Chromium-mordanted oats and 4N-HCL insoluble ash as indicators of fecal output, dry matter digestibility and dry matter intake of horses

by David John Barbisan

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
A pen trial and a grazing trial were conducted to evaluate the use of chromium-mordanted oats (CMO) and 4N-HCL insoluble ash (4N-AIA) as indicators of fecal output (FO) and dry matter digestibility (DMD), respectively. When FO and DMD are known dry matter intake (DMI) can be calculated.

The pen trial utilized eight mature geldings randomly assigned to two treatments of the pulse dose method (PDT1 and PDT2) . In PDT1 = four horses received a one-time dose of 15 grams chromium. In PDT2 = four horses received a one-time dose of 30 grams chromium. The five day steady state method had two treatments (SST1 and SST2). In SST1 = four horses received 3 grams chromium daily. In SST2 = four horses received 6 grams chromium daily. All CMO doses were fed via nose bags. Horses were individually housed in 5 x 15 meter, partially covered pens with ad libitum access to water and salt. Daily intake was restricted to two equal feedings per day. Total daily intake was 8.1 kg dry matter for PDT1 and PDT2 and 9.03 kg and 9.15 kg for SST1 and SST2, respectively. Total fecal collections (TFC) were conducted during the pulse dose method for 72 hours post dosing.

Mean actual FO from TFC was 3.60 kg DM/hd/day, with a range of 2.92 kg to 3.89 kg. Dry matter digestibility from TFC and 4N-AIA was 54% and 53.6%, respectively. Mean FO collected was not different (P>.05) from estimated FO for PDT1. A difference (P<.05) was detected between actual and estimated FO for PDT2. Estimated SSFO was different (P<.05) from TFC during the PD portion of the trial.

The grazing trial utilized six mature geldings, steady state dosing and TFC methods. Pasture dry matter available, crude protein, grosse energy, and 4N-AIA were measured. Representative forage samples for apparent DMD determination were obtained from pasture composition, mouth forage, or hand-pluck sampling. Daily steady state dosing was 7 grams chromium. Sample collections were on days 6 and 11 following first CMO administration.

Actual FO did not differ (P>. 05) between days of TFC. Estimated FO was different (P<.05) from TFC, resulting in a 20% over-estimation. The DMD of mouth forage samples was the same for days 6 and 11. Two-day averages of forage samples were different for the three methods with DMD being 69.6%, 71.6%, and 79.3% for pasture, mouth, and hand, respectively. Estimated Voluntary dry matter intake differed for each method of determination with ranges of a reasonable 15.9 kg DM/day (3.1% of body weight)" to an unreasonable 29.8 kg DM/day (5.9% of body weight) '.

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David John Barbisan

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

10/27/93
Date

Ray Anderson
Chairperson, Graduate Committee

Approved for the Major Department

10/29/93
Date

Head, Major Department

Approved for the College of Graduate Studies

11/14/93
Date

Graduate Dean

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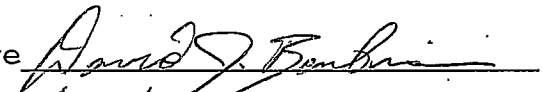
Signature 

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ABSTRACT

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CHAPTER 1

INTRODUCTION

A recent article in Equus magazine (Kopp Du Teil and Strain, 1993) addressed the statement, "What we don't know about horses." According to the article, horse owners ask equine practitioners the most questions about nutrition, followed by hoof care and lameness, behavior, and lastly, exercise and conditioning programs.

Horses evolved to be continuous grazing, free-roaming animals that utilized vast amounts of grassland for their total nutritional needs. Research has investigated behavioral aspects of grazing horses (Carson and Wood-Gush, 1983; Crowell-Davis et al., 1985; Duren et al., 1987b; Kiley-Worthington, 1987; Gagnon et al., 1989), nutritional status of grazing mares (Gallagher and McMeniman, 1988), growth and development of weanlings (Ott and Asquith, 1983; Boren et al., 1987) and yearlings (Heusner and Albert, 1977; Hansen et al., 1987; Webb et al., 1987; Aiken et al., 1989; Webb et al., 1990) and the plant species preferences of grazing horses (Archer, 1973), but relatively little has been done on the actual forage utilization and intake of free-grazing horses. The nutritional utilization studies done on equines have mostly been conducted with small numbers of animals in confinement situations.

In the western United States and, more specifically, the intermountain region, most horses graze improved and native pastures several months of the year or yearlong. This region seems to be experiencing an increase in human population and the development of agricultural and native grasslands into smaller acreages that quite frequently house recreational horses grazed throughout the year. With this increase of private land use also comes an increase of use on public lands. Many recreational horses are used in back-country activities. Unfortunately, the utilization and impact of horses on these pastures and public lands is uncertain.

Ruminant animal forage intake and utilization studies have employed numerous techniques and methods to quantify the parameters of fecal output, dry matter digestibility and dry matter intake (Hardison and Reid, 1953; Harris, 1970; Kartchner & Campbell, 1979; Raleigh et al., 1980; Cochran et al., 1987; Paterson & Kerley, 1987; Pond et al., 1987; Hatfield et al., 1991). The use of accurate estimates of forage intake and utilization by the grazing animal coupled with a knowledge of forage availability and nutritional composition can provide a method of objectively meeting the requirements of the animal and the pasture needed to support these requirements.

Can external and internal marker methodologies be used accurately enough in horses to estimate the parameters of

dry matter fecal output, apparent dry matter digestibility and dry matter intake?

Objectives of this research were to:

1) Compare pulse dose (PD) and steady state (SS) external marker methodologies with total fecal collection (TFC) techniques for the eventual determination of fecal output (FO) by horses.

2) Utilize dual internal and external marker methodologies to estimate dry matter fecal output (DMFO), dry matter digestibility (DMD) and dry matter intake (DMI) of horses in a controlled confined situation and a free-grazing pasture situation.

CHAPTER 2

LITERATURE REVIEW

Horse Grazing Behavior

Members of the genus Equus are large, nonruminant herbivores (Ralston, 1984) which evolved to be continuous grazers under range conditions. The stomach of the horse is just slightly larger than the animal's heart. According to Hayes (1982), the stomach has a capacity of 7.6 to 15 liters and the best physiological conditions for digestion occur when it contains about 7.6 to 9.5 liters, or is distended to two-thirds of its capacity.

Several studies (Feist, 1971; Francis-Smith et al., 1982; Gagnon et al., 1989) have shown that horses graze in bouts of two to four hours, grazing a total of twelve to sixteen hours per day. Food ingested by the horse passes from the mouth to the ceacum within an hour and another 35-70 hours is required for the ingesta to reach the annus (Linerode, 1968). Horses secrete gastric juice continuously during fasting and the sight of oats does not cause any increase in its flow (Hayes, 1982). In addition, the horse requires to be fed often, and long fasts tend to induce gastric disorders. Ralston (1984) states the normal feeding pattern of horses and ponies is one of small meals at frequent intervals. Meals are initiated when there are

still large amounts of ingesta remaining in the gastrointestinal tract, usually before microbial fermentation of the ingesta has produced significant amounts of volatile fatty acids (VFAs) from the previous meal.

Many factors, both internal and external, interact to affect the grazing animal and, consequently, the pasture on which it is grazing. External factors are numerous, including weather, season, management, palatability, structure, abundance, digestibility, and monotony of the pasture, as well as the presence of feces and urine patches, social factors and size of the pasture. Some internal factors affecting grazing behavior are the selectivity of the grazing animal, its nutritional requirements, the condition of its teeth, bite size and frequency as affected by the animals hunger and physical limitations, together with its age, sex, and past experience (Carson and Wood-Gush, 1983). Observations by Crowell-Davis et al., (1985) showed that foals begin grazing almost at birth and, by twenty-one weeks of age, spend 47 $\pm$ 6% of their time grazing.

Duren et al. (1987b) considered the effect of exercise on grazing behavior in yearling tethered horses. Bite rates were significantly higher during the first twenty minutes of grazing for the non-exercised than the exercised horses. Forage intake, however, did not differ during the three-hour grazing session for exercised and non-exercised horses. It

was concluded that exercised horses modified their grazing behavior by taking fewer, but larger, bites.

Archer (1973) found that horses ate more species of broad-leafed plants, than was previously reported. Horses also grazed selectively and moved quickly over areas of less palatable forages. Kiley-Worthington (1987) theorized that the horse is a selective grazer because of its inability to vomit. Horses initially take small portions of plants and young horses are usually cautious in eating a new substance or plant. Horses apparently learn to select their diet by imitation. The close association of mare and foal allows the foal to learn much of what to eat from the dam.

Regulation and Control of Feed Intake

A study with esophageal fistulated ponies (n=3) was conducted by Ralston (1984) to investigate the relative importance of oropharyngeal versus gastrointestinal cues in the control of feed intake. Results of this study show that ponies sham-fed (esophageal fistula open) pelleted feed consumed immediate meals that are not different in size or duration from those consumed by control fed (esophageal fistula closed) ponies. The mean intermeal interval however, is 59% shorter ($P<.05$) after sham-fed than control meals, indicating fewer minutes of satiety/gram of feed eaten. This suggests eventual gastrointestinal control of total feed intakes (Ralston, 1984). Hill et al. (1952)

reported that in monogastrics a glucose load placed in the jejunum resulted in a reduction of feed intake. Snowdon (1975) found that hyperosmotic solutions placed into the stomach of rats did not reduce feed intake unless they had nutritive value such as glucose. Ralston and Baile (1982a,b) conducted two independent studies in which the effects of plasma and intragastric glucose loading were compared. They found that, in ponies, intravenous glucose loads can prolong the duration of satiety experienced after a meal. Intragastric glucose delayed onset of feeding 113 $\pm$ 65 minutes and ponies exhibited normal satiety behavior during this time. Ralston (1984) concluded that the regulation of feed intakes by horses and ponies is on the following basis: the animals receive stimuli from the products of enzymatic and fermentative digestion both pre- and post-absorption that reflect the status of the body energy stores. These cues are integrated with other internal and external (sight, smell of feed, time of day, social facilitation) factors and influence the initiation of meals by dictating the responsiveness to food-related stimuli. The size and duration of a meal are regulated by the degree of hunger the animal was experienceing when it started to eat (setting initial eating rate) but are controlled primarily on the basis of oropharyngeal and other external cues during the eating bout. The onset of normal satiety in the pony and horse is determined primarily on the

basis of pregastric stimuli such as taste, texture, and odor of feed. The duration of satiety, however, falls under the control of the gastrointestinal, metabolic, and environmental cues.

The feed intake of stalled animals fed long hay forage can be used as a guide to the total amount of forage that may be consumed voluntarily by the animal. However the feeding of mechanically harvested forage or even grazed forage may not accurately reflect the forage consumed under grazing conditions with the associated animal selectivity and the difference in nutrient requirements between confined and grazed animals (Kartchner and Campbell, 1979).

Methods of Measuring Forage Intake

An accurate method of estimating intake and digestibility of pasture has been a problem encountered by researchers for decades. Biomass collection techniques used by Cantillon (1986) and Duren et al. (1987a) have been established as a viable means of estimating forage intake in horses by simultaneously comparing biomass collection to an internal marker (lignin) technique for determining intake. The rate of dry matter intake was estimated by dividing total intake by grazing time. Cantillon (1986) defined intake by the equation: Intake (I) = Intake per bite (IB) x Rate of biting (RB) x Grazing time (GT) and stated that these three ingestive intake characteristics change in

response to sward conditions. When one of the three factors increases or decreases, the other factors compensate in order to maintain intake approximately constant. The tethered grazing techniques used by both Cantillon (1986) and Duren (1989) yielded forage dry matter intakes of 1.5 kg per hour for endophyte-free Kentucky 31 Fescue (Festuca arundinacea Schreb), 1.65 kg per hour for alfalfa (Medicago sativa L.) and 0.615 kg per hour for orchard grass (Dactylis glomerata L.). Duren (1989) further stated that if the value of 0.615 kg per hour dry matter intake is extrapolated to fit a circadian grazing pattern, the estimates are in agreement with published estimates of total DM intake for yearling horses (500 kg mature weight) of 6.00 kg per day (N.R.C. 1978). The studies by Duren (1987a and 1989) and Cantillon (1986) consider the voluntary intake of picketed horses. The study by Duren et al. (1989) was done with picketed yearling horses that received supplemental textured concentrate and free-choice timothy (Phleum pratense L.) hay for a total daily intake of approximately 11.75 kg dry matter per day or 3.1% of body weight. Markers or indicators have been used as an indirect method of quantifying forage intake. According to Kotb and Luckey (1972), markers for nutritional studies need to be an inert substance with no toxic physiological or psychological effects; be neither absorbed nor metabolized within the alimentary tract, and, therefore, be completely recovered

from either raw or processed food. Markers can be classified as two types: external, which are added to the diet or administered orally, e.g. chromic oxide, ferric oxide, silver sulfide, polyethylene glycol, and preparations of chromium, cobalt, hafnium and several of the rare earth metals; and internal, where the marker (indicator) occurs naturally in the diet and is neither digested nor absorbed in the gastrointestinal tract, e.g., fecal nitrogen index, lignin, chromogen, acid insoluble ash, indigestible fiber. If the digestibility of a component of a diet and its fecal output are known, then voluntary intake can be calculated. Fecal output can be measured by fitting a grazing animal with a fecal bag to collect the actual total fecal output, or by feeding the animal an external indicator. The use of an external indicator permits estimation of the fecal output without the use of a fecal bag and harness apparatus which is laborious and may also have an effect on the grazing behavior of animals (Cordova et al., 1978). Parkins et al. (1982) stated that some horses never adjust to having fecal collection bags and harnesses in place even though they are properly padded to minimize discomfort. The fecal bags need emptying four or five times a day and exercise routines are hampered if the apparatus is left in place. Cordova et al. (1978) stated that; in view of objections raised against other methods for estimating fecal output, and eventually intake, it may be concluded that total collection may still

be the procedure of choice under many situations, in spite of its relatively arduous and time-consuming disadvantages. Therefore, fecal output and ultimately intake of the grazing animal can be arrived at by total collection (TFC) techniques or marker methodologies.

When external indicators are fed in fixed amounts to the grazing animal total dry matter fecal output (DMFO) is calculated from the concentration of the indicator in a representative fecal sample (Equation 1):

Equation (1):

$$\text{DMFO (g/day)} = \frac{\text{external indicator consumed (g/day)}}{\text{external indicator in feces (g/g DM)}}$$

Two main approaches have been followed with the use of external markers (Paterson and Kerley, 1987). The first and most common has been the "steady state" or daily feeding of a fixed amount of indicator. The second is the "pulse dose" or one-time-only feeding of indicator at the beginning of the grazing trial. The first method gives only an estimate of fecal output (Equation 1) while the second method can predict fecal output, rate of passage, mean retention time in the gut and fill of undigested dry matter by employing the appropriate non-linear compartmental model (Pond et al., 1987). The concentration of external indicator obtained from frequent fecal collections is plotted with time of

collection to construct a pulse dose fecal excretion curve (Fig. 1).

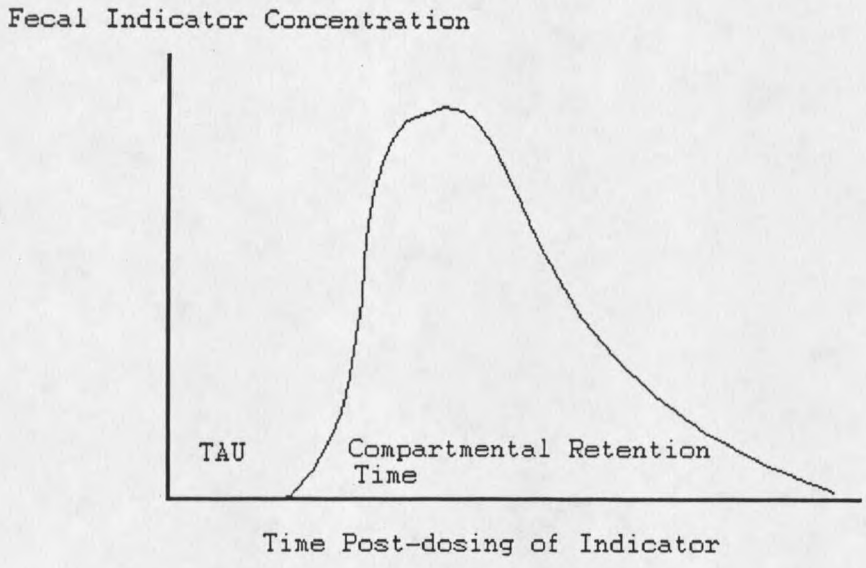

Figure 1. Example of Fecal Excretion Curve Constructed From Concentration of Marker in Feces After Pulse Dose Administration.

A time delay (TAU) occurs between indicator administration and first appearance in the feces followed by an increase of indicator concentration to a peak level then decreasing with time to below detectable levels. Dugan et al. (1993) in a rate of passage and digestibility stall feeding trial utilized three different nonlinear models to fit chromium-mordanted hay passage data to the fecal excretion curve of horses. The models used were: 1) one compartment with gama two, age dependent 2) biexponential, age dependent and 3) single exponential, age dependent, utilizing only data points from the peak through the declining phase of the

curve. Age dependency assumes that the probability for passage increases with time, while age independent models assume equal probability for escape of all particles regardless of size or time spent in the compartment. The single exponential, age dependent model gave the best fit of actual and predicted data points as well as tau for that rate of passage study.

Dry matter digestibility (DMD) of the forage consumed can be calculated using an internal indicator and the indicator ratio technique (Equation 2):

Equation (2):

$$DMD\ (\%) = 100 - \left(100 \times \frac{\%\ \text{internal indicator in feed}}{\%\ \text{internal indicator in feces}}\right)$$

This ratio technique involves the use of a naturally occurring substance in the forage and determination of this indicator in forage and feces. Indigestible dry matter (IDM) is calculated from Equation 3:

Equation (3):

$$IDM\ (\%) = 100 - \%DMD$$

Voluntary dry matter intake (VDMI) can then be calculated by a combination of external and internal indicators (Equations 4 and 5):

Equation (4):

$$VDMI = \frac{\text{external indicator consumed}}{\text{external indicator in feces}} \times \frac{\text{\% fecal DM}}{\text{\% IDM}}$$

Equation (5):

$$VDMI = \frac{\text{DMFO X \% internal indicator in feces}}{\text{\% internal indicator in forage}}$$

Cantillon (1986) discussed the difficulty in accurately estimating intake by grazing animals and suggested that one approach which may increase accuracy is the use of both internal and external indicators. The equations described above and referred to throughout are from Harris, 1970; Theurer, 1970; Kartchner and Campbell, 1979; Maynard et al., 1979; and Pond et al., 1987.

Chromic Oxide as an External Marker in Measuring Fecal Output

Chromic oxide (Cr_2O_3) has been the most commonly used external marker. It has the advantages of relatively cheap cost and simple analysis by atomic absorption spectrophotemetry. Problems of sedimentation in the rumen and sporadic transfer of Cr_2O_3 through the gastrointestinal tract have been discussed by Pond et al. (1987) and Ellis et al. (1982). According to Pond et al. (1987) part of the problem has been reduced with the incorporation of Cr_2O_3 into paper or cellulose. The shredded paper is then administered to the animal resulting in a more representative flow of digesta particles than that obtained

with Cr_2O_3 powder. Hardison and Reid (1953) described the normal phenomenon of diurnal variation in the excretion of Cr_2O_3 in ruminant animals causing an increase in variation of chromium concentration in fecal samples obtained for analysis. Therefore, samples must be taken that represent several time periods throughout a 24 hour period to help reduce the effect of this variation.

The diurnal variation and fecal excretion rates of horses have been examined (Haenlein et al., 1966; Crawford et al., 1974; Parkins et al., 1982). Haenlein et al. (1966) found the daily variation of fecal Cr_2O_3 ranged from 59.8% to 134.8% of the daily mean for six individual horses. The daily mean concentration is represented as the center of the oscillation for the excretion curve. It was concluded that time after administration of Cr_2O_3 to the horses appeared to be the major determinant for the extinction of the excretion curve, but the night curve showed a higher extinction than the day curve. Estimates of dry matter fecal output obtained by chromium content of rectal grab samples taken at 0700 hr. and 1900 hr. for four days were similar to results from the ten-day total collection procedure. Fecal Cr_2O_3 samples taken randomly for ten days gave estimates of dry matter fecal output that did not agree with those from the total collection procedures. A soft gelatin capsule containing 10 grams Cr_2O_3 was administered with a balling gun before feeding at 0700 hr. and 1900 hr.

daily, commencing ten days before fecal collections for this study.

Crawford et al. (1974) concluded that when 0.25% Cr_2O_3 and five percent molasses was added to diets consisting of oats, oats and hay, and hay fed to horses, the excretion of Cr_2O_3 appeared to be less variable for the hay ration than for either oats or oats plus hay. No significant differences were found between horses, energy levels or periods, suggesting that the variation in Cr_2O_3 excretion pattern is comparatively low when the indicator is mixed with diets fed twice daily. In the third study by Parkins et al. (1982) it was reported that giving one 10g capsule of Cr_2O_3 once per day at 2100 hr. resulted in a higher chromium concentration in the fecal output collected overnight. A significant underestimation of digestibility was calculated from samples obtained solely between 0900 hr. and 1800 hr.

Several experiments have quantified the total recovery of chromium in the feces of horses (Haenlein et al., 1966; Crawford et al., 1974; Heusner and Albert, 1977; Parkins et al., 1982). In all of these experiments total recovery of chromium in the feces was very near to 100%.

Raleigh et al. (1980) and Hatfield et al. (1991) made the following recommendations where chromic oxide is to be used in range nutrition studies:

1) Results obtained under pen feeding or drylot conditions should not be presumed to apply similarly to grazing situations.

2) Chromic oxide should be used only for estimating forage intake and/or digestibility on a comparative, not an absolute, basis.

3) Comparisons should be limited to the results obtained within one trial. Comparisons should not be made between trials under different conditions.

4) Animals utilized should be as homogeneous as possible.

5) Chromic oxide can be administered with a carrier; eg. paper, solka floc. Chromic oxide given in powder form results in a more erratic excretion pattern than when given impregnated on paper.

6) Twice daily dosing may produce a more uniform excretion pattern than once daily.

7) A minimum four-day preliminary dosing period should be employed when either paper or solk floc is used as a chromic oxide carrier.

8) Fecal sampling can be done at any time of the day, but provisions should be taken to insure that a sufficient number of samples be taken to average out the day and animal variation.

9) Great care should be taken when analyzing chromic oxide samples (a) be tested under uniform analytical

conditions, (b) be compared to the same standards, (c) be analyzed by the same person, and (d) be analyzed as a group at the same time, if possible.

External Marker Method of Administration

External markers have been most commonly administered to horses either in a gelatin capsule given orally, mixed with a feed supplement, or incorporated as part of a total feed ration. Cr_2O_3 has also been given with paper, cotton strips or salt block as a carrier. Chromium-mordanted fiber can also be fed to the animal. Theurer (1970) stated that recovery of Cr_2O_3 in fecal grab samples was erratic and the loss of the indicator by regurgitation, though not normally a problem in horses, have been the major problems in limiting the use of chromic oxide under grazing conditions with ruminant animals. Haenlein et al. (1966) used gelatin capsules and a balling gun in horses and reported no problems. Parkins et al. (1982) used the same method and had severe problems in the physical administration of the capsule. These included either periodically ejecting the capsule or biting into the capsule before swallowing. The author advises that in future studies it would be better to include chromic oxide in the complete pelleted feed in order to prevent such problems. This method had been used by Crawford et al. (1974) where each ration contained 0.25% Cr_2O_3 mixed with molasses. Martin et al. (1989) and

Gallagher and McMeniman (1988) both used the same method of dosing mares two times daily with 5 g Cr_2O_3 mixed with molasses and oaten chaff. No problems with administration of the marker were reported. The shredded paper technique was used by Corbett et al. (1960) and significantly reduced the variability of fecal excretion in sheep as compared to the powdered form given in an oil suspension. Uden et al. (1980) developed the chromium mordant procedure to attach chromium to the forage fiber with it remaining bound throughout the ruminant digestion process. In a stall feeding trial by Dugan et al. (1993) chromium-mordanted hay mixed with molasses was only minimally acceptable to a group of eight mares with one mare refusing to eat any marked hay. This trial utilized chromium-mordanted bermudagrass hay and chromium-mordanted flaccidgrass hay as pulse dose markers of the rate of passage of solid digesta in the horse. Paterson and Kerley (1987) concluded that methods of mordanting chromium to dietary fiber appeared effective in allowing the marker to flow with the particulate phase of the digesta, provided the amount of chromium was at a rate of six to eight percent of the fiber weight allowing the marked fiber to flow at the same rate as unmarked digesta particles.

Acid Insoluble Ash as an Internal Marker in Measuring Digestibility

The use of acid insoluble ash (AIA) as an internal marker in determining digestibility of equine rations has

been reported (Schurg et al., 1977; Sutton et al., 1977; Schurg and Holton, 1979; and Schurg, 1981). The concentration of an indigestible internal marker eg. AIA, in feed dry matter is compared with the concentration in fecal dry matter in order to obtain a measurement of total diet digestibility (Equation 2). Digestibility is then subtracted from 100 to obtain the percentage of indigestibility (Equation 3). The use of internal markers used in this fashion are responsive to the dynamics of in vivo forage utilization and, therefore, alleviate concerns related to the use of in vitro techniques (Cochran et al. (1987). The use of an internal marker in this manner is commonly referred to as a "ratio" procedure.

Sutton et al. (1977) compared 4N-HCL insoluble ash as an index material with total fecal collection in the determination of apparent digestibilities. The overall conclusion suggested that random grab fecal samples used for AIA determinations of digestibility has potential for use in the determination of digestibility coefficients for horses. Schurg et al. (1977) calculated apparent digestibilities of a whole corn plant pellet total ration fed to horses and rabbits by utilizing conventional total fecal collection techniques or the AIA method. Results of the total collection versus AIA techniques were not different ($P>.05$) for crude protein, acid detergent fiber and cell wall content but were different ($P<.05$) for ether extract. It

was concluded that utilizing the AIA technique for determining apparent digestibilities in horses offers another technique that may be more convenient and easier to use than conventional total fecal collection techniques. Schurg and Holtan, (1979) conducted a subsequent study to further evaluate the potential of AIA as an internal indicator method when fecal grab samples were taken from ponies for the determination of digestibility. Results showed no differences ($P>.05$) in digestibilities determined by total collection or AIA techniques. An evaluation of a compilation of data using total collection, Cr_2O_3, AIA or permanganate lignin as methods to determine digestion of equine rations was performed by Schurg, (1981). There were no significant differences between the total fecal collection, Cr_2O_3 or AIA methods in determining digestibility coefficients. The use of either the 4N-HCL or 2N-HCL marker techniques to determine AIA content of feed and feces were not different. In all experiments where the permanganate lignin method was used, digestibility was underestimated from 12 to 18 percent. The use of this method appears to be unreliable as an indicator ratio technique for determining digestibility in horse rations. Schurg, (1981) concluded that the use of internal and external markers offer advantages of saving time, reducing labor, and allowing the use of a greater number of animals.

The Cr_2O_3 and AIA methods will provide satisfactory results for estimating the digestibility of horse rations.

According to Paterson and Kerley, (1987), major concerns when using AIA as an internal indicator would be: 1. Use when diets contain less than .75% AIA; 2. Obtaining an accurate representative forage sample from animals grazing ad libitum; and 3. The potential for erratic soil ingestion by the grazing animal.

Voluntary Feed Intake

Voluntary feed intake by horses has been quantified for various forms of feed, in pen trials and in grazing situations. Todd et al. (1983) concluded that the voluntary intake of penned horses was significantly higher ($P<.05$) for horses fed cubed than pelleted, chopped or long alfalfa and that processing had no effect on the digestibilities of dry matter, energy, and protein. Total collection techniques of feces and urine were employed along with weigh-backs of feed refusal to determine voluntary intake. In a subsequent study Todd et al. (1987) examined the level of feed intake on nutrient digestibilities and the use of two digestibility markers; dysprosium and chromic oxide. Alfalfa cubes and vitamin-mineral pellets containing the markers were fed twice daily at maintainance and eighty percent ad libitum levels of intake. Results of this study found no differences ($P>.05$) in nutrient digestibilities based on

total collection and chromic oxide. The level of feed intake had no effect on the digestibilities of dry matter, energy and protein. Finally, mean retention times for digesta of horses fed at maintainance and ad libitum were twenty-five and fifteen hours, respectively, indicating a negative relationship between intake level and retention time in the horse. The mean rate of passage of digesta was determined by Sutton et al. (1977) to be 70.2 hours in a study that used cotton strips as indicators of fiber flow through the gastrointestinal tract. Vander Noot et al. (1967) found a retention time of ninety-six hours using Cr_2O_3. These results indicate large variation in retention times and diffusion of some markers is a problem.

Aiken et al. (1987) reported voluntary intake from a pen study with coastal Bermuda grass (Cynodon dactylon L,) hay, to be just over 10 kg per day for both yearling and mature horses. Means for voluntary feed intake differed ($P<.10$) for yearling (2.5%) and mature (2.0%) horses when expressed as a percentage of body weight. Aiken et al. (1987) postulated that voluntary dry matter consumption was more proportional to energy requirements of the rapidly growing yearlings than to gut volume. This agrees with the theory described by Frape et al. (1982) that voluntary dry matter consumption was more proportional to energy requirements than gut volume. Energy requirements of the horse rely upon numerous factors such as individuality, body

composition and age of the animal, environmental temperature and humidity, intensity and duration of work, conditions of the running surface, and degree of fatigue (NRC, 1989).

Rate of feed intake of various sizes of pelleted concentrate; .40, .48, 1.59, and 1.90 cm, by yearling and mature horses was reported by Freeman et al. (1990). They concluded that chew rates were the same for the various pellet sizes, pellet density affected intake as less dense pellets were consumed at a faster rate ($P<.05$) during the initial twenty minutes post-feeding and that the amount of feed offered affected intake response in mature horses. Horses that were offered more total feed consumed a lower percentage of the total offered in the initial twenty minutes post-feeding. Freeman et al. (1990) also postulated that slower intake for horses consuming more concentrate and hay per feeding might be expected if gut fill from the previous feeding had an effect on appetite.

A range experiment by Reiner and Urness (1982) evaluated the use of summer grazing by horses as a method of manipulating big game winter range in northern Utah. This experiment utilized biomass disappearance techniques to measure forage consumption by free-grazing horses along with bite count to determine horse dietary composition within the duration of the grazing trial. Results of this trial were that on this winter range grazing horses primarily utilized grasses with moderate consumption of forbs occurring on the

heavily stocked pastures (80% utilization of grasses) and horses consumed 2.43 and 2.07 kg air dry weight/100 kg body weight on moderately and heavily grazed pastures, respectively.

Duren et al. (1987a) conducted a tethered grazing study to determine voluntary forage intake of yearling horses grazing endophyte-free Kenhy tall fescue over a closely monitored three-hour grazing session. It was found that forage bite rate averaged fifteen bites per minute, bite size averaged 1.15 g/bite and mean dry matter intake of forage was 3.22 kg for the three-hour session or 1.08 kg/hour.

Pasture forage intake by unrestrained grazing horses has been measured using external and internal indicator methods (Meacham et al., 1986; Gallagher and McMeniman, 1988; Martin et al. 1989).

Meacham et al. (1986) used a pulse dose of Yb-stained fescue with fecal grab samples obtained at 6, 12, 18, 24, 30, 36, 48, 60 and 72 hours post-dosing. Fecal output was estimated by Yb concentration in the feces, indigestible neutral detergent fiber was used as an internal marker to determine digestibility and forage dry matter intake was calculated by the formula: Dry Matter Intake = Fecal Output / (100 - % forage digestibility) x 100. Estimated dry matter intake and dry matter fecal output were: 4.5 and 1.7

kg/d, 7.5 and 2.7 kg/d, 6.4 and 2.8 kg/d for three trials in August, January, and June, respectively.

Gallagher and McMeniman, (1988) conducted a study to determine the digestible energy and nitrogen intakes of pregnant and non-pregnant mares grazing frost-affected pastures. Grazing dry matter intake was determined by Cr_2O_3 concentration in feces for an estimate of fecal output along with dry matter digestibility of the diets calculated by AIA content of the composite diet and fecal samples. Dry matter intake for this study ranged from 8.4 to 9.6 kg/day for mares in July and 9.5 to 10.9 kg/day for mares in October. In a subsequent study of pregnant and lactating mares, Martin et al. (1989), using the same external and internal techniques as Gallagher and McMeniman, (1988), found that mares supplemented with two kg of pellets per day (25% protein) had a significantly lower pasture intake ($P<.05$) than unsupplemented mares, 7.6 and 9.0 kg/day, respectively. Lactating mares also consumed more pasture ($P<.01$) than the pregnant mares at 9.6 vs. 7.0 kg/day, respectively. It was concluded that lactating mares have the ability to increase intake to try to meet their additional requirements. Correspondence with Dr. N. P. McMeniman, the senior author on both of these studies indicates that no further work has been done on the subject of forage intake by free-grazing horses and that a method of dosing external marker with mordanted material would be good, provided the marked

material mixed well with the digesta. Dr. McMeniman also stated that the results obtained with the Cr_2O_3 method were reasonably reliable, but that more reliable results could be obtained by slightly altering the technique, eg. mordanted material or radioactive ^{51}Cr, which possibly could not be used with grazing animals.

Methods of Obtaining a Representative Forage Sample

The collection of accurate samples of organic matter ingested by the grazing animal is a complex problem. The total forage available to the animal is not representative of what is actually consumed. Cook (1964) attributed this discrepency to several factors including variations in chemical composition of different plants and plant parts, accessibility of different plants and plant parts and selective grazing by the animal. The method of hand plucking forage samples comparable with that actually grazed has been used in horse studies (Gallagher and McMeniman, 1988; Martin et al. 1989).

Three methods are commonly used in obtaining forage samples of ingested material under pasture and range conditions: hand plucking, harvesting before and after grazing, and sampling from esophageal or rumen fistulated animals (Cook, 1964). Although hand plucking is satisfactory on single species stands it is totally inadequate and

impossible on native rangeland because animals selectively graze the heterogeneous vegetation (Theurer, 1970).

The method of harvesting before and after grazing involves harvesting a random set of plots before grazing and another set can be harvested after grazing, or a given number of specific plant units can be collected from each species before grazing and again after grazing. Both methods use the difference in weight and chemical composition between the before and after grazing samples to obtain the content of ingested forage. Cook (1964) warns that the before- and after-grazing requires little or no growth or shattering of plants take place during the periods when the before and after collections are being made. It seems that trampling of standing forage by the grazing animal would be another factor to consider with this method.

Esophageal fistula collections were considered by Lesperance et al. (1974) to be the best technique available, despite limitations such as salivary contamination and failure to obtain a complete collection of ingested forage. Most investigators have reported higher ash content of fistula-collected forage than of clipped or hand-plucked samples as a result of salivary contamination or soil-ingestion, or both (Kartchner and Campbell, 1979). It is possible to determine the botanical composition of the diet of livestock grazing native ranges using esophageal fistulated animals and from microhistological analysis of

fecal droppings as described by Theurer et al. (1976). Archer (1973) described the plant species preference of grazing horses in an observational study conducted on improved pasture. No information is available concerning actual forage samples obtained by esophageal fistulated horses in a free-grazing situation.

CHAPTER 3

PEN TRIAL

Materials and Methods

Eight mature geldings (500 Kg mean weight) were selected for the trial. Prior to the trial, all geldings had been previously maintained in outdoor dry lot pens with a once daily feeding of mixed grass hay and no grain supplementation. Throughout the trial each gelding was individually housed in a 5 X 15 meter pen with *ad libitum* access to water and trace mineralized salt. All geldings were treated orally for internal parasites with an anthelmentic paste[1] the day prior to being brought into the individual pens. Teeth condition was evaluated earlier in the year as a regular horse herd management procedure and floating was done as needed. Visual observations of health and condition of each gelding were made daily.

Following a five day adjustment period, each gelding was randomly assigned to one of two pulse dose treatments in a completely randomized design. Four geldings received a one-time pulse dose of 15 grams chromium (PD15) and four geldings received a one-time pulse dose of 30 grams chromium (PD30). Chromium (Cr) was mordanted to whole oat fiber by the process developed by Uden et al. (1980). Chromium

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[1] Zimecterin™ (ivermectin) Paste 1.87%, Farnum Companies, Inc., Phoenix, AZ.

content of the Chromium-mordanted oats (CMO) used throughout this trial was 2.5% as determined by the methods of Williams et al. (1962). The concentration of Cr was read by induction coupled plasma (ICP) procedures at the soils testing laboratory of Montana State University. The pulse dose of CMO was fed to each gelding via canvas nose bags and mixed with 1000 g of regular whole oats. Complete ingestion of the required dose was obtained within a three hour time period by all of the geldings. Most geldings ate the CMO-oats mixture readily but two were hesitant at first and required additional time to completely ingest the required dosage. The one-time pulse dose of 15 g Cr for the PD15 group of geldings consisted of 650 g CMO, 2,5% Cr, mixed with 1000 g regular whole oats for a total dosage of 1650 g. Likewise, geldings assigned to PD30 received 1300 g CMO mixed with 1000 g regular whole oats for a total dose of 2300 g. Chemical composition of the mixed grass hay fed throughout the pen trial is shown in Table 1.

Table 1. Chemical Composition of Mixed Species Grass Hay Fed Throughout the Pen Trial.

Item	Pulse dose trial	Steady state trial
Dry matter, %	89.15	90.05
Crude protein, %	6.04	6.04
4N-AIA, %	2.22	2.06

Daily feed intake was restricted to two equal feedings at 0600 hour and 1800 hour per day for a total daily intake of 8.1 kg dry matter per gelding. Total fecal collections (TFC) were obtained by collecting voided feces from the floor of the pens every four hours throughout a 72 hour period post-dosing. Total wet fecal collections were then weighed and an aliquot subsample was taken. Subsamples were placed on individual aluminum pie pans, weighed and dried at 60°C then weighed again to gravimetrically determine the dry matter percent of the feces (A.O.A.C., 1980). Actual total dry matter fecal output (DMFO) obtained by TFC was then calculated for each gelding over the 72 hour period as well as the mean for each 24 hour period. Dried fecal subsamples and a composite hay sample obtained from the hay fed throughout the trial were ground in a Wiley mill to pass through a 1mm screen as preparation for laboratory analysis of Cr concentration, 4N-HCL insoluble ash (4N-AIA), dry matter and crude protein.

Chromium analysis of each fecal subsample from each gelding was prepared by the Cr digestion procedures of Williams et al. (1962). Inducton coupled plasma was used to read the Cr concentration of the sample. The composite hay sample was also checked for possible Cr contamination. The CMOs were analyzed by the same methods prior to conducting the trial in order to determine Cr concentration and dosage rates.

The analysis of 4N-AIA was conducted on the composite hay sample, CMO and whole oats as fed, as well as the 0 and 4 hour fecal subsamples from each gelding. Digestibility for the mixed grass hay fed throughout the trial was determined by TFC and the internal indicator 4N-AIA.

Fecal Cr excretion curves for PD15 and PD30 groups were fitted to a one compartment single exponential model (Pond et al. 1987) using the non-linear regression option known as the Marquardt method of SAS (1985). Estimated fecal output from the pulse dose method was also calculated from this model.

General linear models procedures of SAS (1985) were used to test for dose effects. Methods of SAS paired T-test analysis were used to determine differences between the actual DMFO obtained from TFC and the estimated DMFO calculated by the one compartment single exponential model procedure. Dry matter intake (DMI) was calculated using the 4N-AIA analysis and the DMFO values determined by TFC or Cr estimation. Differences between actual amounts of DMI calculated from TFC values of DMFO and 4N-AIA analysis of feed and feces and estimated amounts of DMI calculated from Cr estimate values of DMFO and 4N-AIA analysis of feed and feces were analyzed using the paired T-test procedure of SAS (1985).

The steady state portion of the trial was initiated after a four day clean-out period following commencement of the pulse dose method. During the clean-out period all eight geldings previously used in the pulse dose method were maintained on the same mixed grass hay (Table 1) fed throughout at a level of 8.1 kg dry matter/gelding, divided into two equal feedings/day.

Geldings were randomly assigned to one of two steady state treatments in a completely randomized design. Four geldings received a steady state dose of three grams chromium daily (SS3) and four geldings received a steady state dose of six grams chromium daily (SS6).

All CMO doses were mixed with 454 grams of whole oats and administered 2X daily (0600 hour and 1800 hour) in canvas nose bags.

Total daily dry matter intake was restricted to 9.03 kg and 9.15 kg for SS3 and SS6 groups, respectively. Daily DMI was different for the two groups due to the two levels of daily steady state dosing. A two day preliminary dosing period preceded the four days of fecal collections. Steady state fecal collections were initiated at 48 hours after the first steady state dosing with subsequent collections at 72, 96 and 120 hours for a total of four representative fecal collections for each gelding throughout the steady state method. Total fecal collections were not made during the steady state method. The fecal samples obtained were

collected at the hours mentioned above by observation of defecation within the collecton time period and manually taking a mixed subsample of the freshly voided feces/gelding at that time. Moisture and dry matter content of wet fecal samples was determined gravimetrically (A.O.A.C., 1980).

The 4N-HCL insoluble ash (4N-AIA) analysis was conducted on the composite hay sample, CMO dosages as fed and the 48 hour fecal subsamples from each gelding. Chromium concentration of these fecal samples was subtracted out to give the actual 4N-AIA determination of hay digestibility.

Estimated SS3 and SS6 dry matter fecal outputs (DMFO) per horse per day were determined by the ratio technique of Equation (1):

$$\text{DMFO (g/day)} = \frac{\text{external indicator consumed (g/day)}}{\text{external indicator in feces (g/g DM)}}$$

Daily DMI was then calculated for each gelding using the 4N-AIA digestibility coefficient and estimated DMFO, Equation (5):

$$\text{VDMI} = \frac{\text{DMFO X \% internal indicator in feces}}{\text{\% internal indicator in forage}}$$

Estimated DMI was compared to actual amounts fed using SAS paired T-test statistical analysis with differences being significant at the $P<.05$ level. The general linear

model procedure of SAS was used to test for dose effects. It was not possible to weigh back feed refusals for this trial due to the semi-open design of the individual pens and the extent to which the horses scattered the mixed grass hay individually fed in hay nets. Hay scattered or dropped to the floor was mixed between semi-open pens making an individual amount of hay refused or wasted for each gelding unobtainable. However, daily observations of feed intake indicated that the daily ration fed to each gelding was mostly consumed within the 24 hour period and wastage or refusal was minimal.

Results and Discussion

Three day means and standard errors of dry matter fecal output obtained from total fecal collection and chromium estimation for each gelding during the pulse dose method are shown in Table 2.

Table 2. Means and Standard Errors of Pulse Dose Kg Dry Matter Fecal Output (DMFO) / Gelding, from Total Fecal Collection (TFC) and Chromium Estimation (Cr Est).

Gelding	1	2	3	4	5	6	7	8
PD Trt	15	30	15	30	15	15	30	30
TFC	2.924	3.829	3.886	3.682	3.635	3.815	3.861	3.193
SE	.196	.375	.200	.176	.271	.366	.387	.196
Cr Est	4.392	3.244	3.307	3.199	6.053	3.650	2.800	1.594
SE*	.519	.207	.301	.171	.855	.005	.376	.565

*Standard Errors of Cr Est Means and TFC Means.

Individual animal variation of kg DMFO excreted per day is shown by the standard errors associated with total fecal collection data in Table 2. Geldings 2,5,6 and 7 have the greatest daily variation in kg DMFO. Group means from the individual animal measurements were compared and are shown in Table 3. Mean actual fecal output values from total fecal collections were 3.603 kg DM/hd/day for all eight geldings (Table 3).

Table 3. Least Squares Means and Standard Errors of Pulse Dose Daily Dry Matter Fecal Output (DMFO), kg/day, from Total Fecal Collections (TFC) and Chromium Estimation (Cr Est).

	Overall	SE	PD15	SE	PD30	SE
TFC	3.603[ac]	.166	3.565[ac]	.191	3.641[ac]	.134
Cr Est	3.496[ac]	.430	4.283[ac]	.564	2.710[bd]	.334

[ab]Means within columns with different superscripts differ P<.05.
[cd]Means within rows with different superscripts differ P<.10.

Mean chromium estimated fecal output was different (P<.05) from actual fecal output for the PD30 group of geldings. Numerical differences exist between the PD15 and the PD30 CrEst fecal output with differences from SAS GLM procedure approaching significance (P=.0824). The estimated mean fecal output for the PD15 group of geldings did not differ (P>.05) from the actual fecal output obtained with TFC techniques. However, the relatively high standard error value associated with this estimate should be noted. These actual and estimated DMFO values are greater than the estimated values of 1.7, 2.7 and 2.8 kg/day reported by Meacham et al. (1986) in which a pulse dose of Yb-stained fescue and fecal grab sampling techniques were used with horses.