COMPARISON OF THREE DIGESTIBILITY MARKERS IN BEEF CATTLE FED FINISHING RATIONS CONTAINING DIFFERENT SOURCES OF SUPPLEMENTAL FAT

An Undergraduate Research Scholars Thesis

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ABSTRACT

Comparison of Three Digestibility Markers in Beef Cattle Fed Finishing Rations Containing Different Sources of Supplemental Fat. (May 2014)

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Beef cattle production is profoundly dependent on the diets provided to the cattle and their ability to promote growth. Dietary markers significantly aid beef cattle nutritionists by providing an index to measure diet efficiency and feed bioavailability throughout the digestive system. This study compares two external dietary markers, chromium oxide (Cr_2O_3) and titanium dioxide (TiO_2), and one internal marker, acid detergent insoluble ash (**ADIA**) with the objective of validating titanium oxide as a marker. Fecal and duodenal samples were collected from four ruminally and duodenally cannulated steers, over four trial periods with each period having duration of twelve days. External dietary markers were hand-mixed and administered daily in a fat-supplemented concentrate diet. The interaction between marker and treatment was significant ($P<0.05$) for OMD and ADF. Digestibilities determined by TiO_2 and Cr_2O_3 were significantly different ($P<0.05$) for all nutrients observed; however TiO_2 and ADIA differed significantly only for organic matter digestibility (OMD). This project did not provide definitive data regarding the suitability of any of the markers for measuring digestibility.

CHAPTER I

INTRODUCTION

Ruminant digestion studies

Digestion studies with ruminants are integral to the process of understanding nutritive value of feedstuffs. This form of experimentation assists in the approximation of the disappearance and flow of nutrients within the ruminant digestive system. A digestion study necessitates ruminants cannulated in multiple locations along the digestive tract; for example one ruminal and one intestinal cannula (Titgemeyer, 1997). Digestion studies are imperative to the beef cattle industry as thorough comprehension of a particular feedstuff allows the most efficient and economical method of feeding.

Specifically, digestion studies are designed to measure the change in nutrient content after digesta has passed through the various segments of the digestive tract. This is particularly significant in ruminants due to the breakdown of nutrients between the rumen and the abomasum and small intestine. A feed is metabolized in the rumen by microbial fermentation, and, conversely, in the abomasum and small intestine by enzymatic and hydrolytic processes. Microbial fermentation, primarily of carbohydrates, is responsible for the production and absorption of volatile fatty acids (VFA's), wheras protein is fermented to ammonia (NH_3), VFA's, and used to synthesize microbial crude protein (Merchen, 1988).

The results of a digestion study are derived from the comparison of the feed consumed and samples of digesta at various points throughout the gut. Traditionally, an accurate digestion

study required total fecal collection in order to compare the amount consumed to amount excreted. The introduction of inert dietary markers enabled sampling of fecal matter on a smaller scale, as well as sampling of ruminal and duodenal fluid, which ultimately increased the efficiency of digestion studies.

Dietary markers

A dietary marker is a substance that is not metabolized by the animal, and thus maintains a constant quantity throughout the digestion process. This can then be used to determine the proportion of feed digested, and accordingly, the proportion that is excreted as waste. Introduction of markers simplified digestion trials by reducing the need for total fecal collection (Merchen, 1988). Digestion studies which employ markers are less intensive, less expensive, more practical, and more sanitary than those utilizing total fecal collection.

It is vital to the digestion study that the marker used provides accurate results. Proposed by Kotb and Luckey, the "ideal" marker must meet the following criteria: must be inert, have no toxic effects, cannot be lost in digestion, cannot add considerable mass, must be easily and equally mixed into feed and remain so in digesta, have no effect on digestive constituents such as secretions, microbes, and muscle motility, and must have chemical properties that allows for efficient determination of concentration (1972).

Digestion markers are classified as internal or external. Internal markers are indigestible components originally existing within a feed that can be identified and measured before and after the digestion process. Examples of internal markers include lignin, acid-insoluble ash (AIA), acid detergent insoluble ash (ADIA), and silica. Lignin has, however, been deemed as an

unsuitable marker due to inconsistent fecal recovery (Titgemeyer, 1997). Internal markers are particularly useful in grazing diets when animals are consuming high levels of indigestible fiber and cannot be isolated to provide a supplementary ration. External markers are defined as diet additives which are not metabolized during digestion. Methods of external marker administration include: gelatin capsules, intraruminal injection, and mixed and fed through as a powder. Utilization technique varies based on the type of feed (for example, forage or concentrate) being consumed. External markers are ideal for use with high concentrate diets that are low in the indigestible components used as internal markers (Merchen, 1988; Pond et al., 1987).

Dietary markers aid considerably when the following calculations are desired: fecal dry matter (DM) output (1), digestibility of nutrient (2), apparent crude protein digestibility (3), and DM intake (4). Merchen presents the equation of each calculation in his review *Digestion, Absorption, and Excretion in Ruminants* (1988).

1. $Fecal\ DM\ output\ \left(\frac{g}{d}\right) = \frac{marker\ consumed\left(\frac{g}{d}\right)}{marker\ concentration\ in\ feces\ \left(\frac{g}{gDM}\right)}$
2. $Digestibility\ of\ nutrient\ (\%) = 100 - (100 * \frac{(\%\ marker\ in\ feed)}{(\%\ marker\ in\ feces)} * \frac{(\%\ nutrient\ in\ feces)}{(\%\ nutrient\ in\ feed)})$
3. $Apparent\ crude\ protein\ digestibility =$

   $100 - (100 * \frac{(\%\ marker\ in\ feed)}{(\%\ marker\ in\ feces)} * \frac{(\%\ crude\ protein\ of\ feces)}{(\%\ crude\ protein\ of\ feed)})$
4. $DM\ intake = fecal\ output * \frac{100}{\%\ indigestibility\ of\ DM}$

Chromium oxide

Chromium oxide (Cr_2O_3) is the predominantly utilized dietary marker in cattle digestion studies. It is an insoluble, intensely green-colored metal oxide that generally yields satisfactory recovery rates in fecal matter (Fenton, 1979; Raymond et al., 1955; Scurch et al., 1950; Titgemeyer, 1997). Schurch accounts that Cr_2O_3 was proposed as a dietary marker by Edin in 1918, and has been used in digestion trials with sheep, horses, cattle, pigs, fish, and humans. (1950). A summarized procedure of chromium oxide concentration determination in a sample calls for combustion of sample at 450°C, acid digestion of residual ash, and spectrophotometric reading at 440 nm (Fenton, 1979).

It has been observed that recovery of Cr_2O_3 varies greatly among individual animals and often deviates from 100%. Titgemeyer reports an average fecal recovery rate of 94% in nine studies using Cr_2O_3 as an external marker (1997). There are also health concerns regarding the carcinogenic properties of Cr_2O_3 (MacRae, 1974; Merchen, 1988; Norseth, 1986; Pond et al., 1987; Titgemeyer, 1997).

Acid Detergent Insoluble Ash

Acid detergent insoluble ash (ADIA) is an internal marker consisting of the residue after a sample has undergone acid detergent digestion followed by ashing (Van Soest, 1994). This residue may be composed of inorganic cellular structural components, such as silica (SiO_2). Due to the ease of its recovery method, ADIA use as an internal marker is becoming more frequent. ADIA is reported as an effective internal marker in beef cattle consuming unsupplemented, forage-based diets, with recovery rates of 99.3% and 97.5% in two trials (Bodine et al., 2002).

ADIA was also found to accurately predict fecal output and dry matter digestibility (DMD) for cows on hay diets (Kanani et al., 2014).

Titanium dioxide

Titanium dioxide (TiO_2) is a highly manufactured metal oxide that is often used in the production of sunscreen lotions, toothpastes, paints, and pigments. Although it is suspect to increase the risk of cancer, studies of human exposure to TiO_2 in the workplace do not indicate a carcinogenic effect on workers (Bofetta, 2004). In addition, it is a feed additive approved for use by the United States Food and Drug Administration, and is less expensive to obtain than Cr_2O_3. Upon combustion and acid digestion with hydrogen peroxide, organic samples containing TiO_2 turn a deep yellow color, which allows titanium concentration to be determined by spectrophotometer. On these bases, TiO_2 has recently been introduced as a dietary marker in livestock species.

Although they are few, digestion studies in cattle, poultry, pigs, sheep, rats, and fish support TiO_2 as an alternative to Cr_2O_3 (Short et al., 1996; Titgemeyer et al., 2001; Myers et al., 2004; Monforte-Braga et al., 2006; Myers et al., 2006; Glindemann et al., 2009). In studies of sheep, Myers reports duodenal recovery of TiO_2 to be consistent with and fecal recovery greater than that of Cr_2O_3 when administered intraruminally (2004). Glindemann also confirmed and developed a method for the use of TiO_2 in to estimate fecal excretion in grazing sheep (2009). Titgemeyer conducted studies of TiO_2 fecal recovery in three diets of beef cattle; one forage-based and two corn-based. The average TiO_2 concentrations in fecal samples from each diet were 93%, 95%, and 90% respectively (Titgemeyer et al., 2001). Limited prior study warrants further

experimentation and methods development to support regular use of TiO_2 as a valid dietary marker in beef cattle.

Objective

There are numerous factors regarding the use of Cr_2O_3 as a dietary marker which necessitate development of a suitable substitute. TiO_2 is a potential inert marker found to have comparable success and higher efficiency than Cr_2O_3. TiO_2, juxtaposed to Cr_2O_3 and ADIA as dietary markers in beef cattle, will determine that TiO_2 yields similar or greater recovery rates in fecal samples.

Summary

An effective dietary marker is a key element in the development of successful diets for beef cattle. Recently, cause has been found to establish a replacement for Cr_2O_3, the current leading dietary marker. This study aims to verify TiO_2 as an inert dietary marker in beef cattle. It will apply specifically to duodenal and fecal content in four beef steers over four 12-day periods.

CHAPTER II

MATERIALS AND METHODS

Experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at Texas A&M University.

This study will evaluate efficacy of TiO_2, Cr_2O_3, and ADIA as dietary markers through the collection and analysis of substance recovery in fecal samples. Four steers were previously fitted with ruminal and duodenal cannulas and maintained in pens inside a ventilated barn. Steers were offered one of four different high-fat diets (AF, IP, Phos, VOP) and used in a 4x4 Latin square, with twelve-day periods. The steers were provided ad libitum access to water and a concentrate diet, which was supplemented with forage and provided daily at 0730 hr.

Days 1-6 of each period were allotted to the steers for dietary adjustment. 10 grams of TiO_2 and 10 grams of Cr_2O_3 were hand mixed into the feed of each steer from days 2 through 11. Orts were weighed daily, and collected before feedings of days 7-11. Feed samples from days 7-10 were also collected and weighed. Feed and ort collections were dried in a forced-air oven at 60°C for 96 hours. At the end of the period, an equal quantity of sample was taken from each daily collection, and composited per steer to create a single sample representative of that steer's feed from days 7-11. Quantities of orts proportional to that which was collected on days 7-11 were combined to produce a single composite sample for each steer per period. Fecal and duodenal samples were taken days 7-11 every 8 hours, advancing 2 hours each day, and stored in

refrigeration. At the end of each period, fecal samples were thawed, composited in equal amounts by steer, and dried at 60°C. A total of 4 feed, 4 orts, and 4 fecal samples were developed per period. Samples were ground to pass through a 1 mm screen and sealed in plastic bags. Assays performed on samples were dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP= total nitrogen × 6.25), following methods defined by AOAC.

Cr_2O_3 recovery:

Method of Cr_2O_3 recovery was defined by Williams et al (1962).0.5 g of sample were weighed and deposited in 50 mL beakers. The samples were ashed at 450°C for 8 hours. When cooled, 3 mL acid manganese sulfate and 4 mL of potassium bromate are added to the beakers and digested at 200°C for approximately 7 minutes. Then, 12.5 mL calcium solution was added and the sample was rinsed into a 100 mL plastic cup. The total solution weight was brought up to 100 g with deionized water and allowed to settle for at least 24 hours. Samples were sent to Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory (College Station, TX) to be read with an atomic absorption spectrophotometer.

TiO_2 recovery:

TiO_2 recovery methods were followed according to procedure of Short et al (1996). 0.5 g of sample were weighed and deposited into 50 mL beakers. The samples were then ashed at 450°C for 24 hours. Following ashing, 10 mL of sulfuric acid (H_2SO_4) was added to each beaker. These samples were digested at 200°C for approximately 1 hour, allowed to cool, and transferred into a plastic sample cup. 10 mL of 30% hydrogen peroxide (H_2O_2) was added to the cup, and the total

liquid volume was brought to 100 mL with 2 fractions of deionized water. Samples were mixed, and allowed to settle for a minimum of 24 hours. The UV-visible spectrophotometer was calibrated using a set of standards, and samples were read at 410 nm for absorbencies.

ADIA Recovery:

ADIA was recovered according to Van Soest (1991). Sample was digested in an acid detergent solution and ashed. Remaining residue was ADIA content.

Calculations and statistical analyses

Marker absorbencies were read in parts per million (ppm) and converted to grams per day. Marker consumed was calculated as:

$$Marker\ consumed = \frac{marker\ in\ feed(g)}{marker\ in\ orts\ (g)}$$

Fecal output (DM) was calculated as:

$$Fecal\ output\ (DM) = \frac{marker\ consumed(g)}{marker\ in\ feces\ (\frac{g}{g}DM)}$$

Digestibility was calculated as:

$$Digestibility\ of\ nutrient\ (\%)$$

$$= 100 - (100 * \frac{(\%\ marker\ in\ feed)}{(\%\ marker\ in\ feces)} * \frac{(\%\ nutrient\ in\ feces)}{(\%\ nutrient\ in\ feed)}$$

Fecal production and digestion coefficients were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment, marker, treatment × marker, and period, with steer as the random effect. Treatment means were calculated using the LSMEANS option and pairwise comparisons.

CHAPTER III

RESULTS

Interactions ($P < 0.05$) between treatment and marker were observed for OMD and ADF, but were approaching significance for DMD and NDF ($P=0.07$ and $P=0.06$, respectively).
In all analyses, significant differences in digestibility were observed between TiO_2 and Cr_2O_3, and ADIA and Cr_2O_3 ($P<0.05$). All three markers were significantly different for OMD digestibility.

Prior to feeding, 10 g of both Cr_2O_3 and TiO_2 were mixed by hand into the feed. According to the proportions of each element in their respective formulas, the steers were offered 6.84 g chromium and 5.99 g titanium daily. By analysis of feed samples, the average value of chromium in feed was 5.51 g/day, indicating an 80.5% recovery rate. Average titanium found in feed was 8.79 g/day, yielding a 146.7% recovery.

Table 1. Estimates of daily fecal production, dry matter digestibility (DMD), organic matter digestibility (OMD), ADF digestibility, starch digestibility, and EE fat digestibility determined by Cr_2O_3, TiO_2, and ADIA.

Fecal Production (kg/day DM)

Diet	Cr	Ti	ADIA	SEM	Diet	Marker	D × M
AF	3.64	2.75	2.96	0.45	0.64	0.01	0.11
IP	3.41	3.09	2.01				
Phos	3.29	3.45	2.80				
VOP	4.12	2.40	3.22				

DMD Digestion

Diet	Cr	Ti	ADIA	SEM	Diet	Marker	D × M
AF	0.62	0.69	0.71	0.04	0.66	< 0.01	0.07
IP	0.63	0.67	0.78				
Phos	0.65	0.63	0.71				
VOP	0.75	0.57	0.67				

ADF Digestion

Diet	Cr	Ti	ADIA	SEM	Diet	Marker	D × M
AF	0.26	0.44	0.40	0.09	0.26	< 0.01	0.03
IP	0.16	0.24	0.50				
Phos	0.24	0.19	0.36				
VOP	0.07	0.45	0.27				

OMD Digestion

Diet	Cr	Ti	ADIA	SEM	Diet	Marker	D × M
AF	0.54	0.35	0.71	0.04	0.66	< 0.01	0.07
IP	0.54	0.17	0.78				
Phos	0.52	0.07	0.72				
VOP	0.50	0.41	0.67				

Starch Digestion

Diet	Cr	Ti	ADIA	SEM	Diet	Marker	D × M
AF	0.88	0.91	0.90	0.02	0.01	0.01	0.19
IP	0.91	0.92	0.95				
Phos	0.89	0.89	0.91				
VOP	0.84	0.91	0.88				

EE Digestion

Diet	Cr	Ti	ADIA	SEM	Diet	Marker	D × M
AF	0.86	0.90	0.88	0.03	0.07	0.03	0.39
IP	0.81	0.83	0.89				
Phos	0.84	0.85	0.86				
VOP	0.79	0.88	0.84				

CHAPTER IV

CONCLUSION

Recovery data was inconclusive, and it cannot be determined if TiO_2 would be a suitable replacement for Cr_2O_3 (Table 1). Nutrient digestibilities calculated from TiO_2 varied significantly from those calculated by Cr_2O_3. Except for OMD, TiO_2 behaved similarly to the internal marker ADIA. Although ADIA is most useful in high-forage diets, the consistency between ADIA and TiO_2 may support TiO_2 to be more reliable marker than Cr_2O_3. Recoveries of markers in feed samples that were inconsistent with the measured marker added imply that marker recovery procedures were erroneous.

Dietary markers are used to calculate estimations of difficult-to-measure variables, such as nutrient digestibility or fecal output. Although an ideal marker is 100% recoverable, most markers have a tendency to be inaccurate due to the many factors involved and scale of digestion trials. It is imperative that the marker is appropriate for the diet in experimentation, the marker is consumed by the animal, feed and feces samples are representative of the whole, and chemical analyses are performed correctly (Titgemeyer, 1997). A potential way to improve future trials of marker validation would be to use the same diet for each steer, as there were interactions between diet and marker for OMD and ADF digestibilities. Additionally, a more thorough strategy should be developed to mix the external marker with the feed, or to ensure that the steer consumes all of marker. This could be accomplished by offering the entire marker dosage in a pellet or biscuit at the time of feeding, administering orally with a syringe, or adding it directly into the rumen if the subject is cannulated (Pond et al., 1987). In this trial, feed was not sampled

before the addition of TiO_2 and Cr_2O_3. This should be considered in the methods of future experimentation to verify that there is no preexisting TiO_2 or Cr_2O_3 content in the feed, and to create a baseline of comparison for the samples containing marker residue.

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