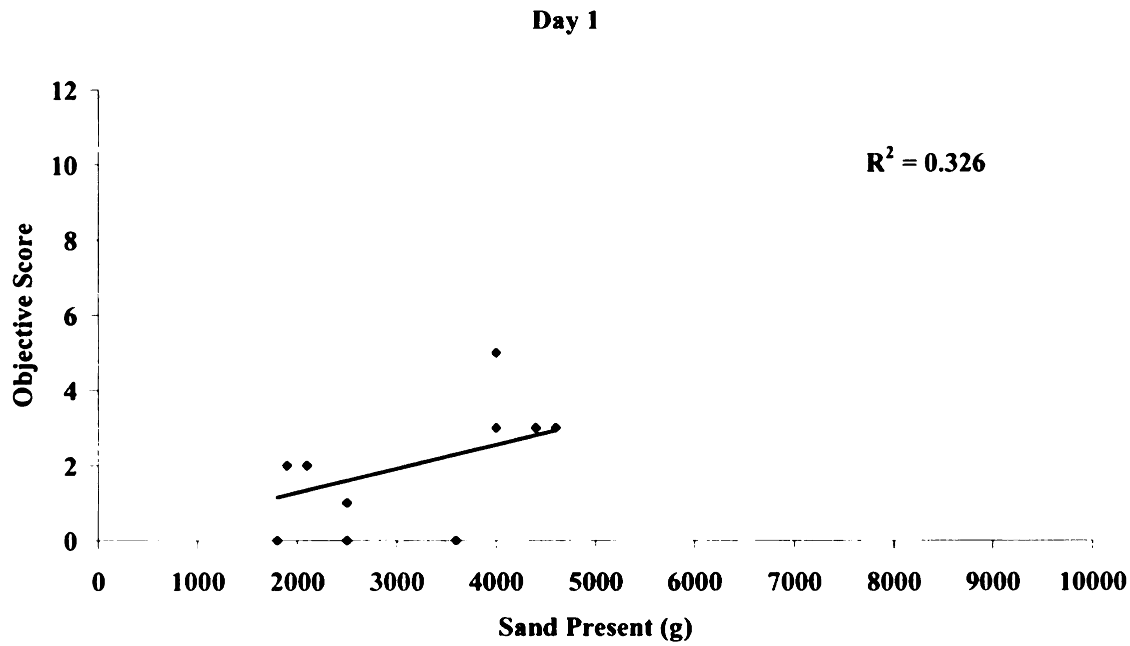
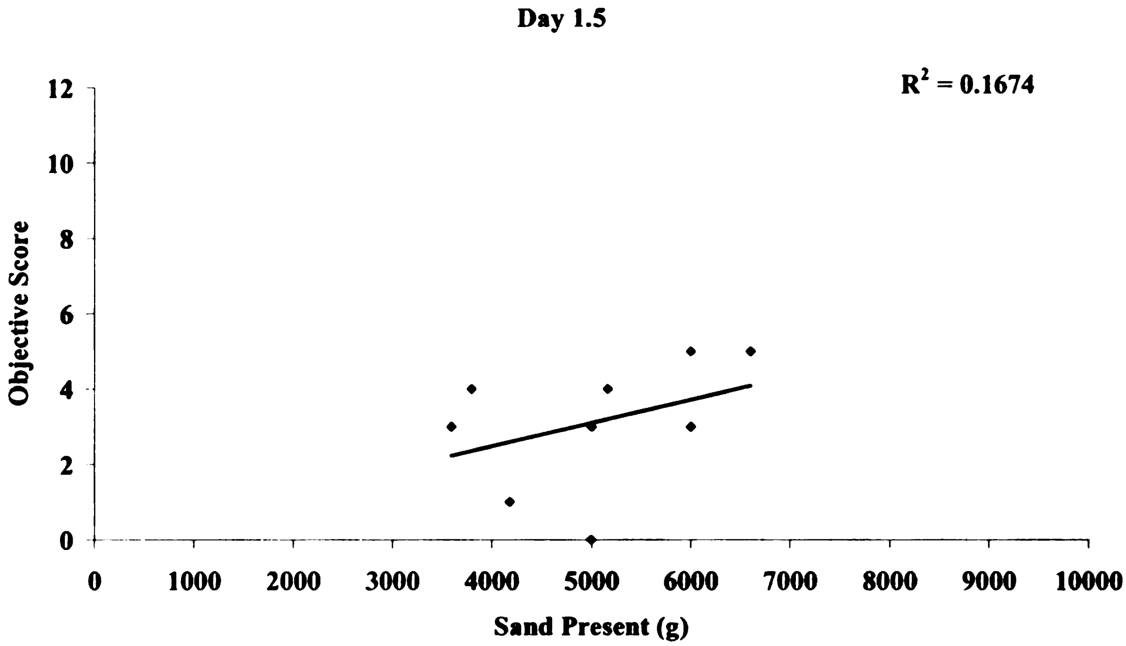


Figure 2-23 (con't).



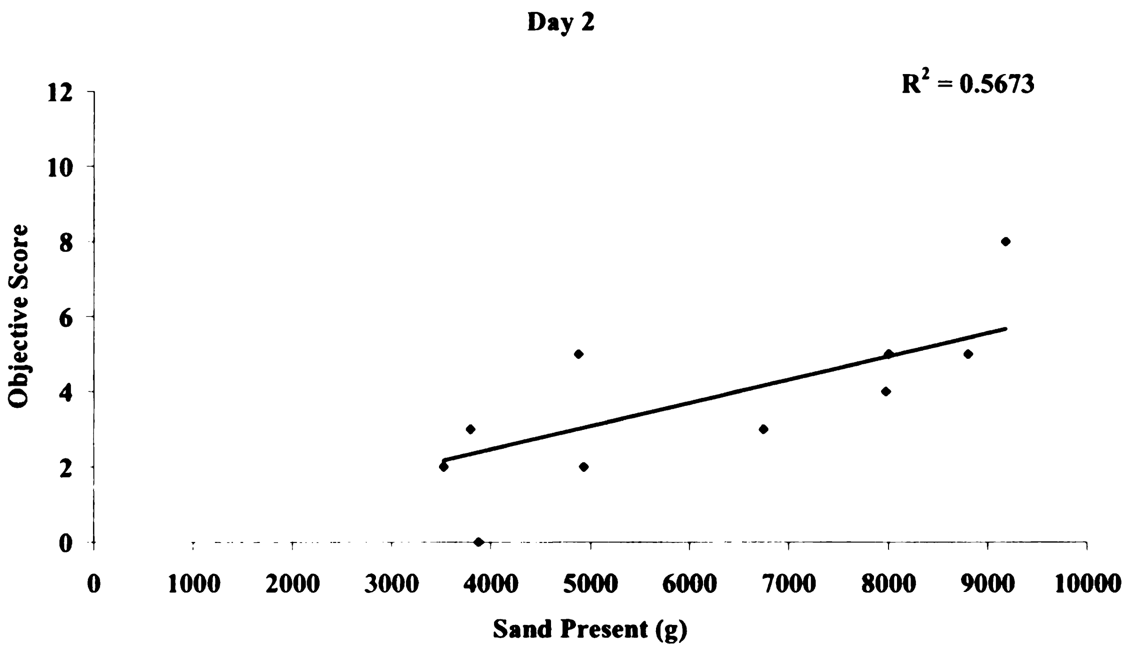
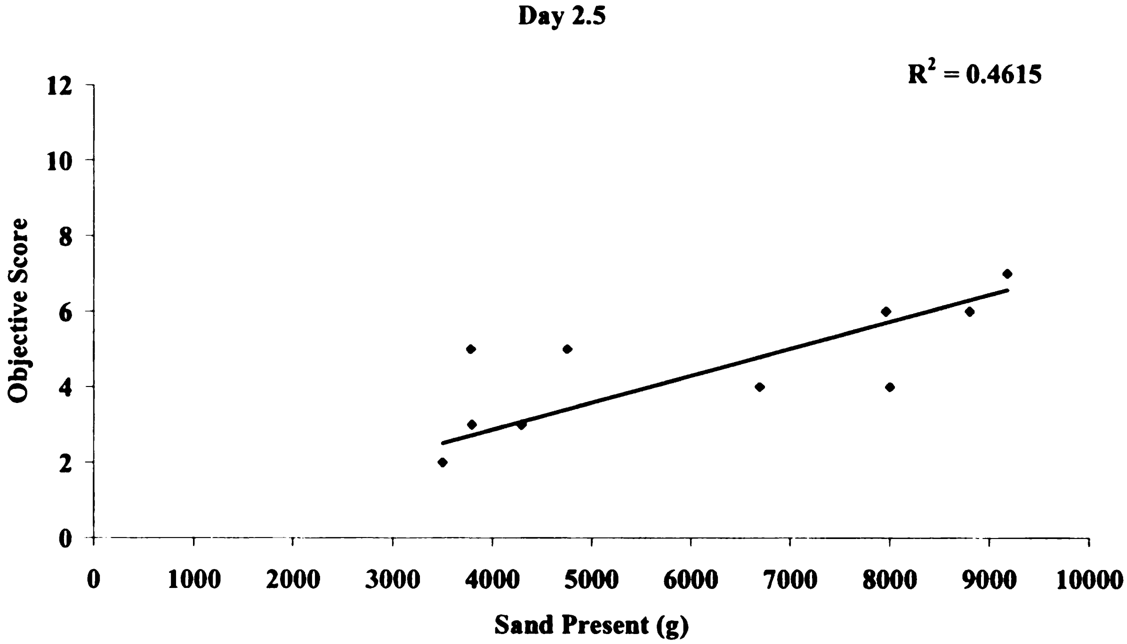


Figure 2-23 (con't).



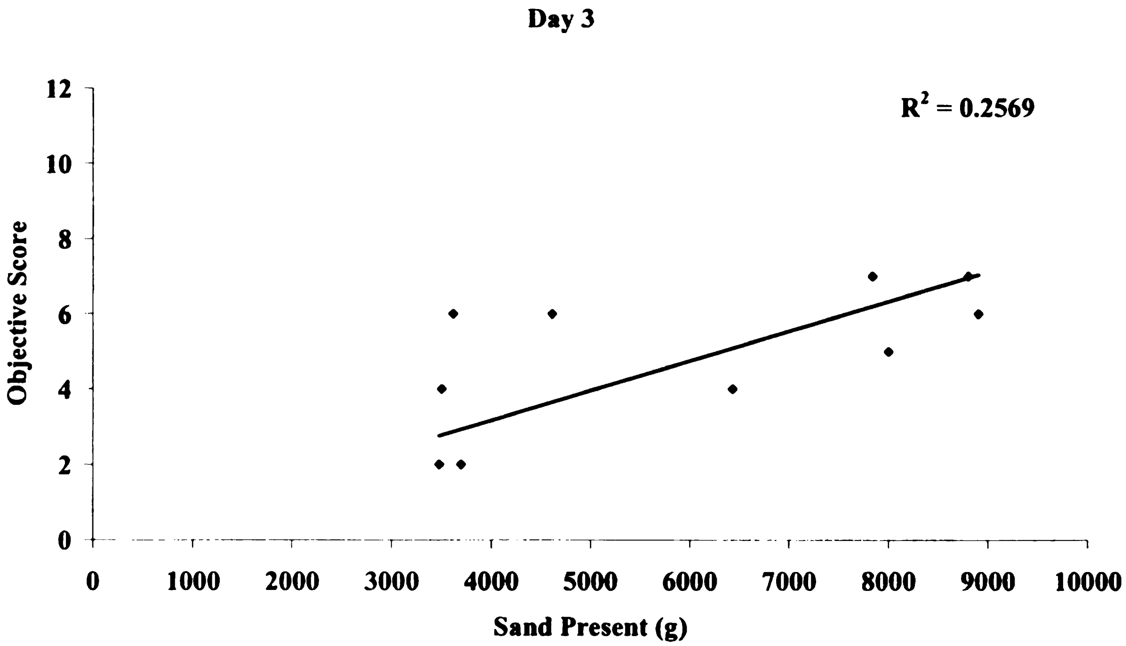
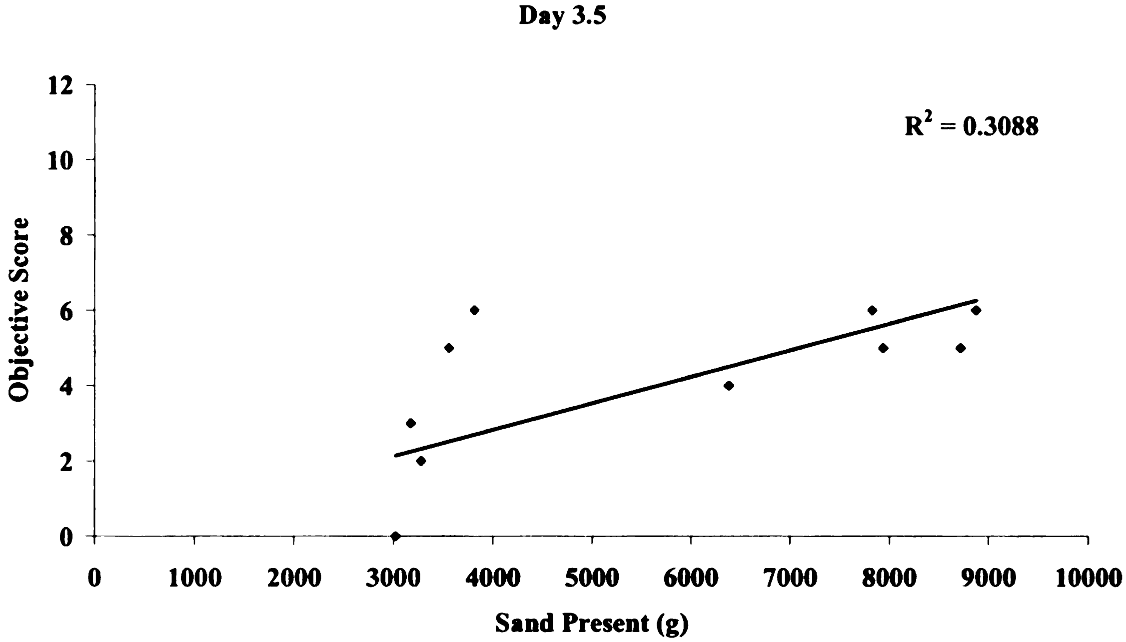


Figure 2-23 (con't).



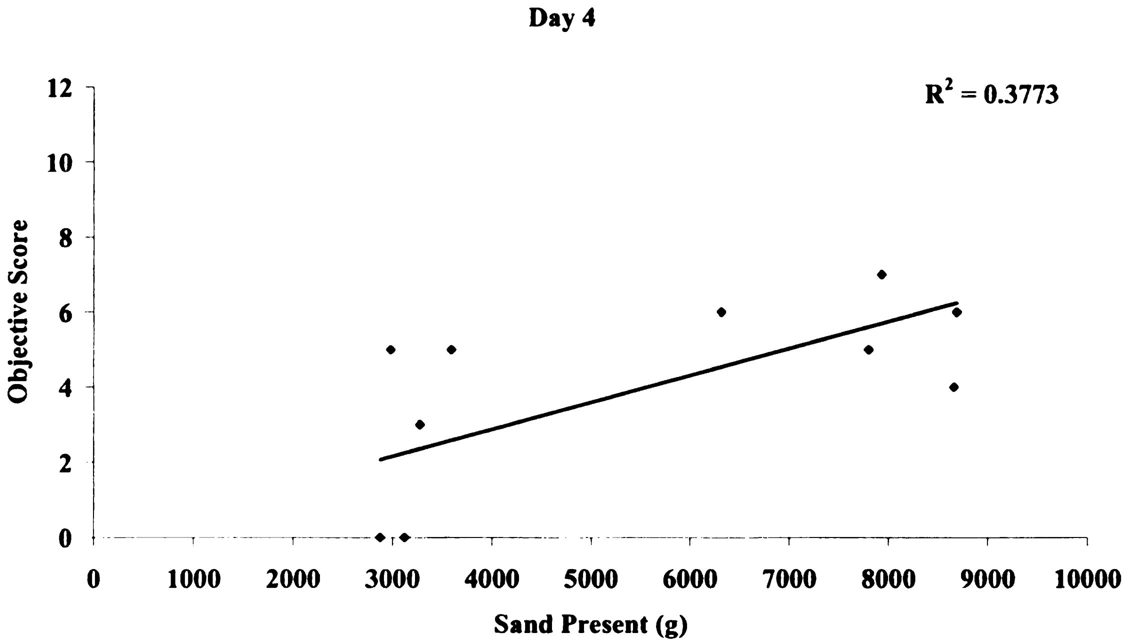
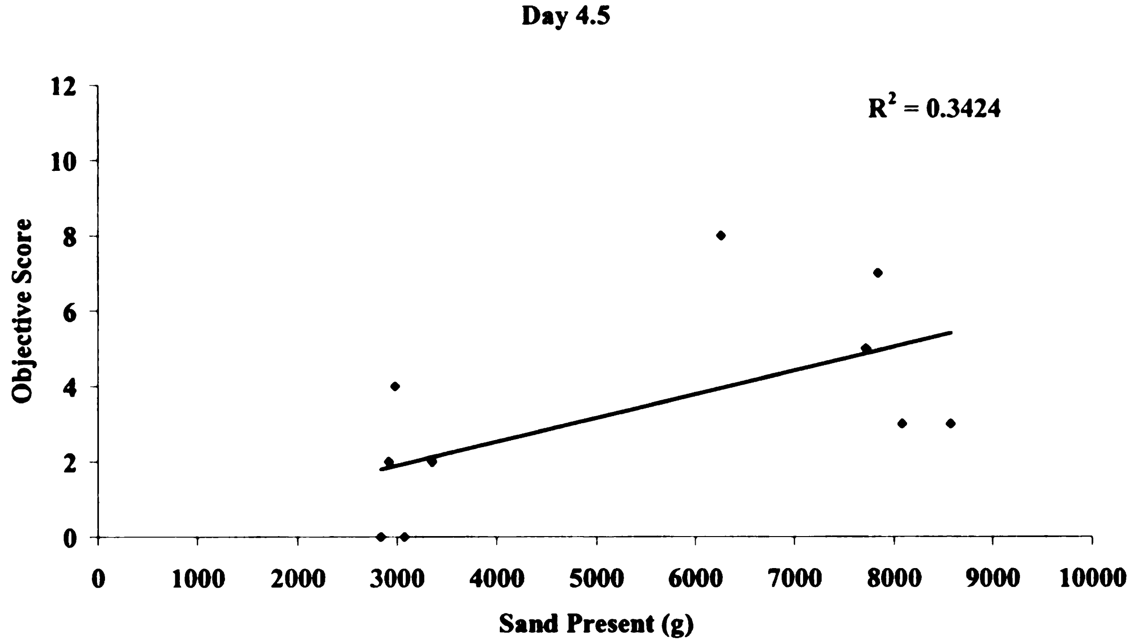


Figure 2-23 (con't).



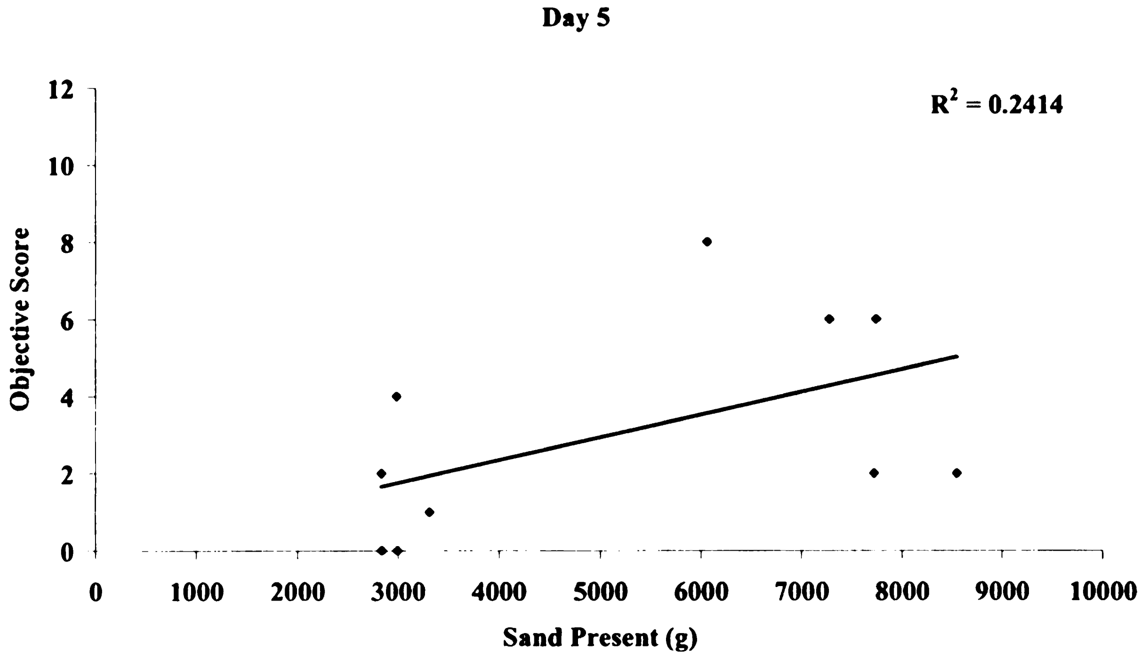
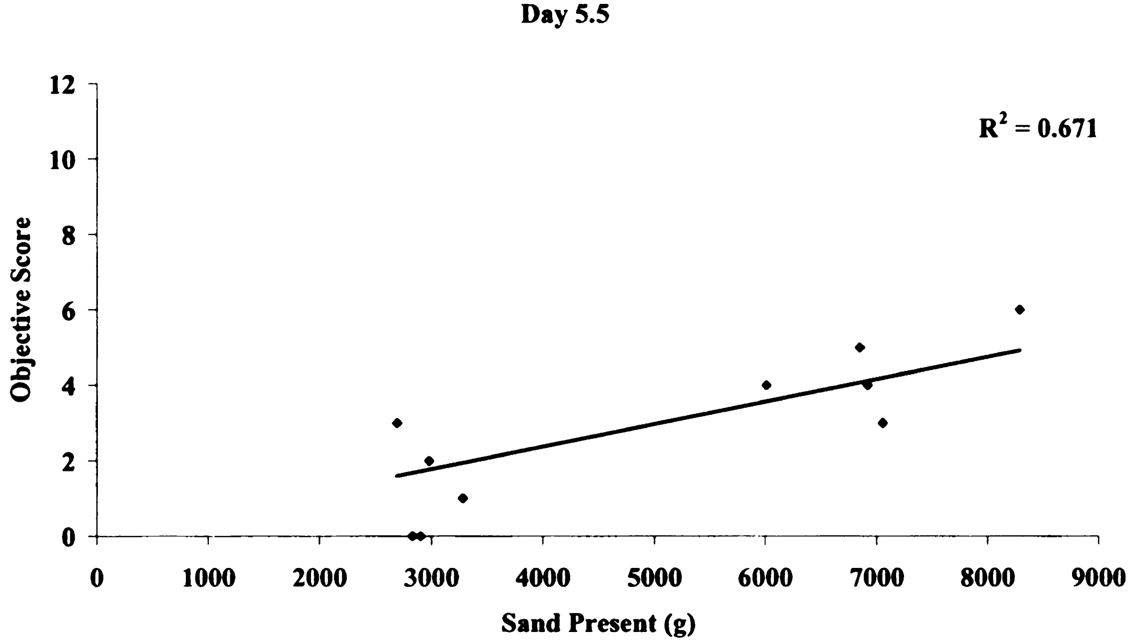
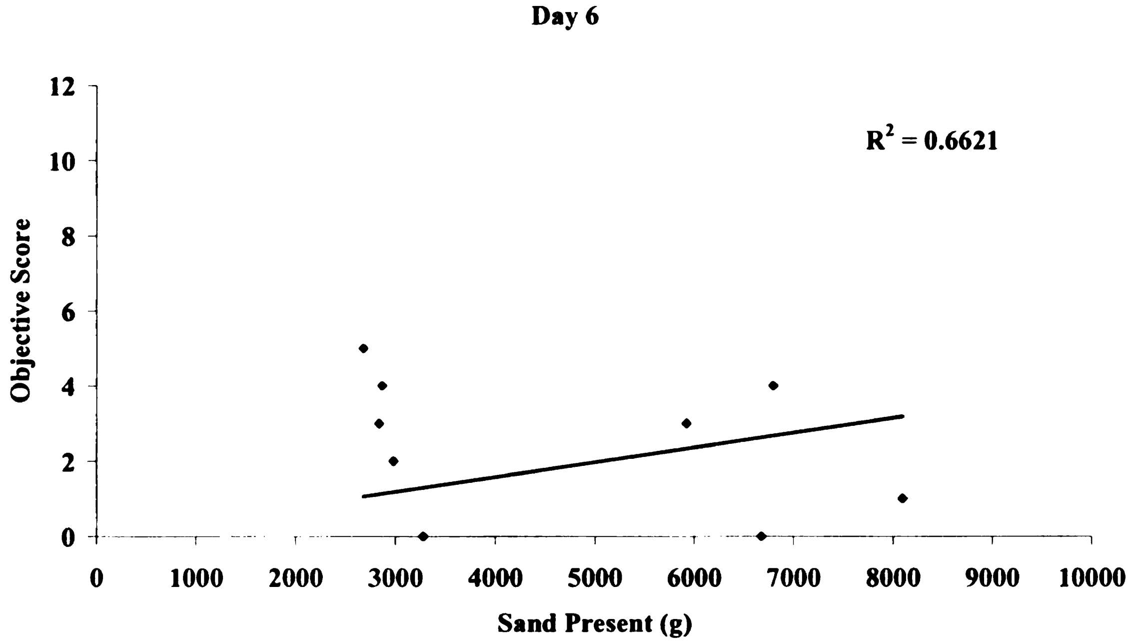


Figure 2-23 (con't).

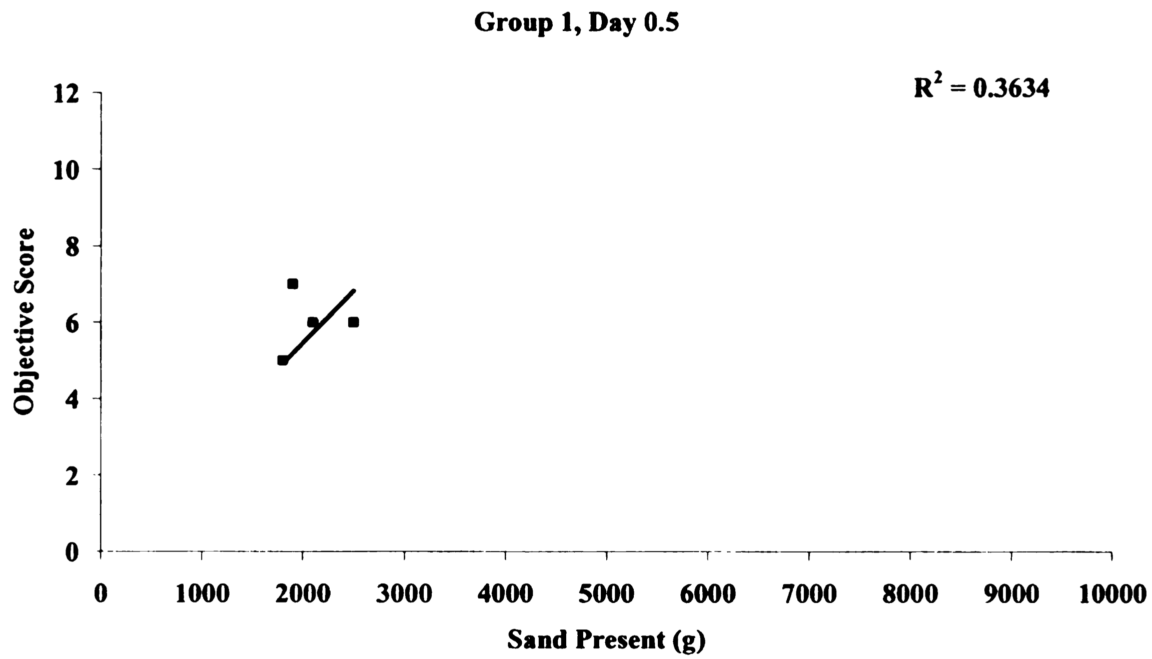




APPENDIX G

Figure 2-24

Individual group objective scores based on weight of intra-abdominal sand on a given day.



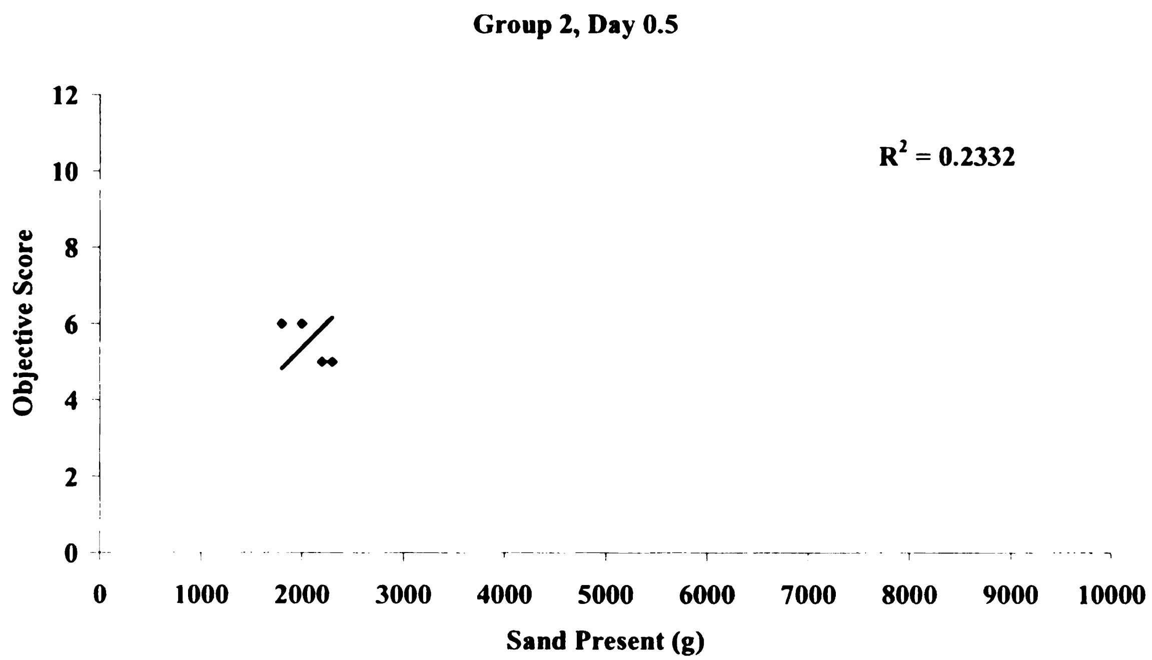
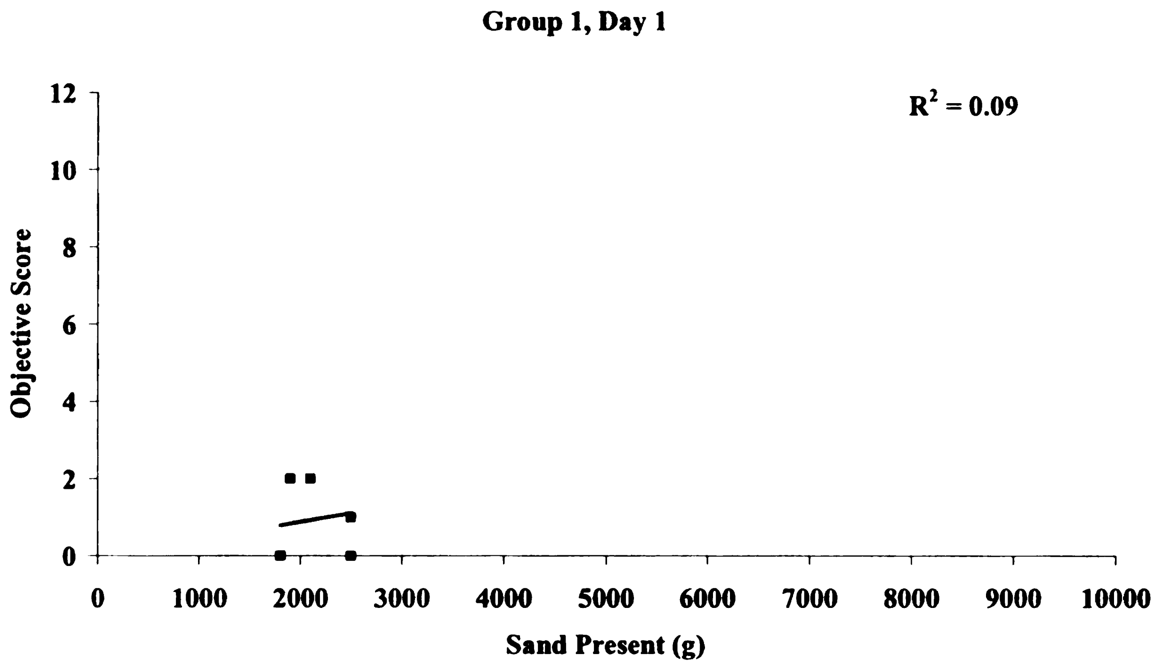


Figure 2-24 (con't).



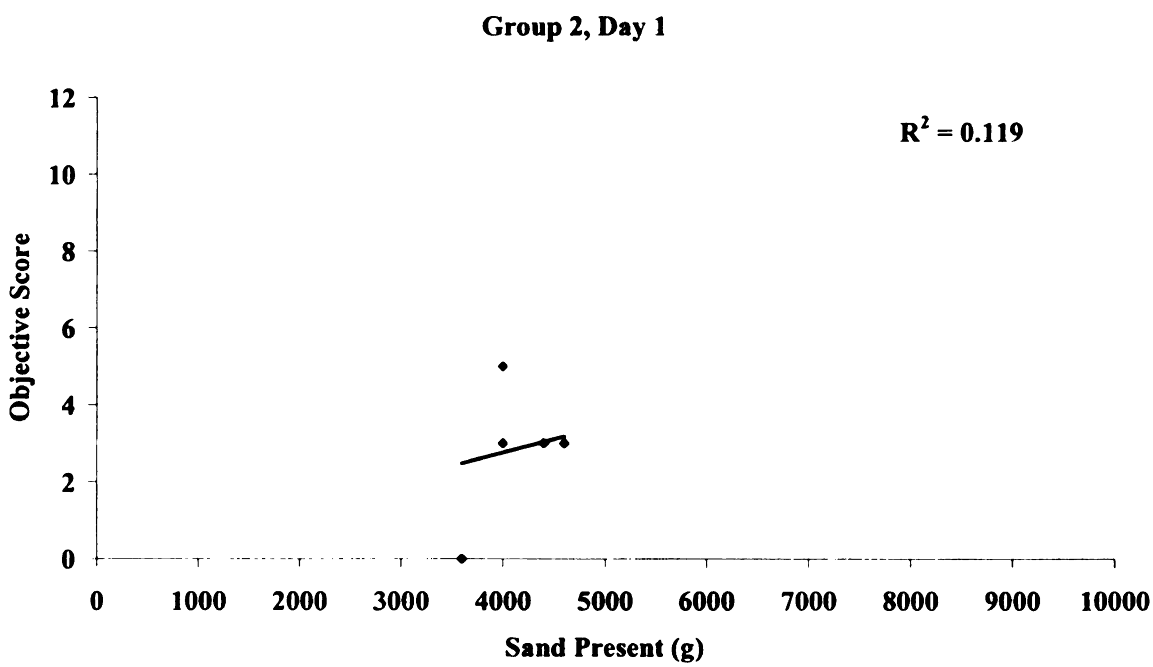
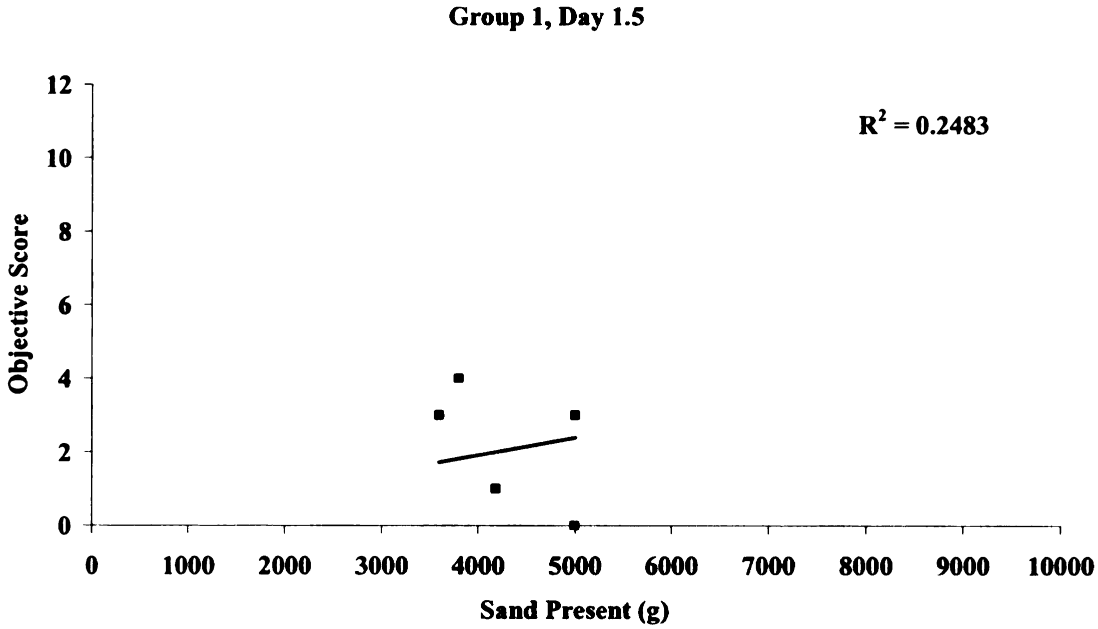
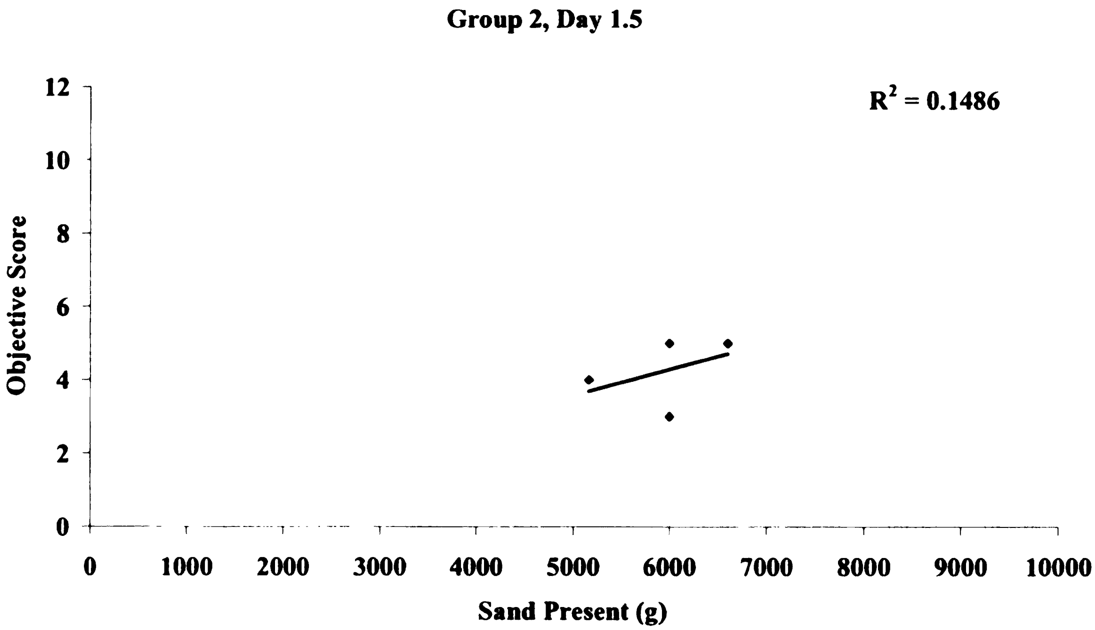


Figure 2-24 (con't).





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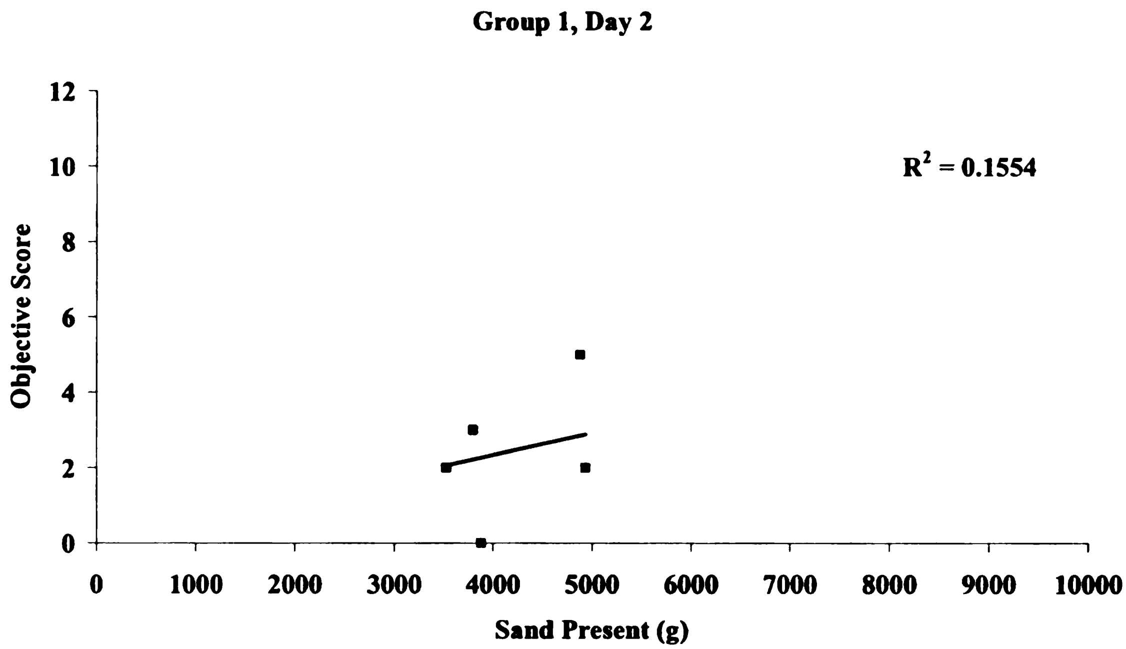


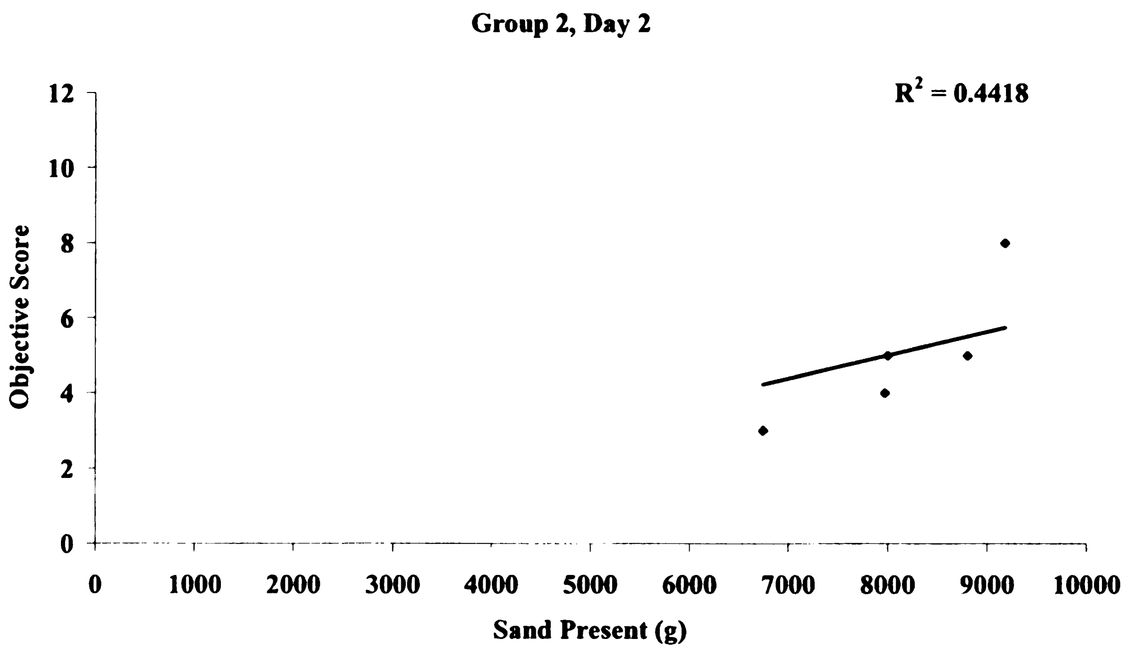
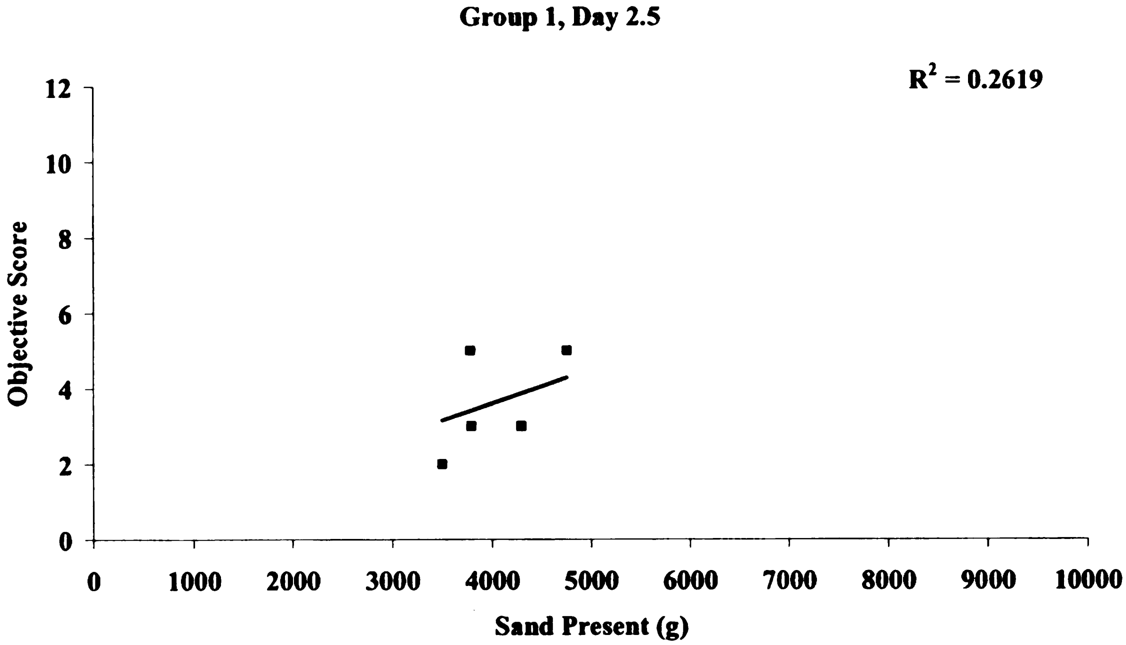


Figure 2-24 (con't).



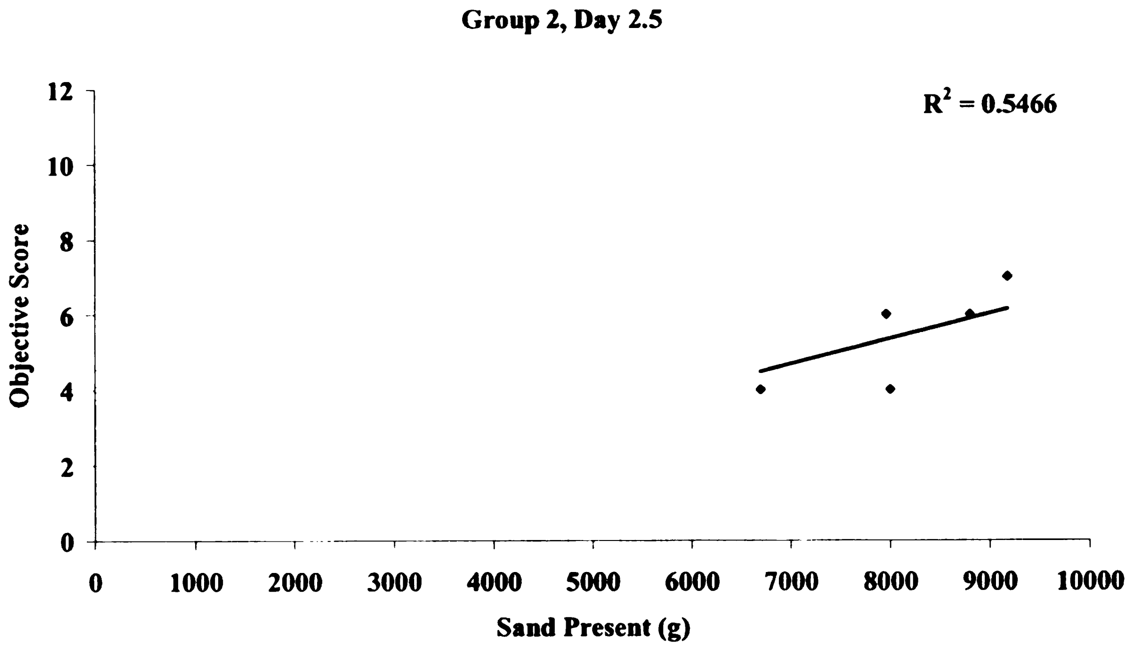
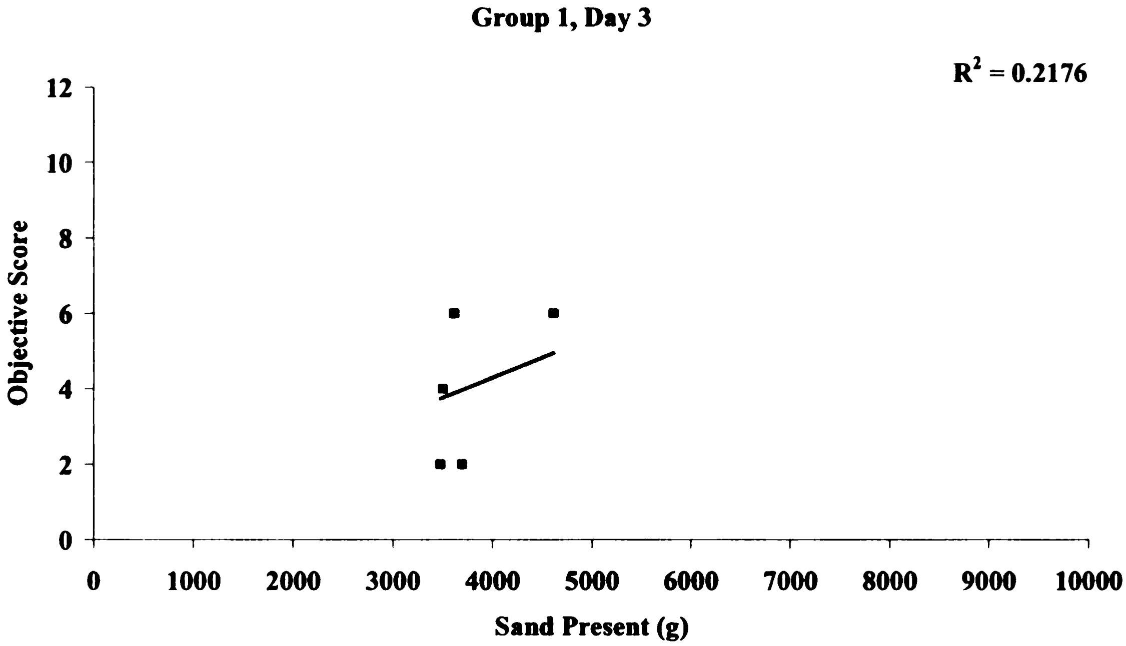


Figure 2-24 (con't).



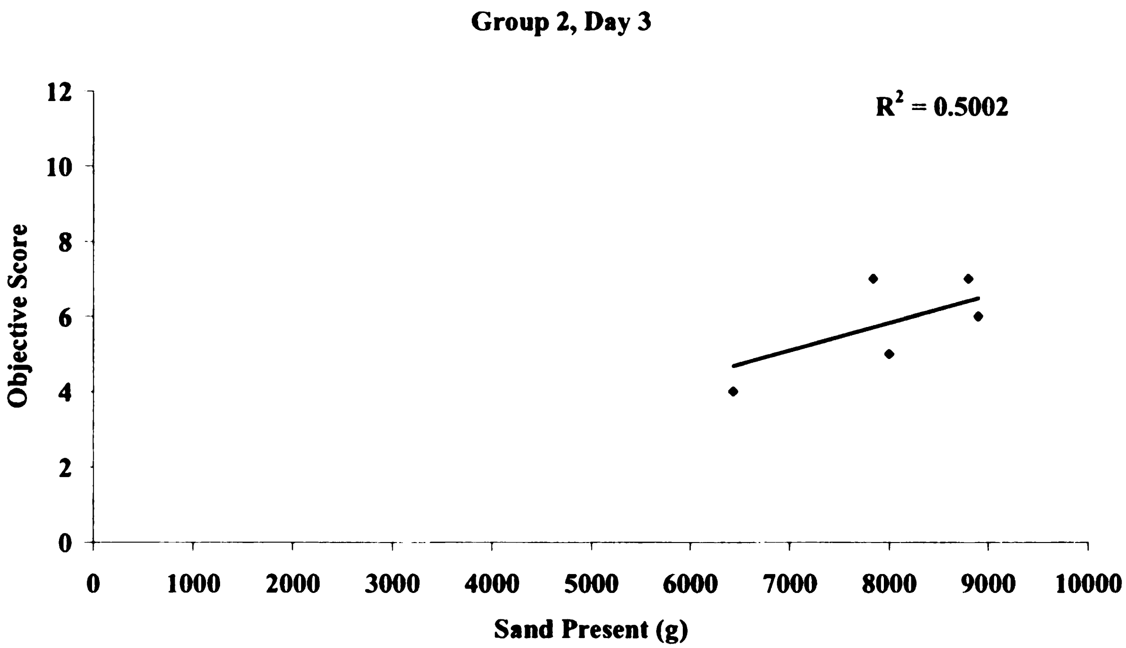
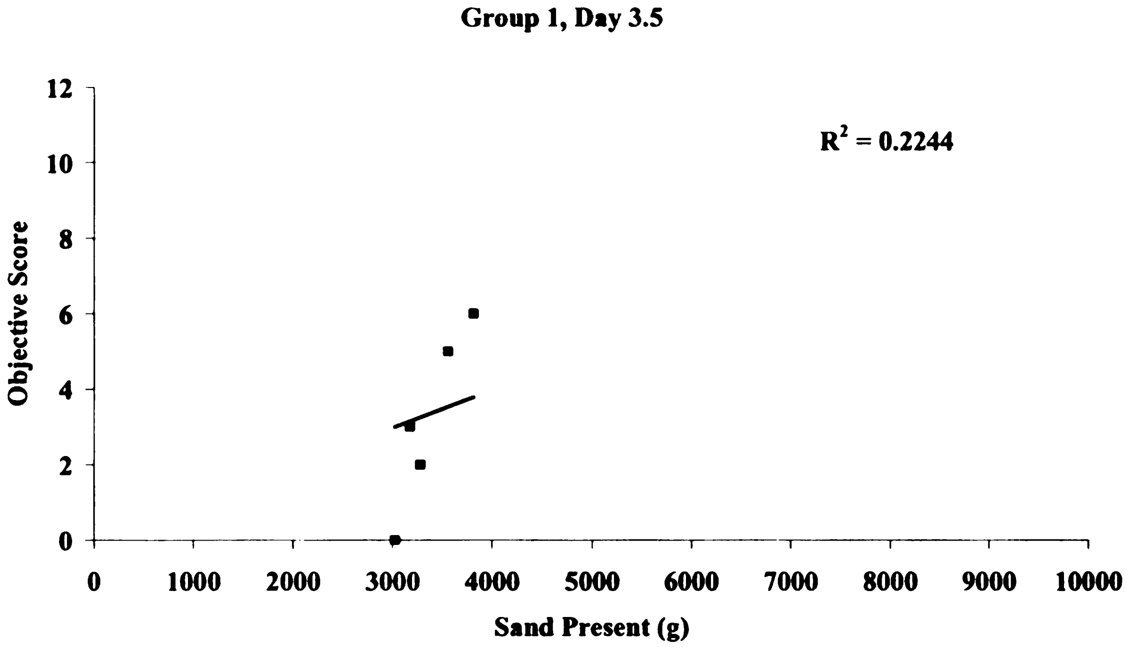
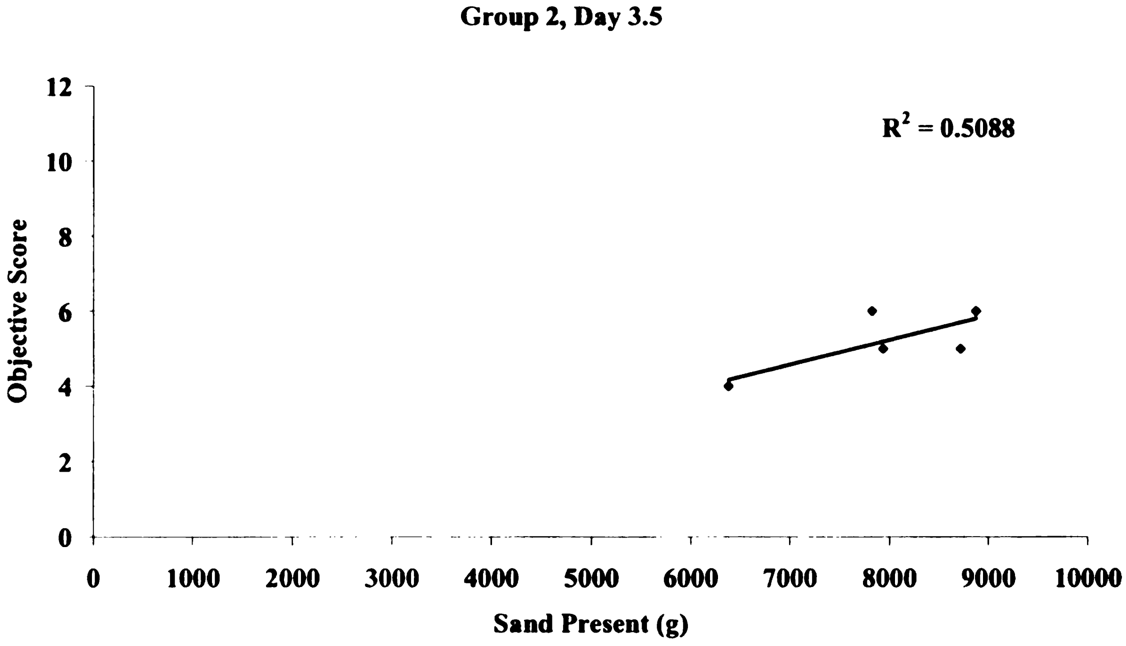


Figure 2-24 (con't).





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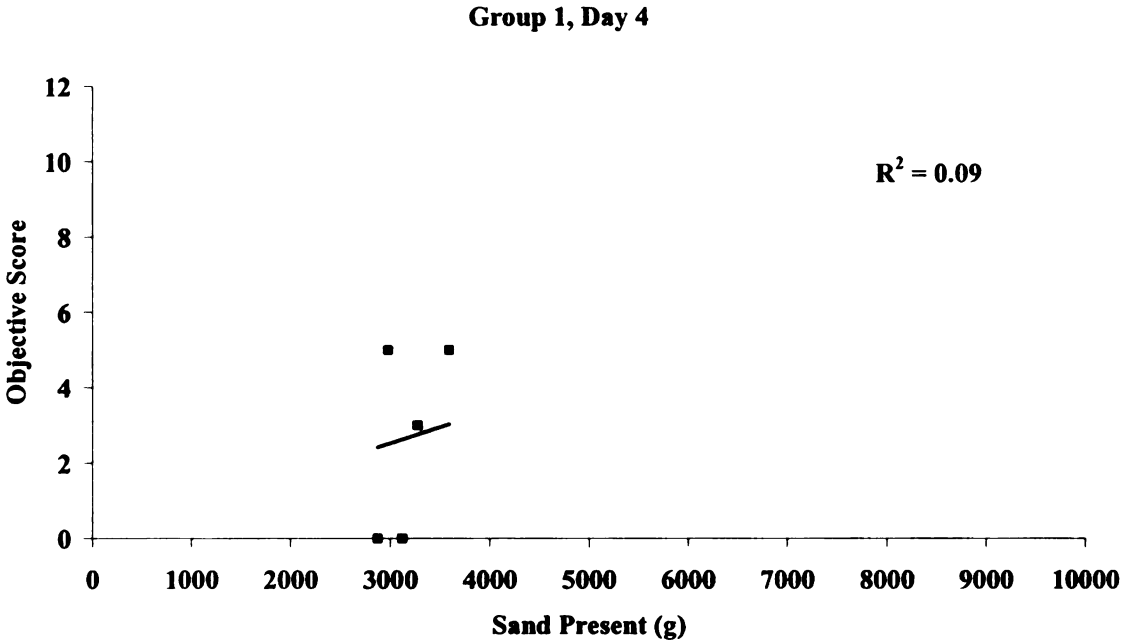

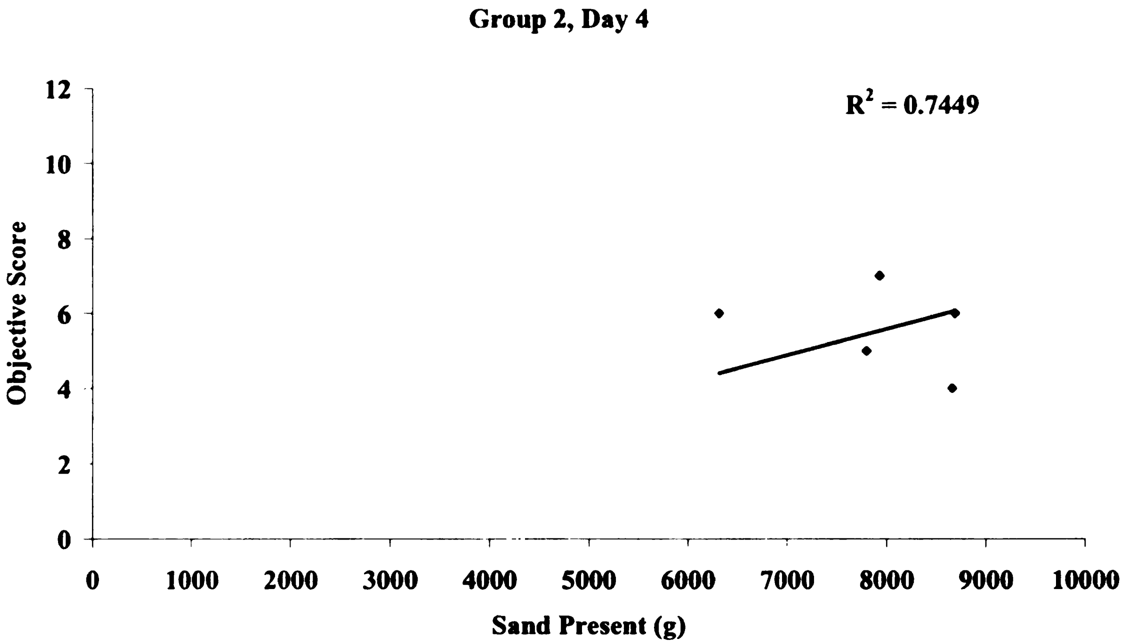

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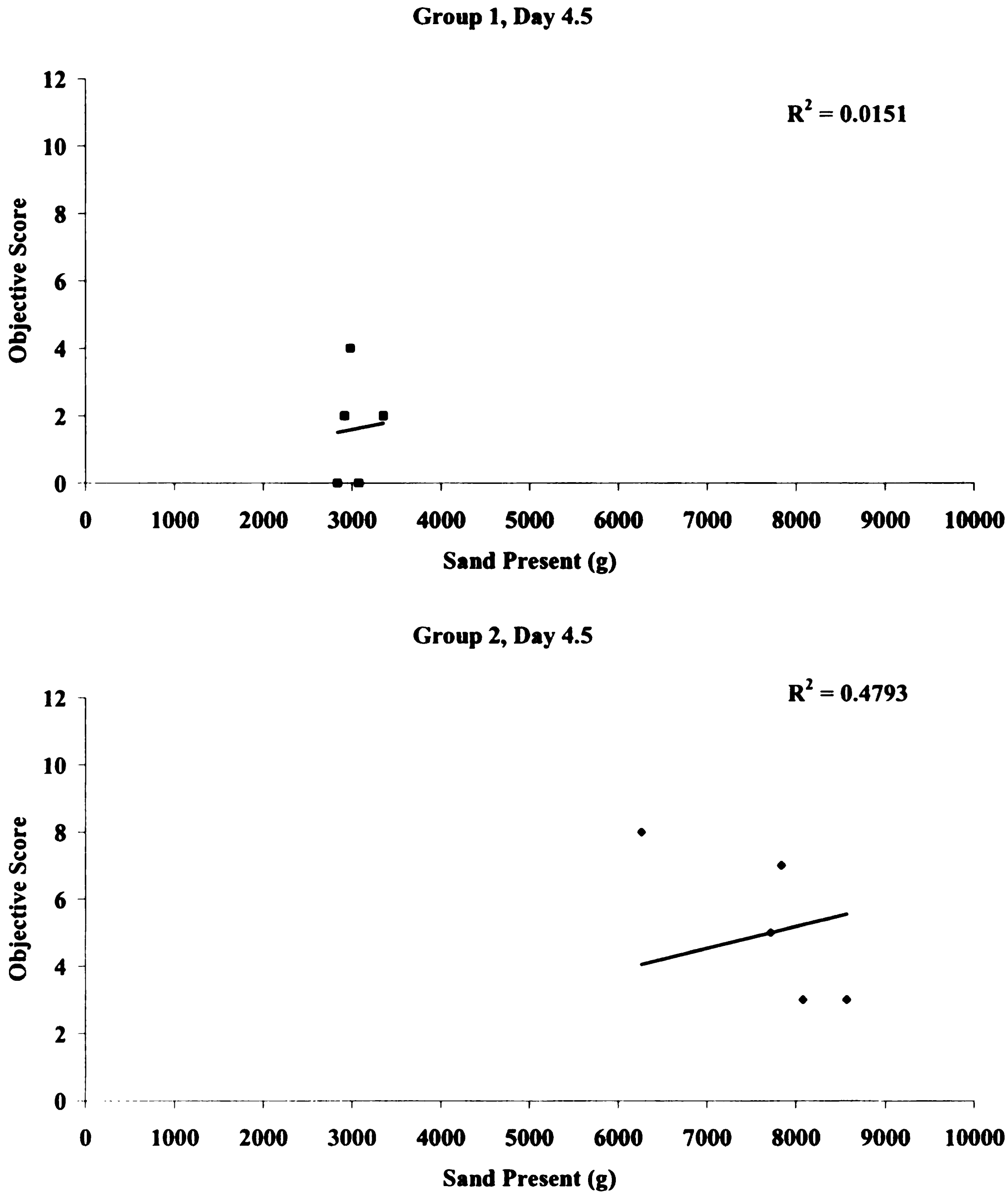
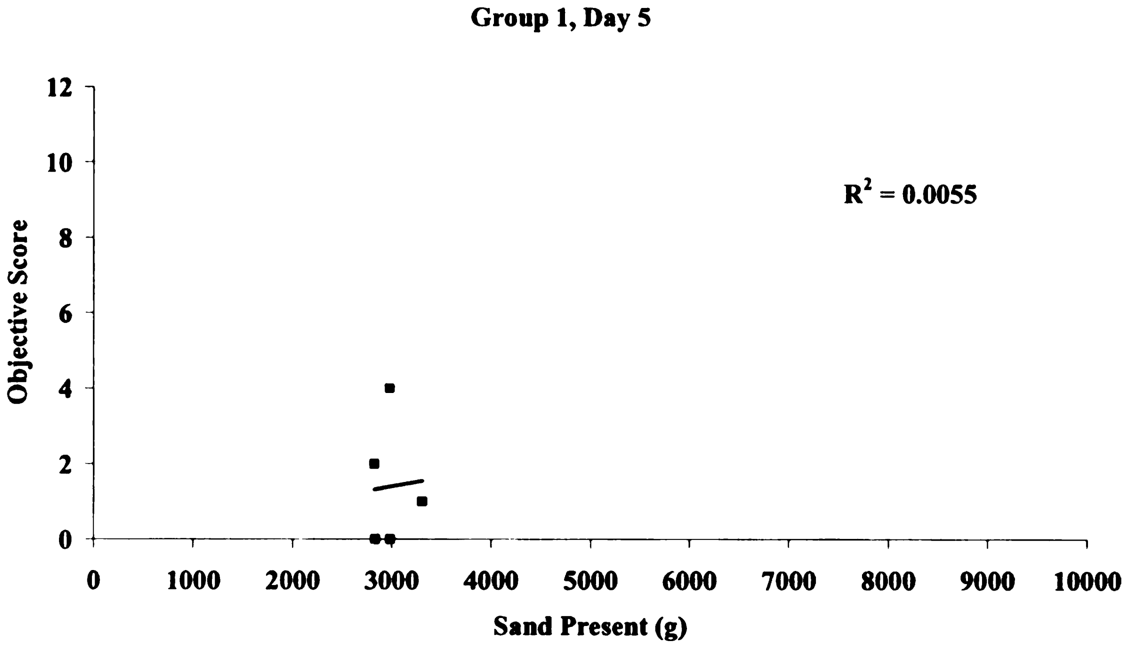
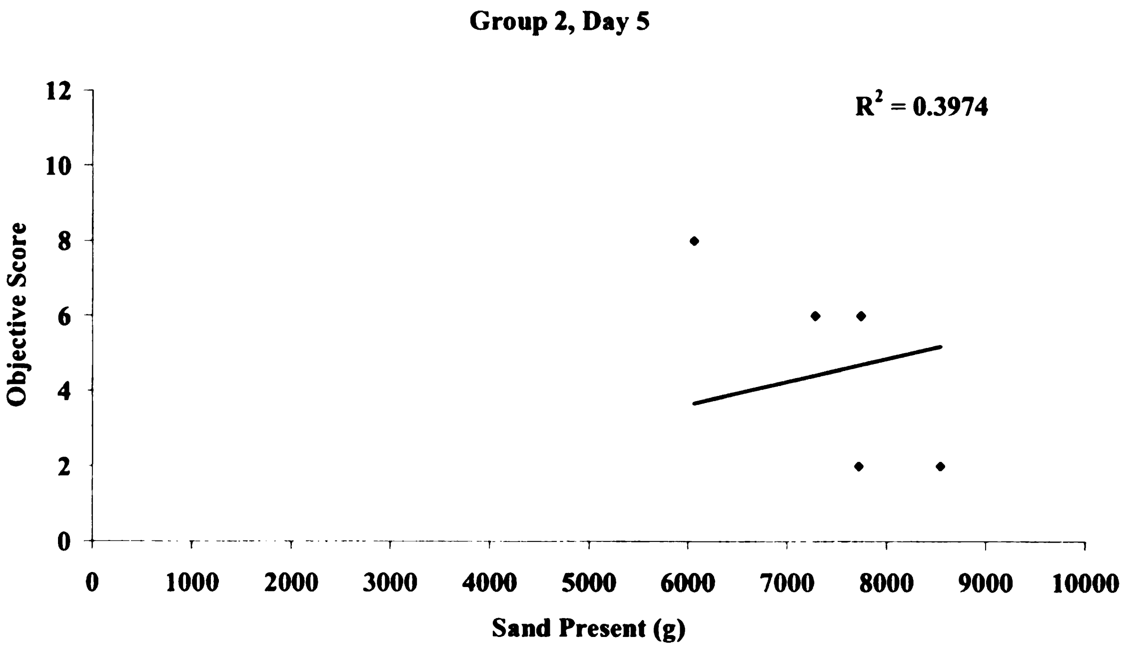


Figure 2-24 (con't).





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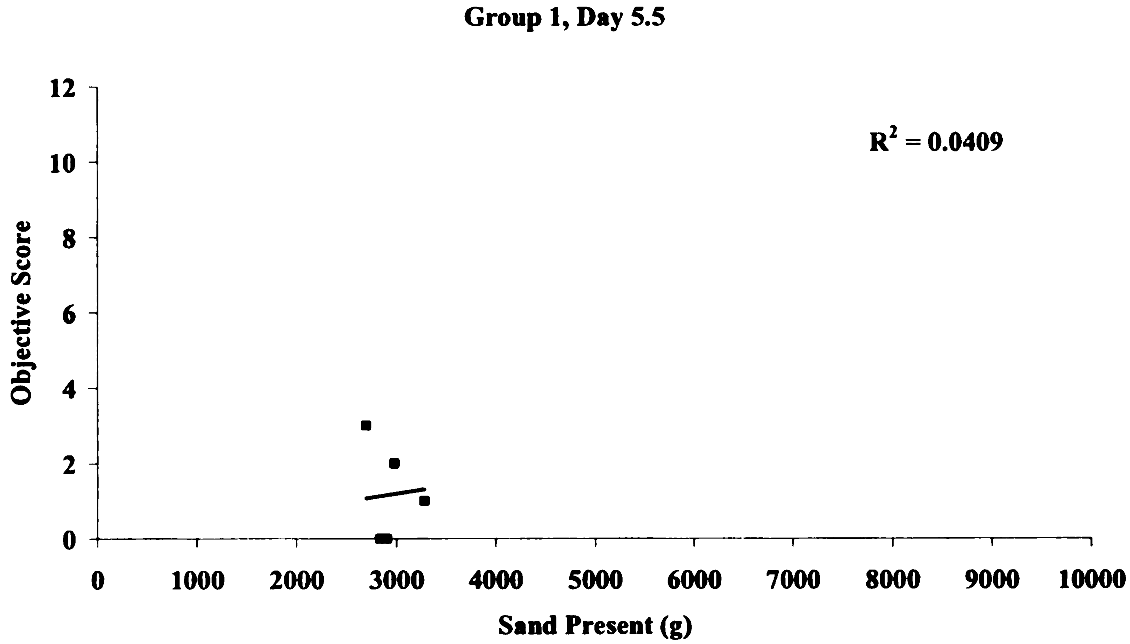

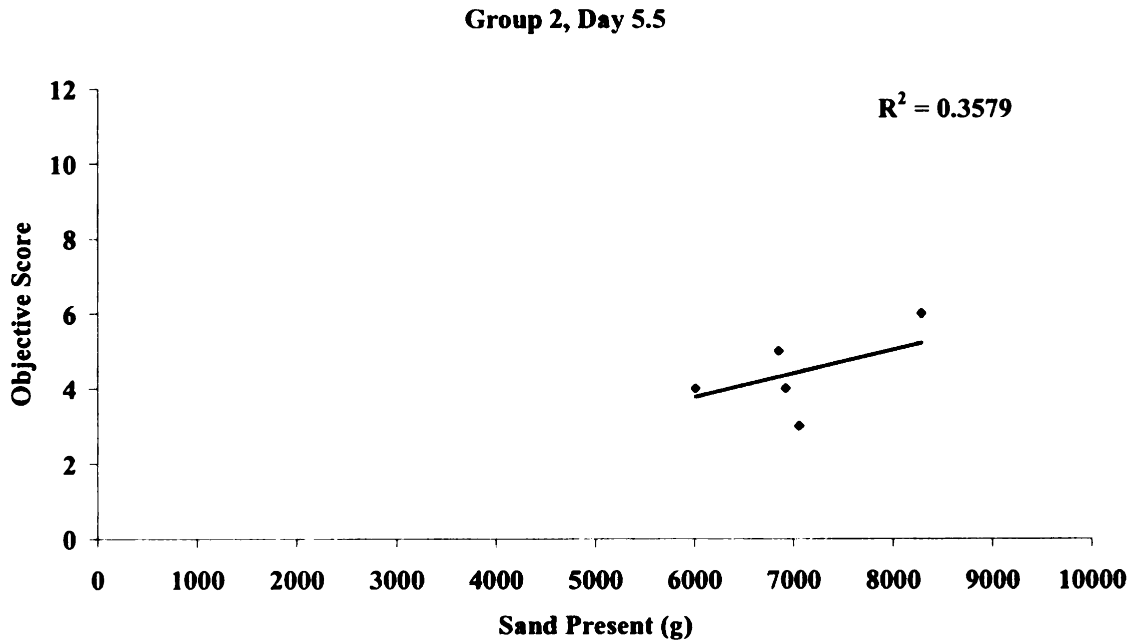

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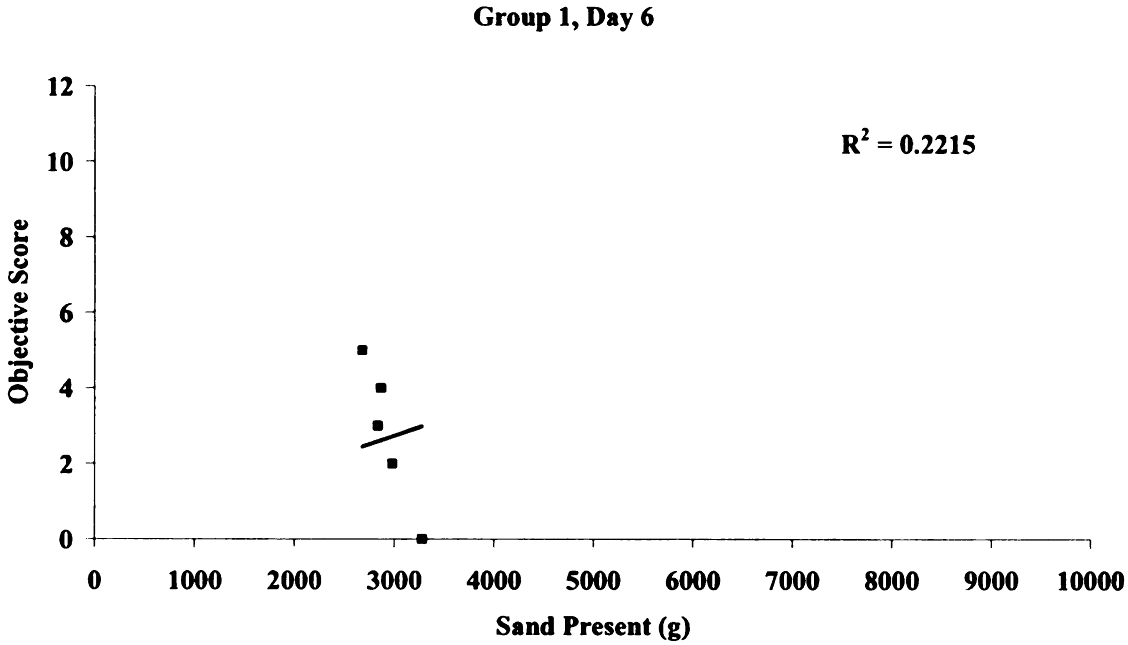
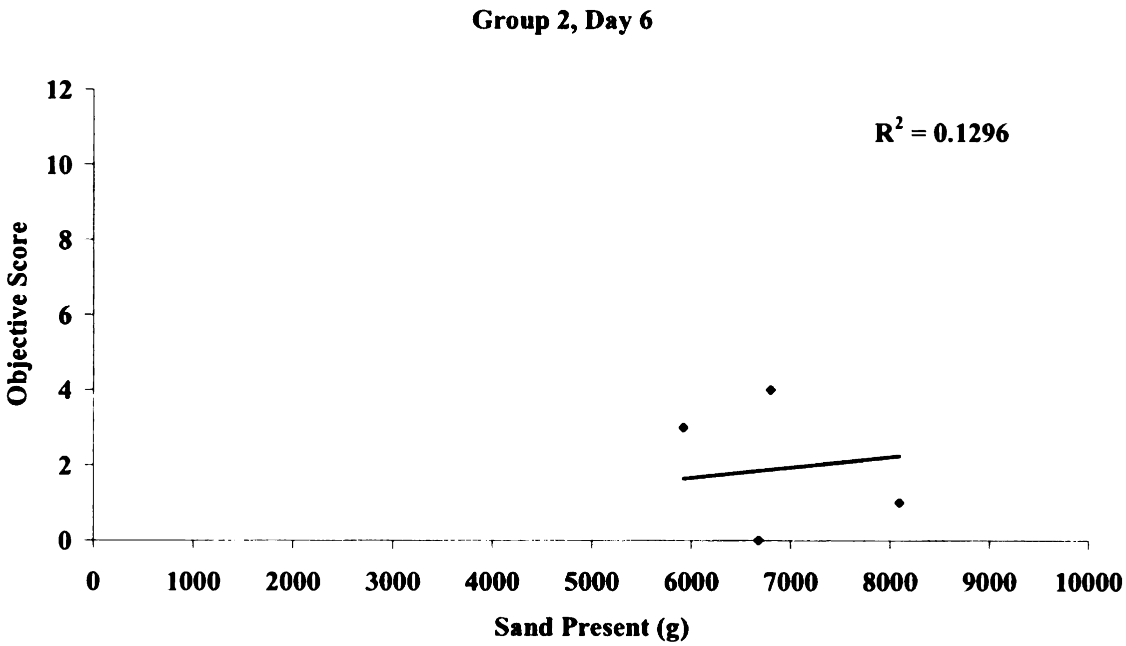
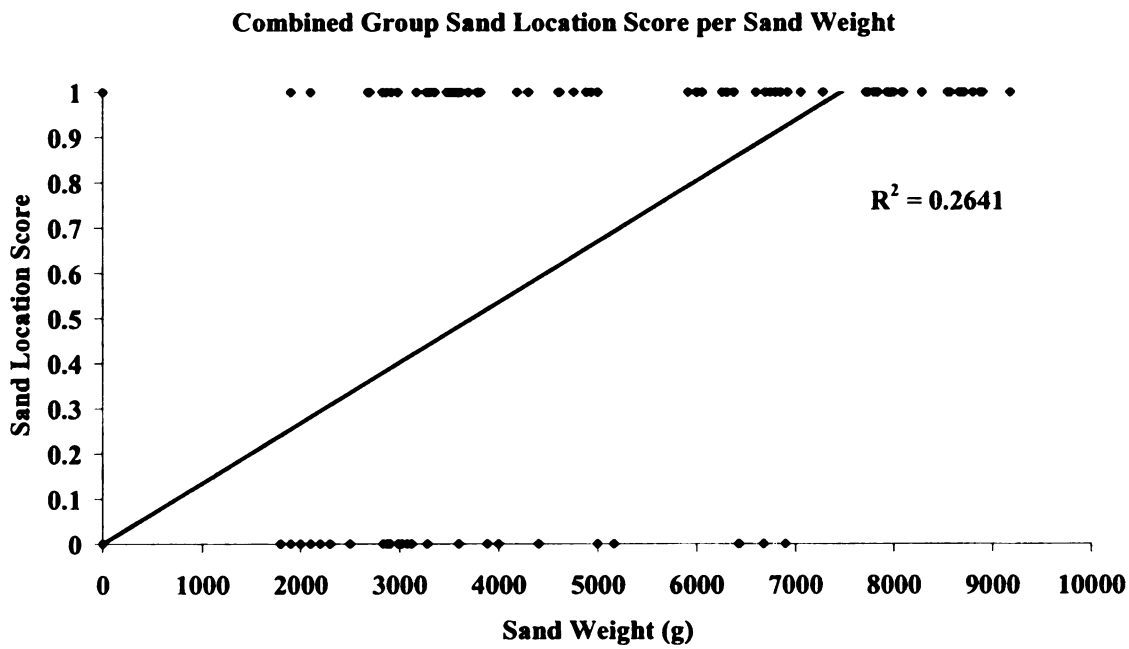




APPENDIX H

Figure 2-25

Individual objective parameter scores based on weight of intra-abdominal sand.



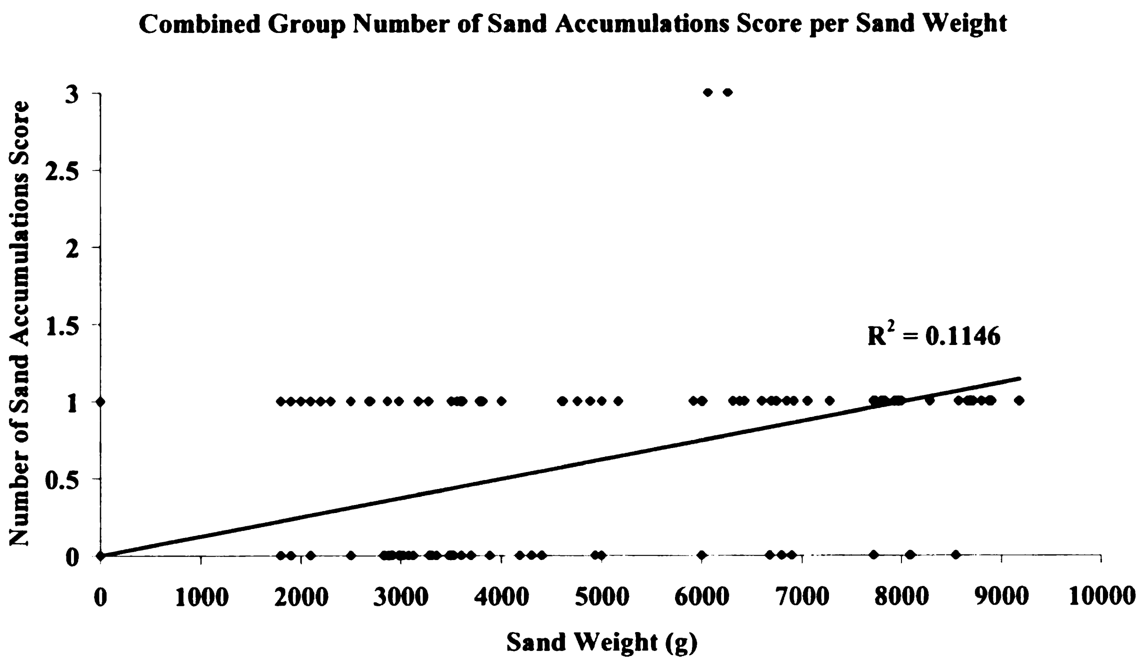
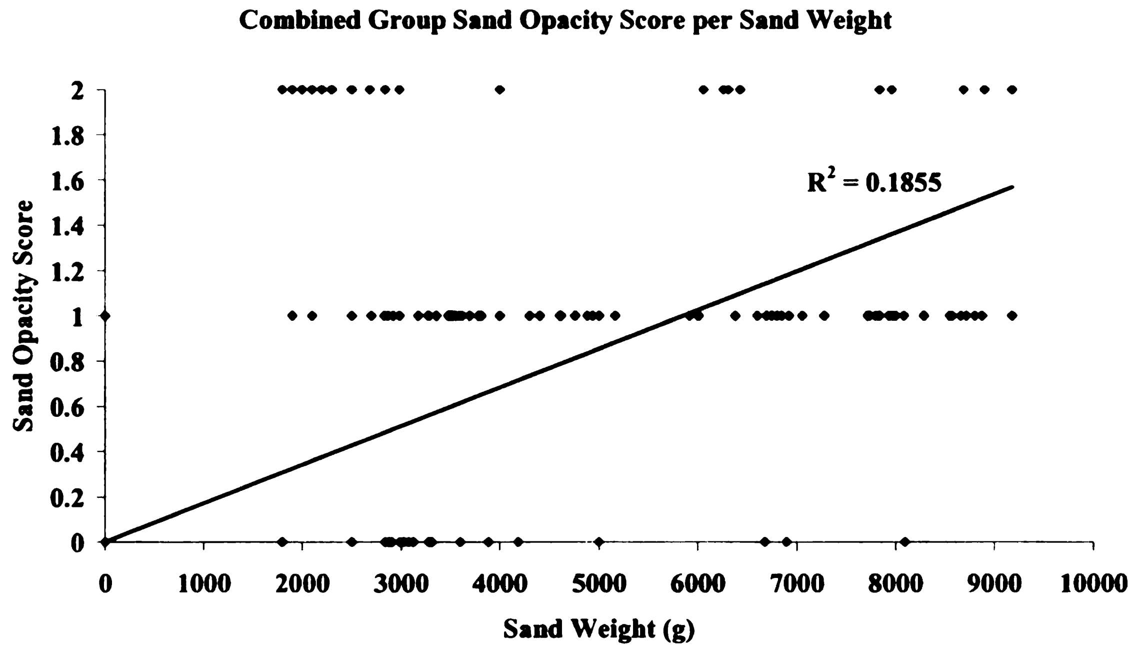


Figure 2-25 (con't).



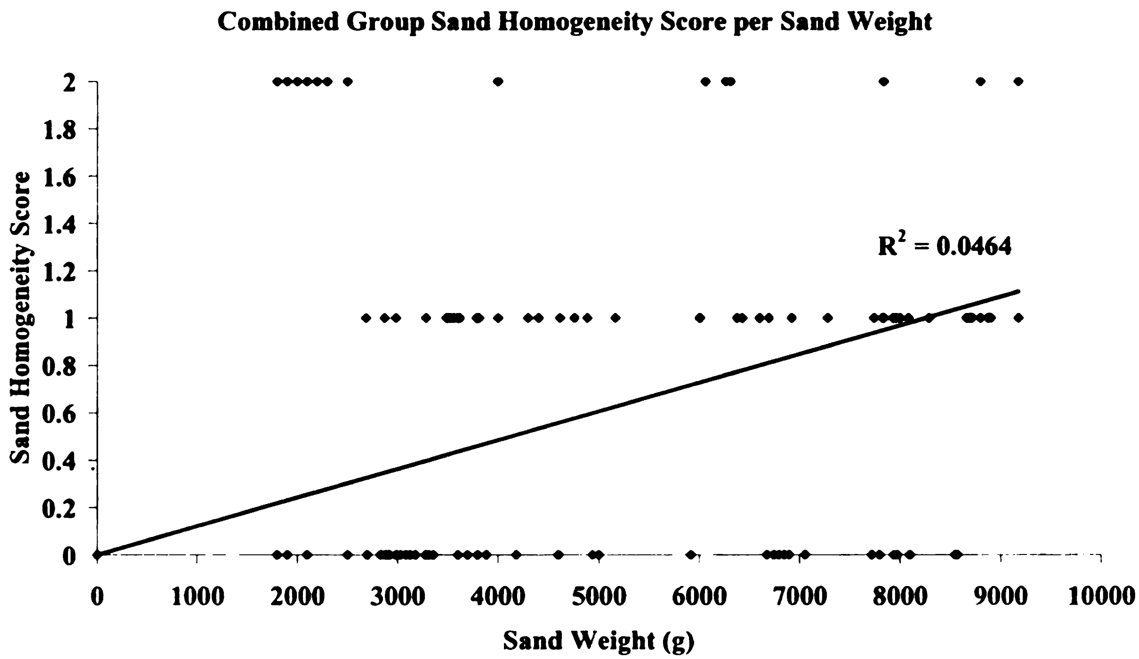
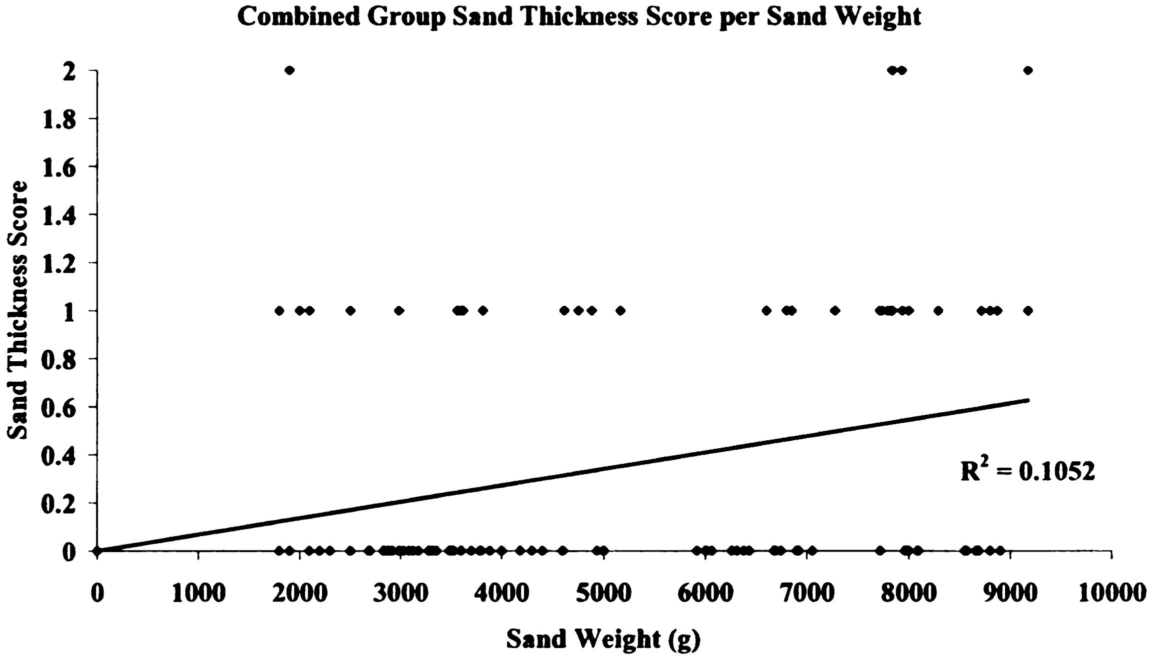
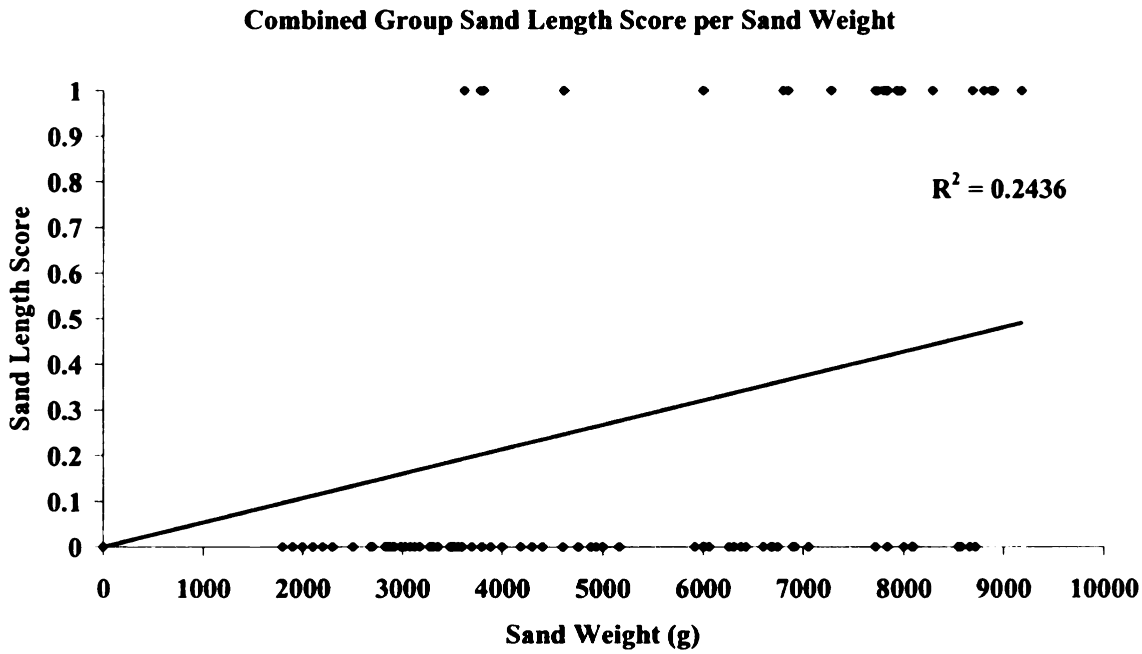


Figure 2-25 (con't).

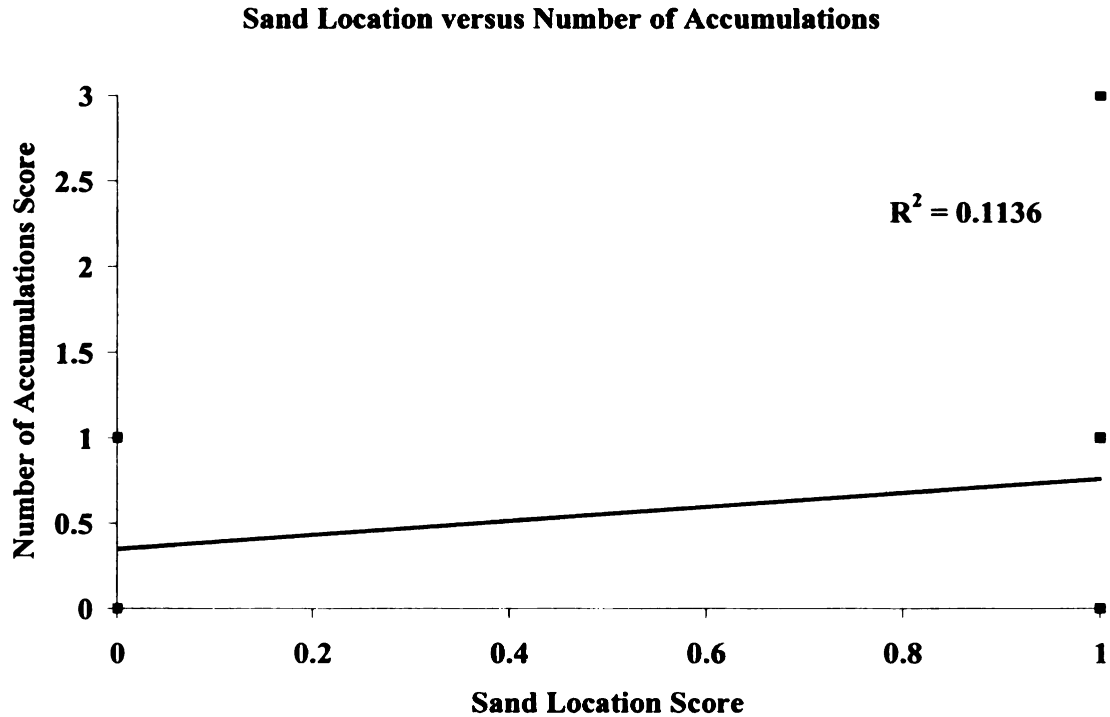




APPENDIX I

Figure 2-26

Scatter plots comparing scores of each objective parameter to another.



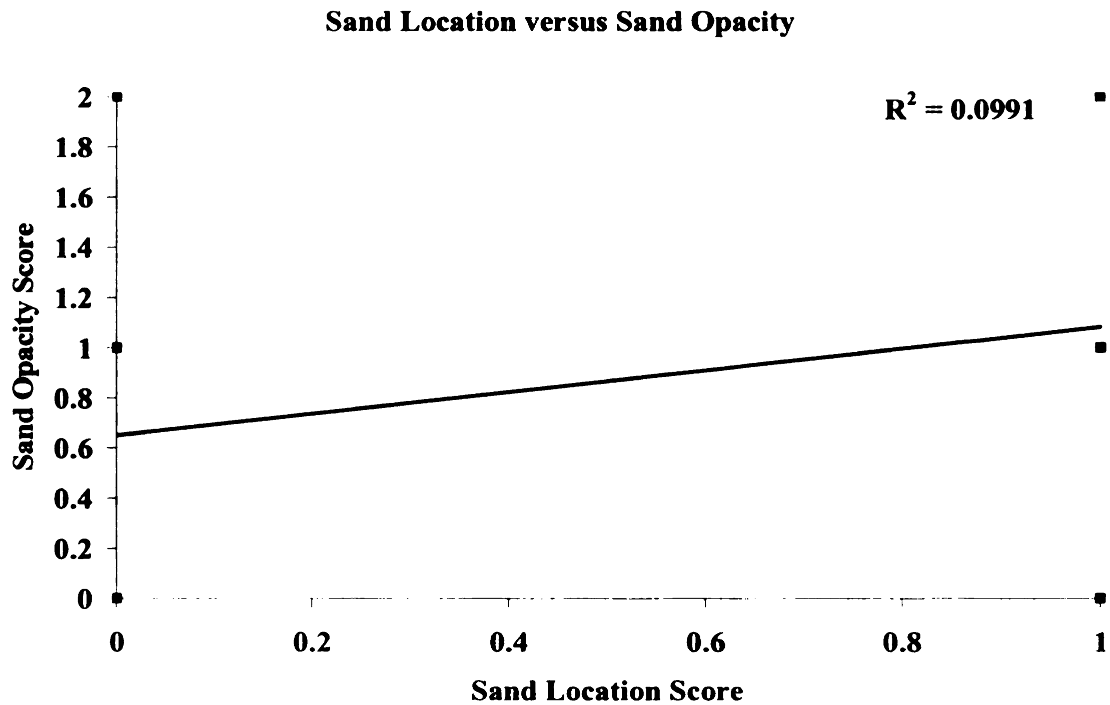
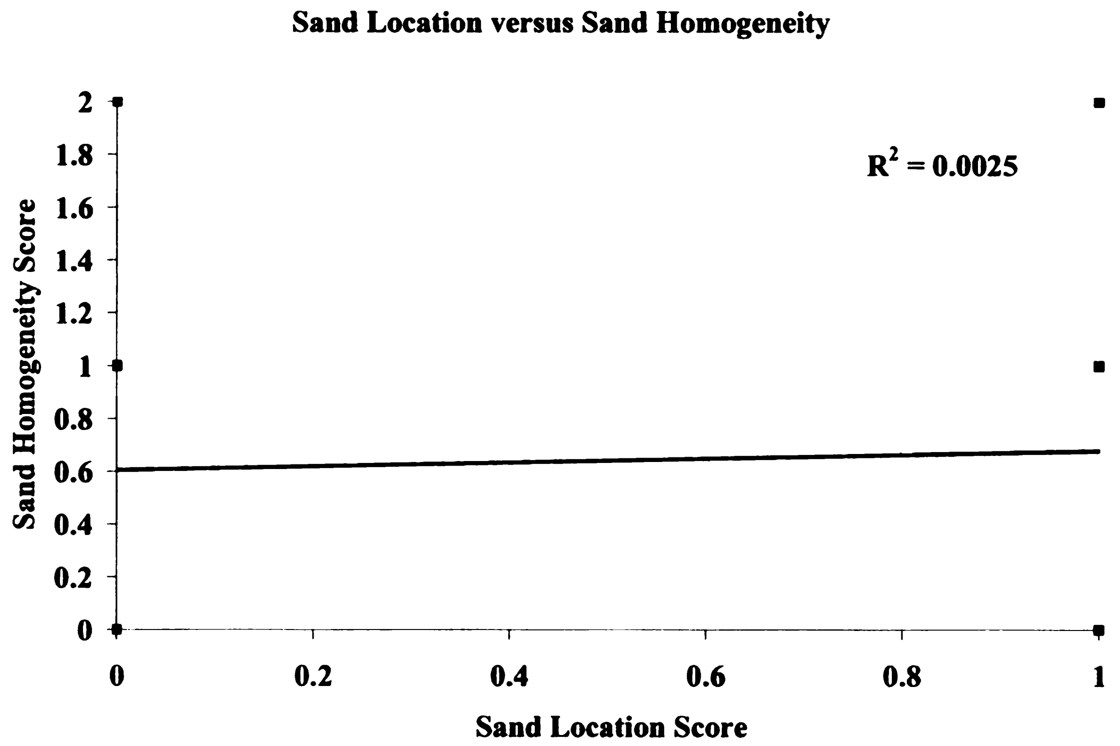


Figure 2-26 (con't).



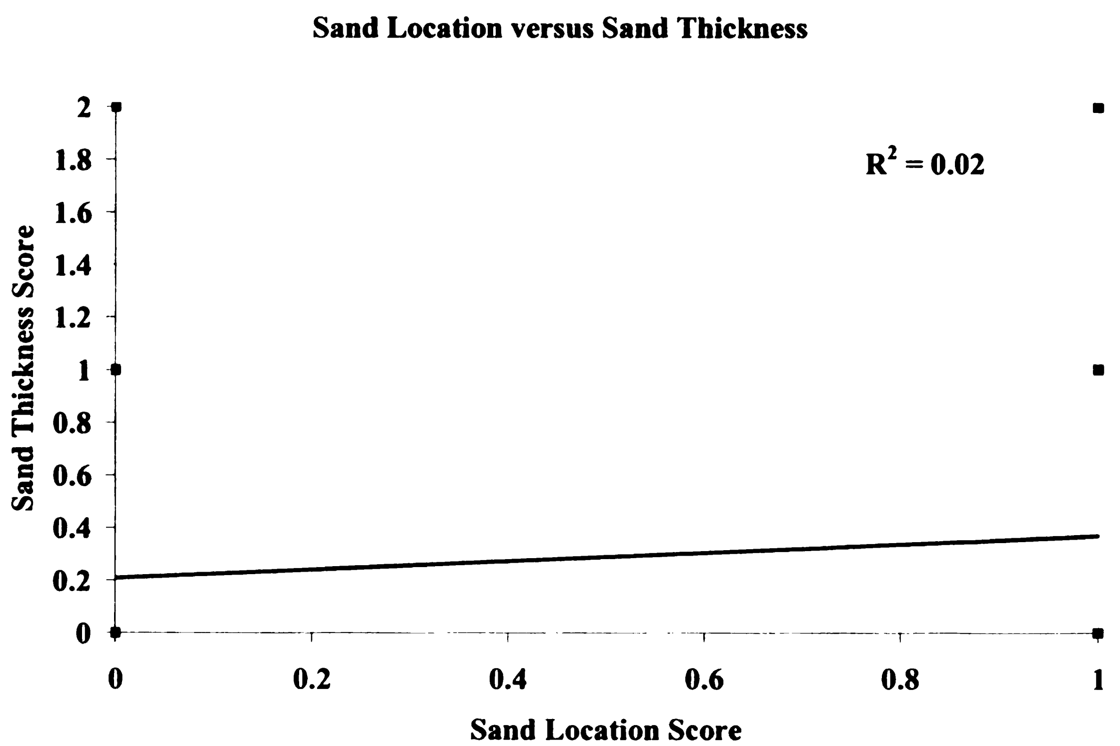
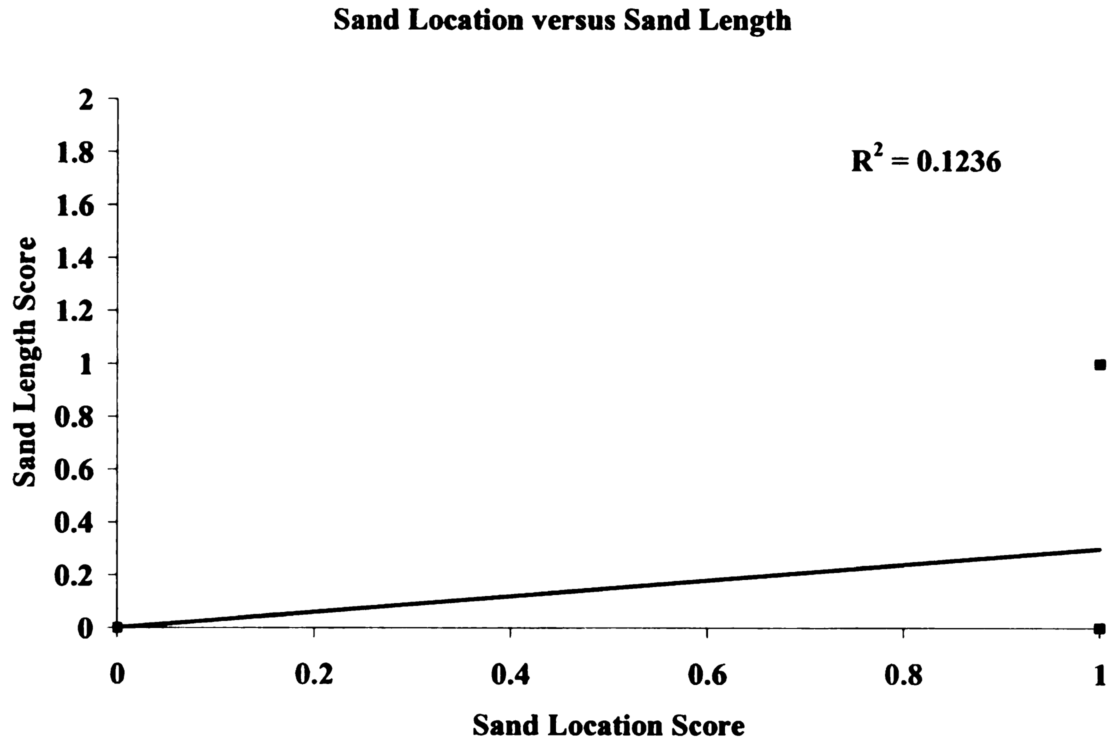


Figure 2-26 (con't).



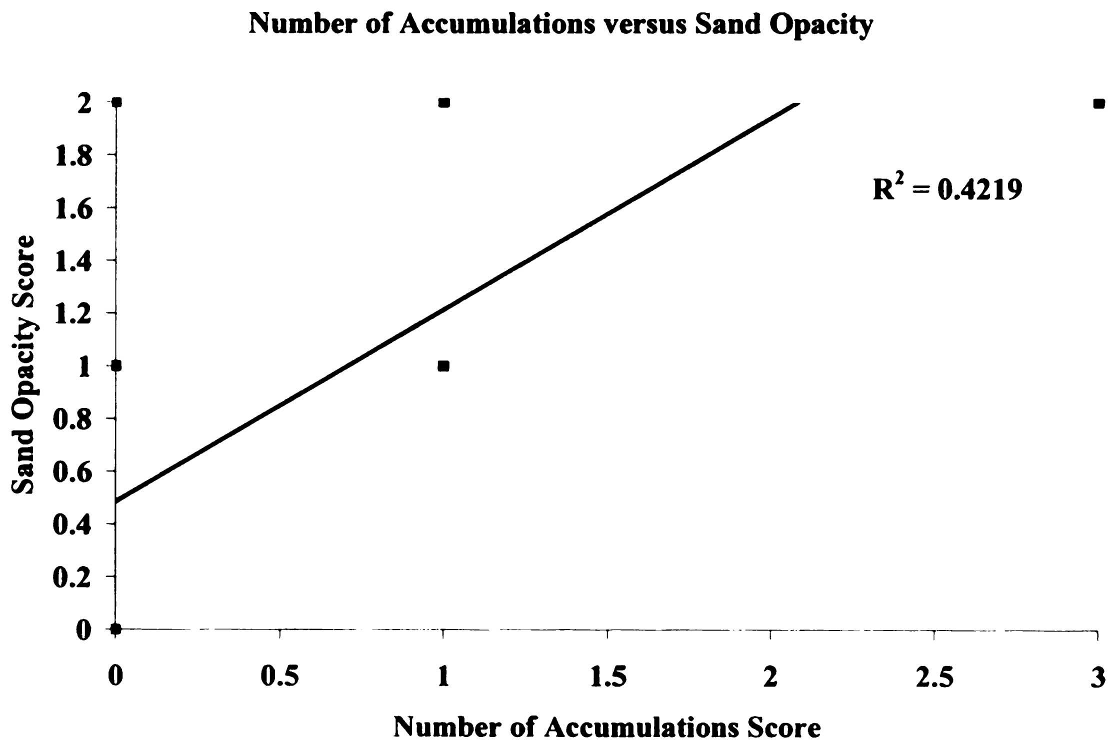
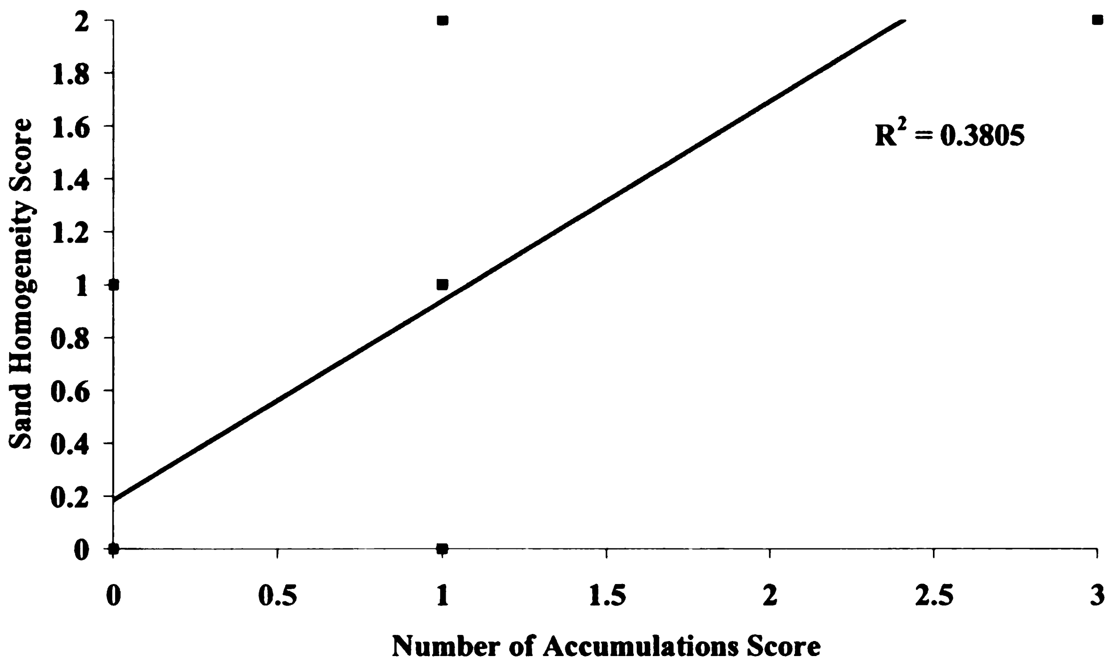


Figure 2-26 (con't).

Number of Accumulations versus Sand Homogeneity



Number of Accumulations versus Sand Thickness

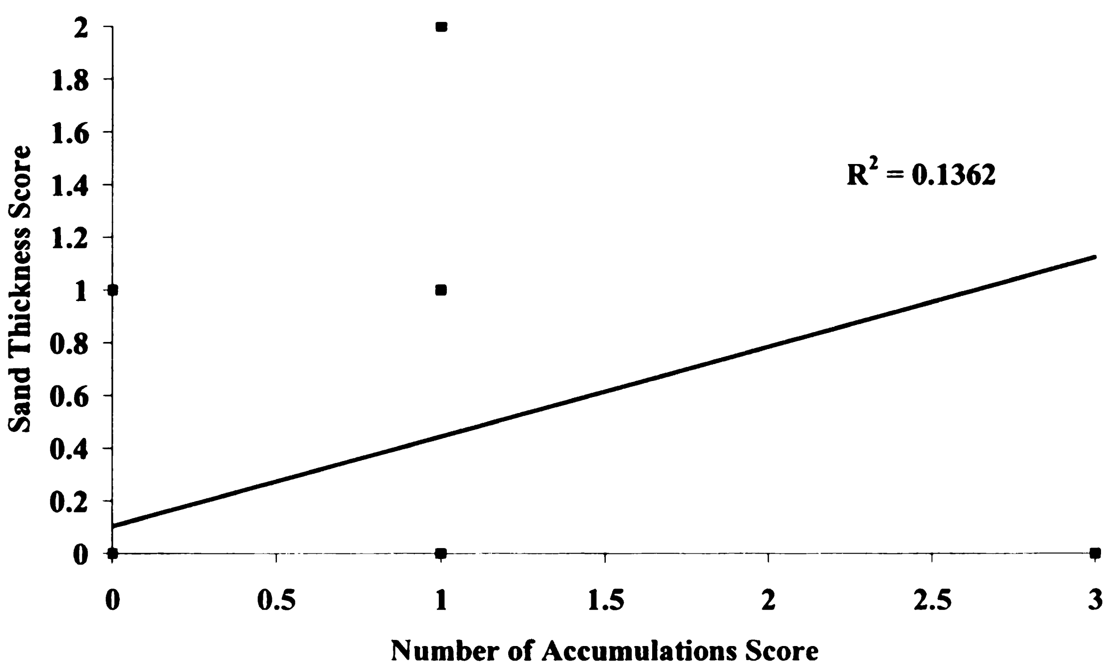
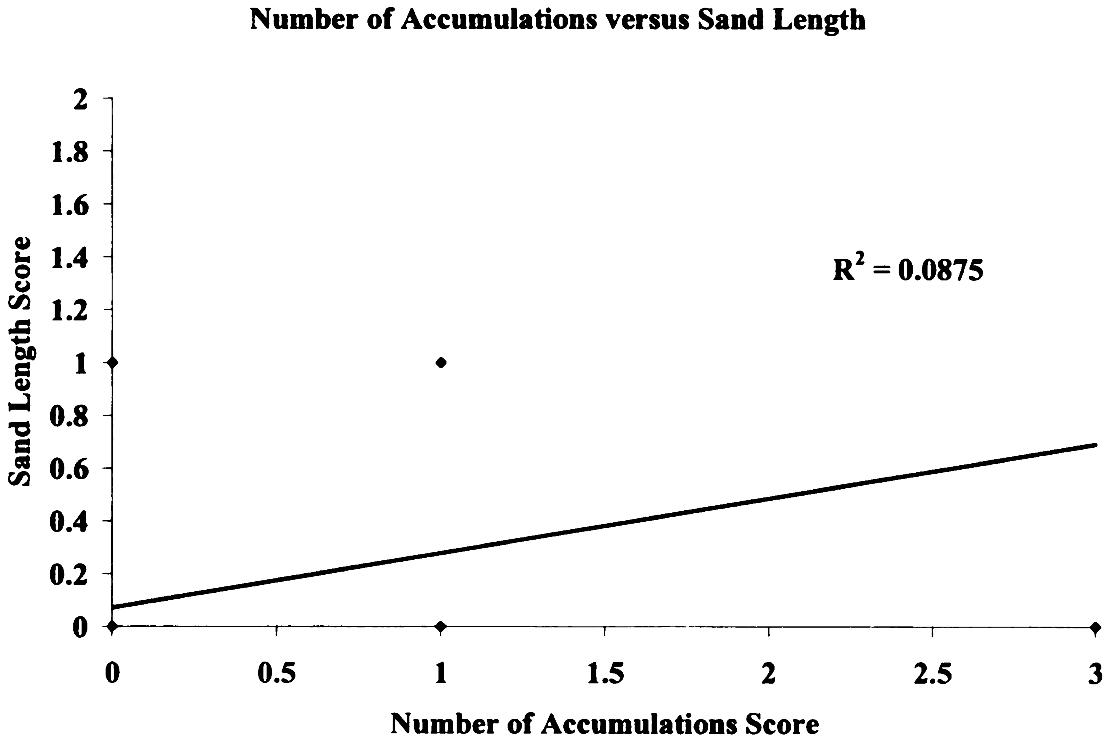


Figure 2-26 (con't).



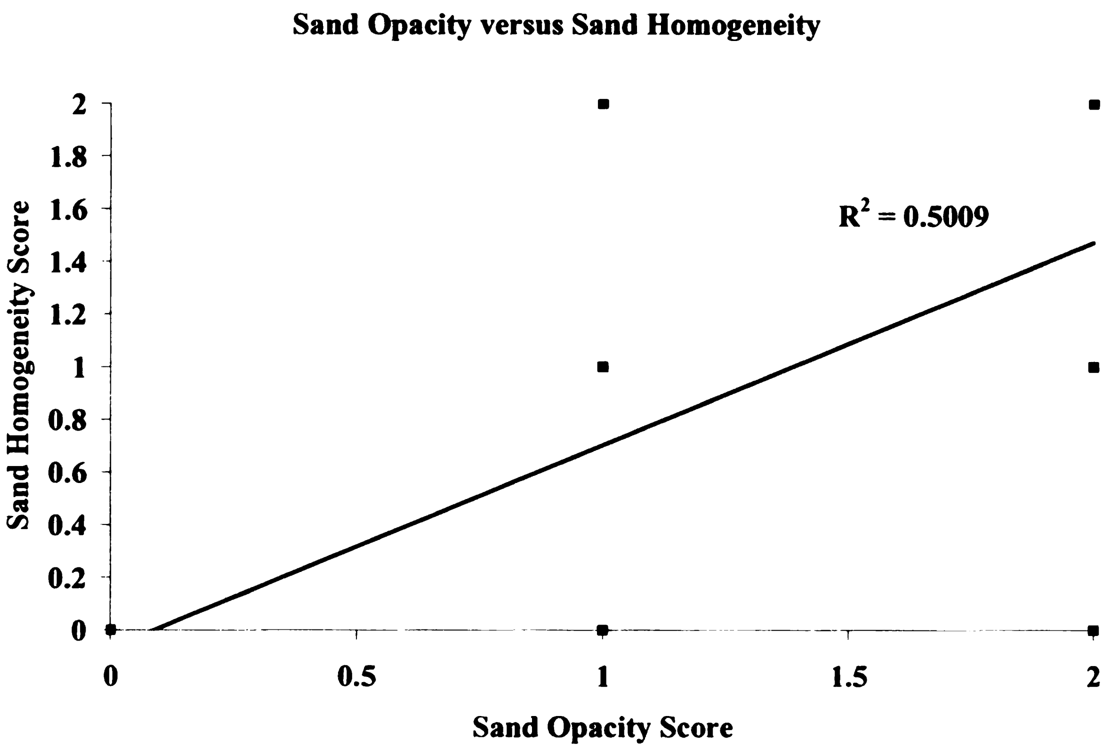
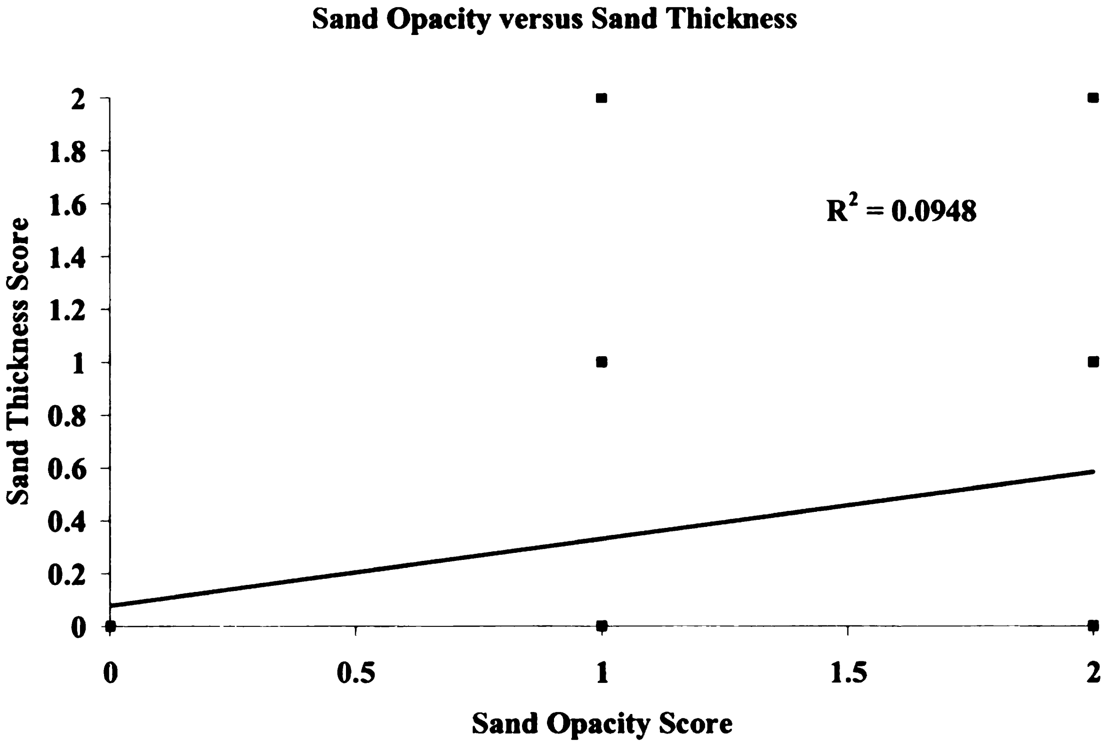


Figure 2-26 (con't).



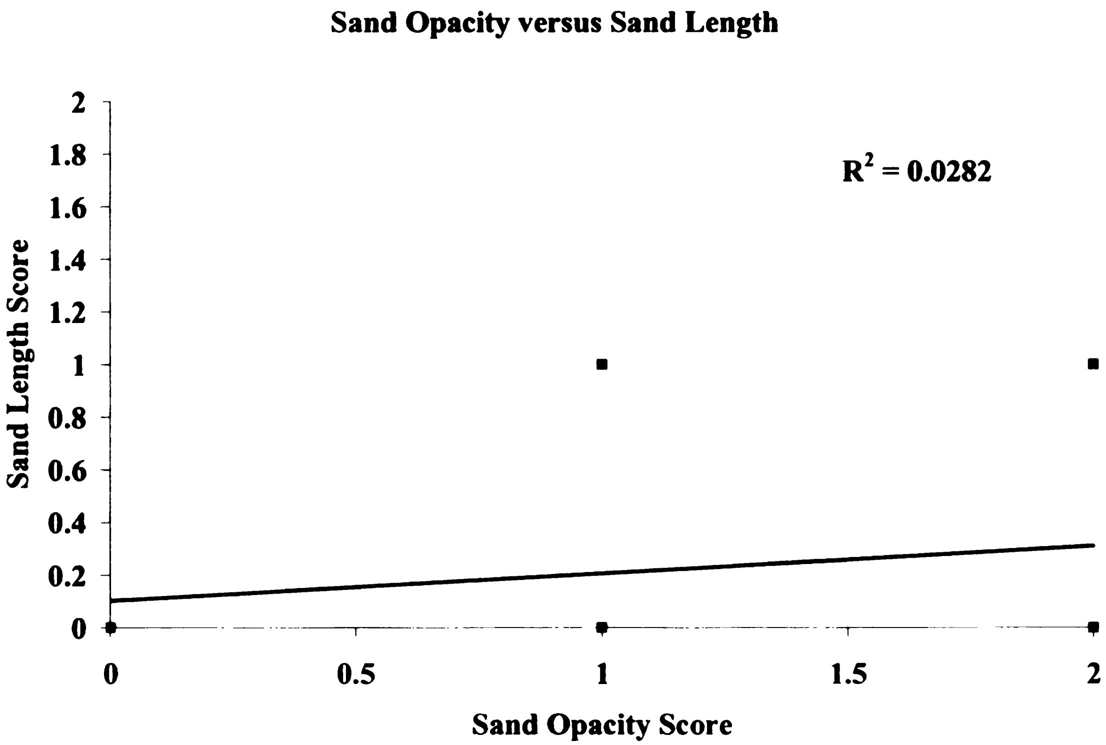
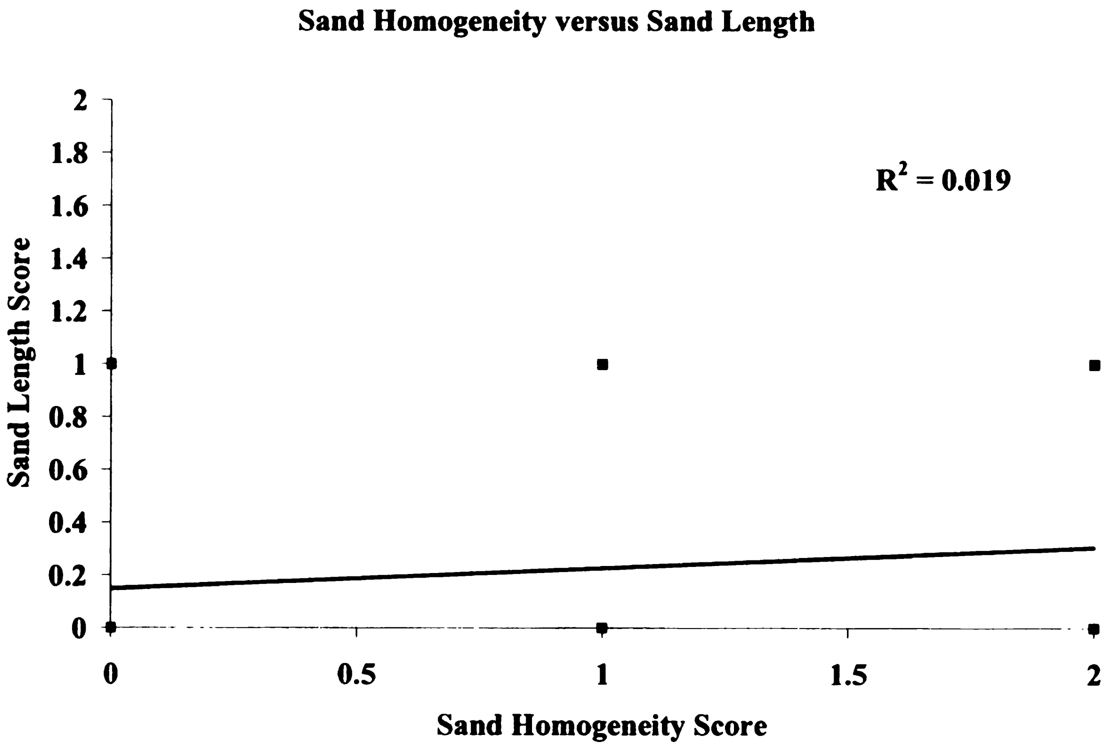


Figure 2-26 (con't).



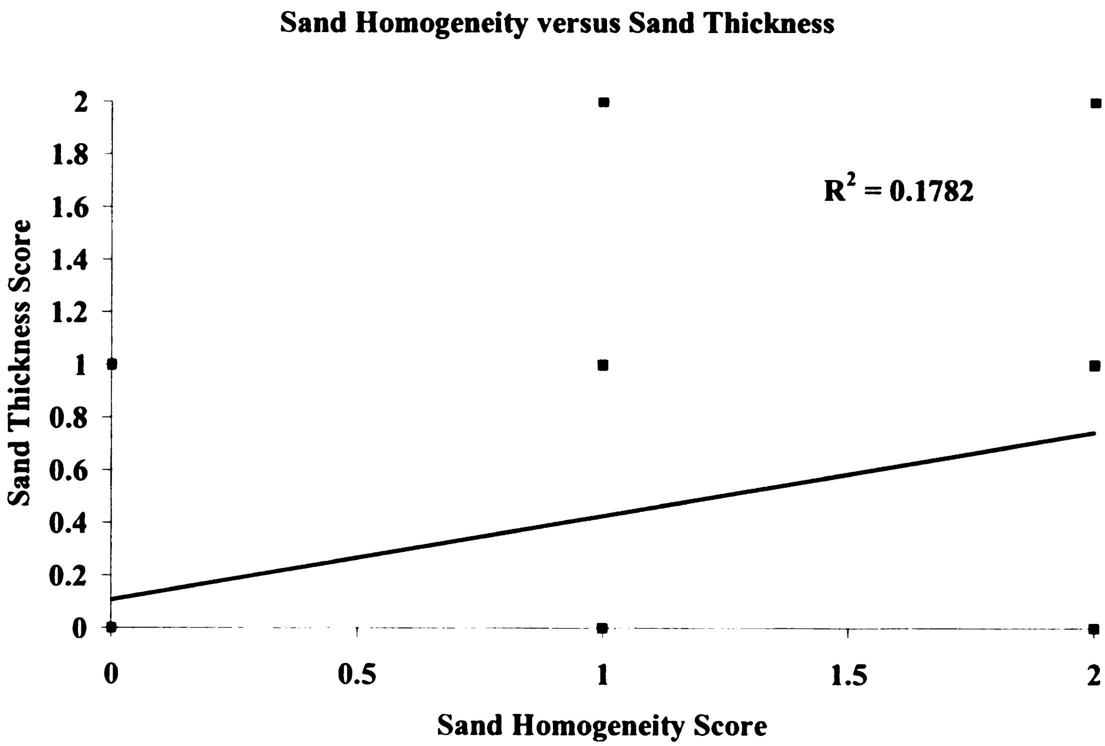


Figure 2-26 (con't).