

# Evaluation of Four Internal Markers and an Intra-Ruminal Chromium-Releasing Device for Use in Predicting Diet Digestibility and Intake by Beef Steers<sup>1</sup>

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## Summary

Indigestible acid detergent fiber would be acceptable for use as an internal marker in fescue hay diets similar to the one used in this study. The Captec® boluses provided a reliable and relatively simple method of administering an external marker for use in estimating fecal output.

## Introduction

Intake prediction of grazing ruminants requires accurate estimates of diet digestibility and fecal output. Internal markers (indigestible materials occurring naturally in feeds) are used to determine digestibility, while fecal output is usually determined via total fecal collection. However, because this can be very labor intensive and impractical under certain conditions, external markers (indigestible materials either added to the diet or intra-ruminally dosed to the animal) are sometimes used to estimate fecal output. Consequently, diet intake and digestibility by grazing ruminants can be estimated without total fecal collection if internal and external markers are used.

The choice of which internal marker to use can be affected by variables such as forage type and maturity. Most research has shown that internal marker recovery is not quantitative across different diets. Therefore, researchers must determine the most appropriate marker to use (and define its recovery) with a particular diet. An external marker can be administered frequently, continuously, or in a single pulse-dose. Theoretically, a continuously released marker would minimize variation often associated with external markers and allow for a more accurate estimate of fecal output. As with internal markers, recovery and validation with the proposed diet is required. Therefore, the objectives of the current study were to evaluate acid insoluble ash (AIA), acid detergent lignin (ADL), the alkane hentriacontane (HTC), and indigestible acid detergent fiber (IADF) as internal markers for use with fescue-based diets. In addition, administration of an external marker (chromium; Cr) by an intra-ruminal releasing device (Captec bolus) was assessed.

## Materials and Methods

Four Angus steers (970 ± 22-lb body weight [BW]) were offered a 100% fescue hay diet (15% crude protein [CP], 77% neutral detergent fiber [NDF], 43% acid detergent fiber [ADF], and 4.8% acid detergent lignin [ADL]; dry matter [DM] basis) to evaluate AIA, ADL, HTC, and IADF as internal markers for determining diet digestibility. In addition, a Captec bolus (listed

mean release rate of 1,013 mg Cr/d) was placed into the rumen of each steer at 0800 on d 1 to validate Cr release rate. The experimental period was 17 d, with 10 d for diet adaptation and 7 d for collection of feces. Fescue hay was offered in two equal portions daily (0700 and 1900) at 110% of the previous day's intake for d 1 to 5 and then at 95% of ad libitum intake for d 6 to 17. All steers consumed their daily allotment of hay. Fescue hay was sampled and composited d 8 to d 15. Intake estimates were based on d 9 to 15 and total feces collected d 11 to 17. Daily fecal output for each steer was weighed, thoroughly mixed, and 1% of fresh weight sampled and composited. Composited fecal samples were dried (130° F) for 96 h while composited fescue hay was dried (130° F) for 48 h. All samples were ground through a 1-mm screen and analyzed for NDF, ADF, AIA, ADL, HTC, IADF, and Cr. Marker recovery (%) was calculated as [marker excreted in feces (g/d)/marker intake (g/d)] \* 100.

## Results and Discussion

Steer dry matter intake (DMI) and diet, neutral detergent fiber, and acid detergent fiber digestibilities are presented in Table 1. Steer DMI ranged from 10.6 to 13.3 lb/d, with dry matter digestibility ranging from 51.3 to 56.5%. Similar results were observed for ADF (51.1 to 55.0%); however, NDF digestibilities were slightly greater (55.6 to 62.0%). Mean digestibilities were 53.4, 58.9, and 53.0% for dry matter, NDF, and ADF, respectively.

Fecal marker recoveries were 90.4, 110.5, 90.0, and 99.9% for AIA, ADL, HTC, and IADF, respectively (Table 2). These results indicate little to no disappearance (less than 10%) of these markers within the gastrointestinal tract. Recovery of ADL

**Table 1.** Dry matter intake and dry matter, neutral detergent fiber, and acid detergent fiber digestibilities of steers consuming a fescue hay diet.

Steer	DMI (lb/d) <sup>a</sup>	Digestibility (%)		
		DM <sup>a</sup>	NDF <sup>a</sup>	ADF <sup>a</sup>
A	10.6	56.5	62.0	55.0
B	13.3	51.3	59.3	51.5
C	12.0	51.5	58.8	51.1
D	11.2	54.4	55.6	54.5
Mean	11.8	53.4	58.9	53.0
Standard error of mean	.6	1.2	1.3	1.0

<sup>a</sup>DMI = dry matter intake, DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber.

<sup>1</sup> Intra-ruminal chromium releasing device from Captec LTD (Manu Street, P.O. Box 22-407, Otahuhu, Auckland 6, New Zealand); Thanks to Russell Sandberg and Dr. Don Adams of the University of Nebraska for alkane analysis.

was greater ( $P < .10$ ) than IADF, AIA, and HTC recoveries and was greater than 100%. Consequently, use of ADL would overestimate intake. These results suggest that of the markers evaluated, IADF would be the best internal marker to use with fescue hay-based diets. Indigestible ADF recovery also resulted in less variation between steers compared with AIA, ADL, and HTC (2.4 vs 4.0, 4.5, and 7.9%, respectively).

Average Cr release for d 11 to 17 was  $916 \pm 10$  mg/d. This was slightly lower than the listed release rate of 1,013 mg/d. Nevertheless, Cr release rate was consistent, with negligible between-steer variation. Therefore, Captec boluses performed well and would serve as an acceptable external marker if the following requirements are met: 1) all boluses come from the same production batch, 2) Cr release rate is validated with a similar diet, and 3) fecal sampling should occur at the same time after bolusing compared with the validation trial (to account for potential differences in release rate due to ruminal incubation time).

**Table 2.** Marker recoveries and chromium release rate in steers consuming fescue hay.

Steer	Marker Recovery <sup>a</sup> (%)				Cr <sup>a</sup> Release (mg/d)
	AIA	ADL	HTC	IADF	
A	84.7	119.0	100.5	96.7	896
B	101.4	113.4	106.5	106.5	943
C	91.3	98.0	75.9	100.0	919
D	84.4	111.6	77.3	96.2	905
Mean	90.4 <sup>b</sup>	110.5 <sup>c</sup>	90.0 <sup>b</sup>	99.9 <sup>b</sup>	916
Standard error of mean	4	4.5	7.9	2.4	10

<sup>a</sup>AIA = acid insoluble ash, ADL = acid detergent lignin, HTC = alkane hentriacontane, IADF = indigestible acid detergent fiber, Cr = chromium.

<sup>b,c</sup>Marker recovery means without a common superscript differ ( $P < .10$ ). Marker recovery standard error of mean = 4.1.

## Molecular Identification of Glutamate Transporters in Ruminant Forestomach, Intestine, Liver, and Kidney Tissues

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### Summary

The goal of this research is to understand how dietary proteins are metabolized to support glucose formation in ruminants. Depending on the production state and type of diet fed, 15 to 35% of all glucose in ruminants is derived from the breakdown of proteins. The transport of one amino acid, glutamate, by the gastrointestinal tract, liver, and kidneys is especially important to support this process. This study has identified three proteins (transporters) capable of transporting glutamate in many tissues of sheep and cattle. Sheep and cattle displayed the same tissue-specific pattern of glutamate transporter expression, which differed among tissues and transporter isoforms. For both sheep and steers, two transporters were expressed in every tissue examined (EAAC1, GLT-1). In contrast, a third transporter (GLAST1) was expressed only by the pancreas, whereas a fourth transporter (EAAT4) was not detected in any non-brain tissue of either species. The size and tissue distribution profiles for sheep and cattle glutamate transporters are consistent with those reported for non-ruminants. This study provides the physiologic knowledge necessary to study the role of glutamate transporter expression in ruminant N metabolism. Studies are now underway to understand how the expression of these transporters is altered when different diets are fed. Results from these ongoing studies will guide the formulation of sheep and cattle diets that match the amounts and ratios of dietary amino acids to the physiologic capacity of the animal to efficiently use the amino acids.

### Introduction

All animals require dietary amino acids to meet maintenance and production demands. Knowledge of the capacity for  $\alpha$ -amino acid absorption and metabolism should allow diets to be formulated to provide adequate, but not excessive, amino acids for a given production state. Although considered to be “non-essential,” the absorption and metabolism of anionic amino acids L-glutamate (glutamate) and L-aspartate (aspartate) are critical for nitrogen metabolism in mammalian tissues. The absorption and metabolism of glutamate are especially important for the ruminant to support the large amount of amino acid-derived gluconeogenesis (15 to 36% of total serum glucose). The identity and distribution of the proteins responsible for glutamate uptake in gastrointestinal epithelia, liver, kidney, and pancreas must be known to understand how nitrogen homeostasis is achieved in differing physiologic states.

In non-ruminants, three anionic amino acid transport systems ( $X_c^-$ ,  $X_{AG}^-$ , ASC) have been delineated by their biochemical activities (substrate affinity,  $Na^+$ -dependence, and pH-dependence). The transport of glutamate and aspartate by System ASC occurs only when pH is below 5.2, thus making the physiologic importance of this phenomenon in healthy animals uncertain. System  $x_c^-$  (cystine-inhibitable,  $Na^+$ -independent uptake of glutamate and aspartate) has been reported in the plasma membranes of fibroblasts, the canalicular (bile-facing) membranes of periportal hepatocytes, and the apical and basal membranes of pancre-

atic acinar cells. In contrast, System  $X_{AG}^-$  activity (the D-aspartate-inhibitable,  $Na^+$ -dependent transport of L-glutamate and L-aspartate) is expressed in the apical and (or) basal membranes of many cell types and is capable of anionic amino acid transport at physiologic pH. To date, five different non-ruminant mammalian proteins capable of System  $X_{AG}^-$  activity have been cloned (GLAST1, GLT-1, EAAC1, EAAT4, EAAT5). The subsequent generation of complementary deoxyribonucleic acids (cDNAs) and antibodies has initiated studies to describe the tissue-specific expression of these proteins and the identification of modulators of expression. For ruminants, homologs of EAAC1 and GLAST1 transport proteins have been cloned from immortalized bovine cells, but the distribution of System  $X_{AG}^-$  activity and the proteins capable of System  $X_{AG}^-$  transport have not been described. The objective of this study was to examine the distribution of GLAST1, GLT-1, EAAC1, and EAAT4 in tissues important for the absorption and glucogenic metabolism of glutamate in ruminants.

## Procedures

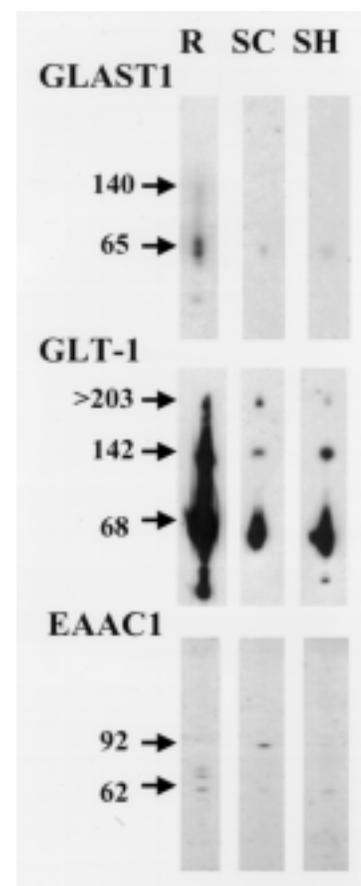
### Care of Animals, Tissue Collection, and Extraction of RNA

Wethers ( $n = 4$ , mean body weight = 28.4 kg) as a group and Angus steers ( $n = 3$ , mean body weight = 939 lb) individually were housed in 2.4 x 2.4 m pens during the experiment in an environmentally controlled room (21° C) with 16 h light: 8 h dark photo cycles. Animals had ad libitum access to water. Sheep were fed a forage-based diet consisting of (as-fed) 76% fescue hay, 7.6% soybean meal, 6.5% soybean meal, 5.4% corn oil, 3.8% blood meal, 3.3% molasses, 3.2% solfa floc, 1.1% sodium phosphate, .54% dicalcium phosphate, .54% ammonium chloride, .54% trace mineral salt, and .04% vitamin mix to meet 1.2 times their  $NE_m$  requirements for at least three weeks. Steers were fed a forage-based diet consisting of (as-fed) 78% fescue hay, 10.8% dry-rolled corn, 8% soybean meal, 2% molasses, .6% limestone, .5% trace mineral salt, and .06% vitamin mix to meet 1.1 times their  $NE_m$  requirements for at least three weeks. On any given day, an animal was anesthetized by intrajugular administration of 80 mg pentobarbital/kg body weight. The liver, rumen, omasum, and intestinal tract were removed and the animal was killed by exsanguination. Tissues were collected from anesthetized animals to mitigate the shedding of epithelial cells and to facilitate mRNA viability. After rinsing in 4° C, .9% saline, the epithelium was scraped from the rumen (cranial ventral sac), omasum (order I and II plies), duodenum (.5 m from the pyloric junction), jejunum (middle of the proximal region), ileum (middle of the distal region), cecum, and colon (.5 m from the rectum) and individually homogenized in a solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate pH 7.0, 0.5% N-lauroyl sarcosine, and 0.1 M 2-mercaptoethanol, using a Polytron homogenizer.

### Isolation of Membrane Proteins and Immunoblot Analysis

Immunoblot analyses for the presence of GLAST1, GLT-1, EAAC1, and EAAT4 were performed. Briefly, 2 g of tissue was homogenized in a 4° C solution containing 0.25 mM sucrose, 10 mM HEPES-KOH (pH 7.5), 1 mM EGTA, and 2 µg/ml each of N-tosyl-L-phenylalanine chloromethyl-ketone, N- $\alpha$ -p-tosyl-L-lysine ketone, leupeptin hemisulfate, aproptinin, and pepstatin A to prevent proteolysis (SEB). A plasma membrane-enriched fraction of cellular proteins was then generated by centrifugation of the tissue homogenate at 500 g for 2 min, followed by centrifugation of the resulting supernatant at 100,000 g for 30 min. The pellet was resuspended in SEB, and the resulting membrane proteins separated by 7.5% SDS-PAGE and then electrotransferred to a 0.45 µm nitrocellulose membrane. For the detection of EAAC1 protein, blots were probed with 43 ng IgG/ml of the EAAC1 polyclonal antibody in blocking solution (1% non-fat dry milk and 2% casein hydrolysate in 10 mM Tris-Cl, pH 7.5, 300 mM NaCl) for 2 h at room temperature with agitation. For the detection of GLAST1, and GLT-1, and EAAT4, blots were probed with 320, 68, and 540 ng IgG/mL, respectively, in blocking solution (1% non-fat dry milk in 10 mM Tris-Cl, pH 7.5, 200 mM NaCl) for 1.5 h at room temperature with agitation. For all antibodies, horseradish peroxidase-conjugated Donkey antirabbit Ig (1:5000) was used to detect immunoreactive bands by visualization with a chemiluminescence kit.

**Figure 1.** Detection of GLAST1, GLT-1, and EAAC1 transporter protein in rat brain (R), sheep cerebellum (SC), and sheep hippocampal (SH) tissue homogenates, using anti-rat GLAST1, GLT-1, or EAAC1 polyclonal antibodies. Immunoblotting was performed as described in procedures.



## Results and Discussion

### *Recognition of sheep brain GLT-1, GLAST1, and EAAC1 by anti-rat GLT-1, GLAST1, and EAAC1 antibodies*

In mammals, expression of System  $X_{AG}^-$  activity is typically greatest in brain tissue. In glial cells, EAAC1 and GLAST1 function to quickly absorb the neuronal-secreted glutamate from the synaptic clefts, thus maintaining strict temporal control of excitatory amino acid neurotransmission. In contrast, EAAT4 and GLT-1 are localized to the plasma membranes of neurons, thus providing glutamate for metabolic needs. To test the specificity of potential sheep glutamate transporters by anti-rat antibodies and to evaluate the potential expression of glutamate transporters in sheep brain tissue, immunoblot analysis of sheep brain vs rat brain tissue was performed (Figure 1). Immunoreactive products were observed for all four transporters in both rat and sheep tissues. The apparent migration weights of the sheep and rat products were essentially the same. The specificity of the immunoreactions was demonstrated when pre-absorption of antibodies with their antigen polypeptides (5 mM) before immunoblotting resulted in the loss of immunoreactive bands (data not shown). A combination of reverse transcription-polymerase chain reaction and Northern analyses initially identified the expression of mRNA for GLAST1, GLT-1, EAAC1, and EAAT4 in sheep brain and indicated that the homology between sheep and rat glutamate transporter protein is very high (data not shown). The similarity of expression and size of the immunoreactive products presented in Figure 1 support this conclusion.

### *Distribution of glutamate transporters in sheep and cattle tissue*

To determine whether glutamate transport proteins are expressed by sheep tissues, immunoblot analysis was performed of proteins isolated from gastrointestinal tract and several peripheral tissues of sheep (Figure 2) and steers (Figure 3). For GLAST1, a single immunoreactive species (~140 kDa) was expressed by only the pancreas in both sheep and steers. In contrast, GLT-1 and EAAC1 was expressed by all tissues evaluated in sheep and steers. For GLT-1, three predominant species were detected (~203, 188, and 142 kDa) in most sheep and steer tissues. For EAAC1, two predominate immunoreactive species were detected (~93 and 62 kDa) in all tissues of both sheep and steers.

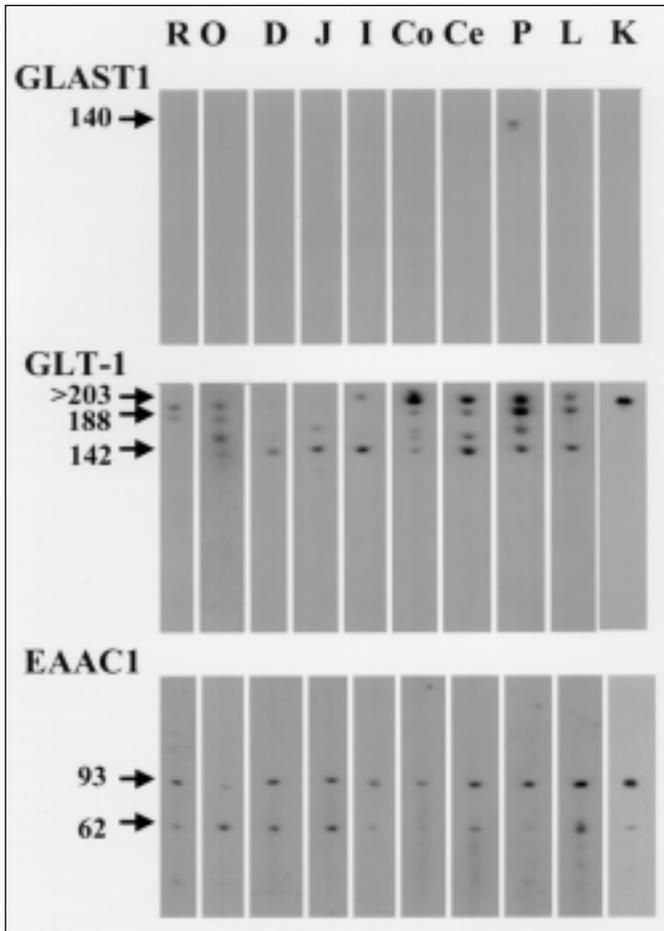
Among transporters, the GLT-1>GLAST1>EAAC1 pattern of apparent migration weights were as observed for the rat. The pattern of multiple immunoreactive GLT-1 and EAAC1 bands has been previously reported in brain tissue homogenates, in cortical synaptosomal, NBL-1 cellular, L cells, and placental syncytiotrophoblast membranes of non-ruminants. The detection of a single immunoreactive species for GLAST1 also has

been observed in rat and mouse placental tissues. The detection of immunoreactive species that are larger than their predicted monomeric form is a common phenomenon among GLAST1, GLT-1, and EAAC1. One theory is that the larger bands represent either functional homodimers and homotrimers that resist separation even under extreme redox conditions or oligomer species that are induced by oxidation of monomers during *in vitro* manipulations. Alternatively, the larger immunoreactive bands could represent a complex with an unknown protein.

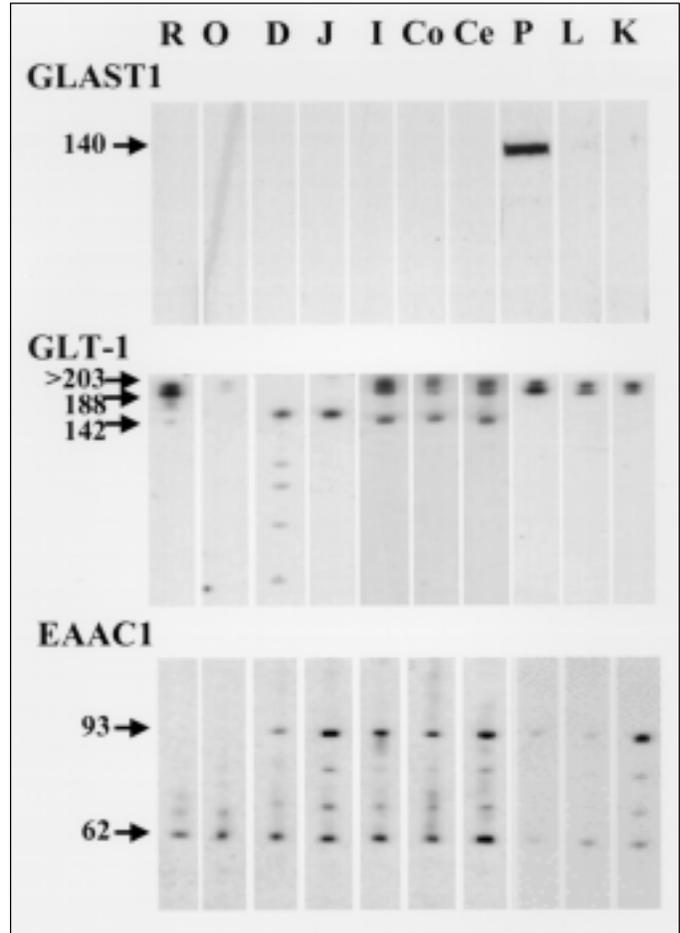
Although the expression of System  $X_{AG}^-$  activity has not been previously characterized in ruminants, the data collected in this study indicate that all tested gastrointestinal tract epithelia (rumen, omasum, duodenum, jejunum, ileum, colon, and cecum), liver, and kidney of both sheep and cattle are capable of  $Na^+$ -dependent high affinity glutamate transport through the functioning of GLT-1 or EAAC1. Also, in the pancreas, System  $X_{AG}^-$  activity is likely mediated by GLT-1, GLAST1, and EAAC1. This study, however, does not indicate the site of transporter localization. Thus, whether the capacity for glutamate absorption by System  $X_{AG}^-$  represents the ability to absorb anionic amino acids from the digesta or blood can not be determined from this study. In the enterocytes of non-ruminants, however, System  $X_{AG}^-$  activity is limited to the apical membrane (digesta-facing). Similarly, in sheep and steer liver, whether the expression of GLT-1 and EAAC1 primarily represents pericentral hepatic System  $X_{AG}^-$  activity as it does in rats remains to be determined. Likewise, in the kidney tissue of sheep and cattle, where glutamate resorption may be especially important to support gluconeogenesis in nutritionally-stressed ruminants, the site and relative contribution to System  $X_{AG}^-$  activity by GLT-1 and EAAC1 is unknown.

An important observation of this research was that all of the sheep and steer tissues expressed more than one glutamate transporter isoform. The physiologic significance of this observation is not clear, given that the substrate recognition and affinity of the isoforms are apparently the same. The significance may relate to the sensitivity of the transporters expression to different stimuli, such that the capacity for glutamate transport is never compromised, regardless of the physiologic state. Alternatively, it may be that the different isoforms are involved in supporting separate metabolic processes, as has been described above for the functional role of GLT-1, GLAST1, EAAC1, and EAAT4 isoforms in brain tissue, and as has been hypothesized for the dual expression of GLT-1 and EAAC1 in rat kidney absorptive cells. In this regard, that GLAST1 expression was limited to the pancreas suggests that GLAST1 supports a unique pancreatic function.

**Figure 2.** Immunoblot analysis of sheep cellular membranes isolated from rumen (R), omasum (O), duodenum (D), ileum (I), colon (Co), cecum (Ce), pancreas (P), liver (L), or kidney (K) tissue for GLAST1, GLT-1, and EAAC1 transport proteins. The blots are representative of the tissue distribution in four animals.



**Figure 3.** Immunoblot analysis of steer cellular membranes GLAST1, GLT-1, and EAAC1 transport proteins. Details are as described in Figure 2. The blots are representative of the tissue distribution in three animals.



## GPS Tracking of Cattle on Pasture

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### Summary

The effects of increasing the Global Positioning System (GPS) fix interval when tracking cattle on pasture to estimate pasture utilization was investigated in two experiments, November 1997 and May 1998. During November, seven collars were set to take readings every five minutes for four days, but four collars provided data. In May, seven collars were set to take readings every five minutes for seven days, during which three collars provided data. Determination of the time spent in each of the segments could be made by summing the location fix times in each segment of the pasture. Results obtained using GPS fix intervals of 10, 15, 20, or 30 minutes instead of five minutes were

simulated by considering only every second, third, fourth, or sixth record, respectively, in the analysis, and this provided a measure of the expected error when compared to the data taken at five-minute intervals.

When all the cow records were included in the analysis, the error was approximately 2/3 of that found for individual cows. Discrete events, such as watering or feeding, may have significant numbers of events that are of lesser duration than the fix interval. These events are at risk of being overlooked if long fix intervals are used. These results will be used to better understand and modify cattle grazing behavior with respect to pasture utilization and activity near streams and ponds.

**Introduction**

The development of the Global Positioning System (GPS) by the U.S. Department of Defense, starting in 1978, has resulted in widespread availability of a reliable, effective, and accurate positioning capability. Originally designed exclusively for military use, it was not until the mid-80's that civilian applications were developed. Since then, the unique power of a system that allows fast and accurate global positioning has been increasingly utilized in civilian applications, until the number of these users far outweighs the number of military users.

One of the fastest growing areas for application of GPS is "precision agriculture." Farmers and researchers recognize that variability exists among the many factors affecting the harvest of crops from a field, and they attempt to optimize inputs to the field to maximize the profit from the harvest. Thus, inputs such as fertilizer and seed, which were uniformly applied over a field, are now differentially applied within the field to those areas which will maximize profits. The GPS technology allows accurate and real-time positioning of a vehicle or implement in a field. Coupled with a Geographic Information System (GIS), decisions can be made and actions taken to manage crop production in the field. The GIS is a computerized database which has stored geographic attributes.

Interest has grown in tracking and monitoring movement of domestic cattle in an attempt to better understand grazing response to the environment. Improved understanding of cattle grazing habits could lead to better performance of cows and pastures. This report investigates the use of GPS collars and GIS to track intensively managed beef animals. Some of the issues facing this new application are investigated and explained, particularly the question of the time interval at which a GPS "fix" needs to be taken in order to maintain an accurate track across a pasture.

**Procedures**

The GPS collars used in this study were GPS\_2000 small animal location system units (Lotek Engineering Inc., Newmarket, Canada). The collar uses an eight-channel receiver, allowing a signal lock on eight satellites simultaneously. Location information (latitude and longitude) is stored cumulatively in random access memory sufficient for 4400 differential locations. In addition to location, each record is stored with a height estimate, date, time of fix, dilution of precision value, fix status (two-dimensional 2D or three-dimensional 3D), the ID numbers of satellites used in getting the fix, ambient air temperature, vertical activity sensor count, and horizontal activity sensor count. Readings can be taken at intervals of 5, 10, 15, 20, or 30 minutes or 1, 2, 3, 4 or 6 hours. The power supply is from a battery pack made up of high-density lithium cells. The pack is non-rechargeable and supports data collection for up to 10 days at five-minute fix intervals. The collar units are compact and sturdy, and weigh less than 2.2 lb. Figure 1 illustrates one of the GPS collars on a cow in the field.

Software has been developed to communicate with the collar, send specific fix schedules, and download data. The collars store the data, necessitating the retrieval of the collars before

data can be downloaded. Proprietary differential correction software (N3WIN, Lotek Inc.) was used to correct the data.

The pasture used in the experiments is located on the University of Kentucky's Animal Research Center (Woodford County) and is located at approximately 38E 06' 02" N and 84E 44' 50" W. The pasture is rectangular in shape, measuring 900 by 726 ft (Figure 2). The total area is 15.0 acres. The center of the pasture along the long axis is divided by an electrified fence, allowing for division of the paddock if rotational grazing were to be used. An opening of 16 ft in the center of the fence accommodates a livestock waterer and allows for free movement of cattle between halves if desired. A pair of utility poles are located in the lower left corner of the pasture. Forage is composed mainly of endophyte-infected tall fescue, but clover, bluegrass, and alfalfa also are present. All other species were considered weeds.

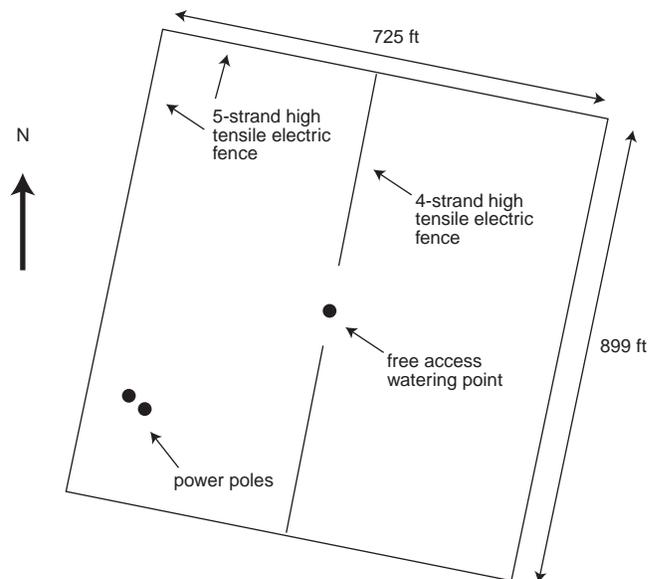
The cattle used in the study were mature Angus and Angus-cross cows averaging 1350 lb. The first data set was taken in November 1997 with eight cows on pasture. Seven cows were collared, and the collars were programmed to take readings every five minutes for four days. The second data set was taken in May 1998 after calving, with the eight cow/calf pairs cograzing with 16 (650-lb) steers. Seven cows were collared, with the GPS fix interval set to take readings every five minutes for seven days.

Data were downloaded from the collars in the proprietary format. Base station data were used from a Trimble GPS base station for differential correction approximately 15 miles from

**Figure 1.** A GPS collar used on a cow in the field.



**Figure 2.** Diagram of pasture layout.



the pasture site. Files from the Trimble base station were downloaded and converted to RINEX format. The RINEX files were used to differentially correct the collar files in N3WIN. The corrected data files were then converted to the UTM coordinate system (zone 16, NAD83) and loaded into ArcView GIS, where the tracks for each collar could be displayed graphically.

A technique was developed for quantifying the extent to which the animals were utilizing the different segments of the pasture. The results of the analysis using five-minute GPS fix intervals were compared to results using intervals of a greater value, such as 10 or 15 minutes. The pasture was divided into a 16-cell grid, four cells by four cells. The GIS database was then asked to determine how many fixes were to be found in each cell of the pasture for each collar. This provided a percentage of time that the animal spent in that segment alone out of the total number of fixes over the study length in the pasture (n=1935) and provides a useful measure for comparison among time segments. Multiplying the number of events in each cell by five minutes (the length of time until the next GPS location fix) provided a measure of the actual amount of time spent by each animal in different sections of the pasture.

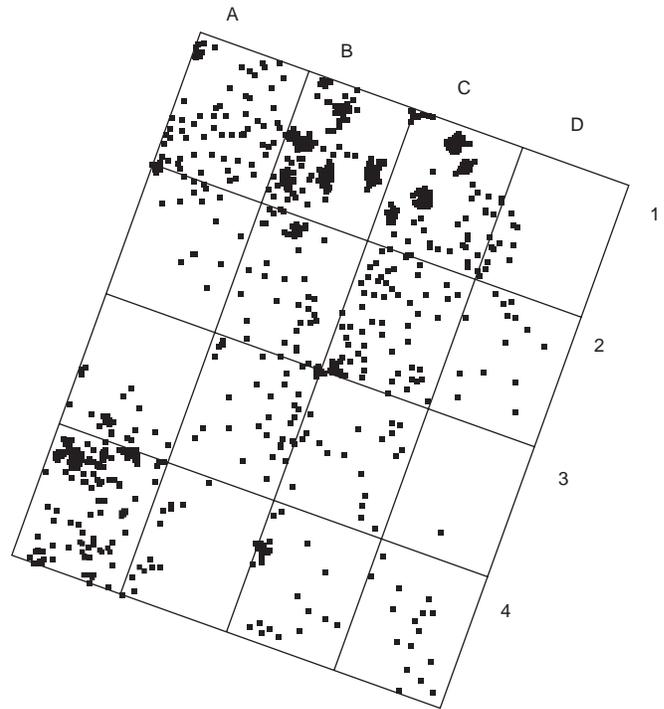
To assess the error introduced in the estimate of pasture use by using greater intervals of time between GPS fixes, we performed the same analysis as above, except that to simulate a 10-minute sampling interval, every second point was considered for analysis. Similarly, to simulate intervals of 15, 20 and 30 minutes, every third, fourth and sixth GPS position fix for the collars were considered, respectively. It became apparent that in segments where there were very few GPS fixes (consecutive GPS fixes < 6), large percentage differences were found when comparing the 30-minute sampling interval to the five-minute interval, as the 1/6 sampling strategy for the 30-minute interval could overlook a high percentage of GPS fixes. Therefore, to meaningfully measure the “error” involved in the different time strategies, it was necessary to weight this apparent percentage error with the amount of time that the animal spent in that segment. Thus the utilization errors in segments where the animals spent little or no time were given a lower weighting than those segments where the animals spent a large portion of their time.

**Results and Discussion**

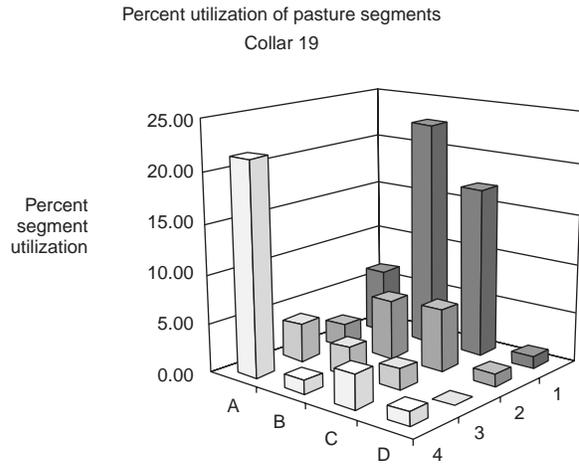
The GPS collar performance was negatively affected by high ambient air temperatures for the duration of the study (up to 100.4° F), resulting in some of the collars not recording data. Results are based on four of seven collars providing data for the first experiment and three of seven for the second experiment. The negative effects of high temperature upon the collars has since been corrected.

Figure 3 illustrates the locations of GPS fixes for a representative cow during the November experiment during the four-day test period. These data were analyzed as described previously to assess pasture utilization. Table 1 shows the percentage utilization of different pasture segments for each cow for the November data set. Figure 4 displays results from Collar 19 in graphical format to give an example of the relative magnitude of segment occupation times. Values range from 0.09% to

**Figure 3.** Plot of fix locations observed for Collar 19 during the four-day November experiment.



**Figure 4.** Utilization of pasture segments by Collar 19—November 1997.



24.73% of the total time spent in identically sized segments. There are significant preferences displayed by the cows for certain areas of the pasture. The D column segments were used similarly by all the cows, in which none of the four cows spent more than 1.42% of their time. Similarly, all cows displayed preferences for the segments B1, C1, and A4. It is interesting to observe the individuality displayed by the cows in some cases, where segments are preferred by some and not by others (cells B2 and B4 for example).

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Table 2 shows the percentage utilization of different pasture segments for each animal in the May data set. The cows again showed preferences for similar cells. However, these cells are different than the cells preferred in the November data set. In May, it appeared that the cows preferred the cells around the watering area (B2, B3, C2, and C3) and those in the bottom left of the paddock (A3, A4, and B4—highest elevation and air flow from wind). Similar to the November data set, the cells in the D column were underutilized, while in sharp contrast to the November experiment, the cells B1 and C1 (south facing and wind protected) were underutilized.

Various theories can be formulated to explain the observed trends, backed up by visual observation during the study periods. The preference for cells B1 and C1 in the November data set can be partially explained by the bedding behavior of the cows in these areas. Every evening, the cows walked to these cells to bed down for the night, the bedded periods lasting up to 10 hours. Therefore, the cows were recorded as spending a large proportion of their time in these cells and not necessarily productively grazing these portions of the pasture. There is no apparent reason why the D cells are so underutilized.

Unseasonably hot weather occurred during the May data set, with the average temperature during the day being around 86° F. This influenced the animals' behavior significantly, as grazing was restricted to the cooler nighttime hours. Daytime hours were concentrated around the watering area in the center of the paddock, increasing the amount of time recorded in segments B2, B3, C2, and C3. During this time the animals were not intensively grazing, but standing or laying in groups. The utility poles, located in segment A3, were an attraction for the herd. During the heat of the day, cows would often stand around the utility poles to benefit from the small amount of shade the poles provided. There is no obvious reason why the D cells continued to be underutilized or why the cells B1 and C1 were underutilized when they were heavily favored in the November data set.

Pasture utilization can be estimated by the amount of time spent in certain segments of the pasture, the GPS fix per pasture segment multiplied by five minutes per fix interval. From this five-minute basis, pasture utilization error can be estimated as GPS fix interval is increased. Table 3 shows the average, proportionally weighted percentage errors associated with increasing the fix interval from five minutes to 10, 15, 20, and 30 minutes. The errors are not large, with a fix time of 30 minutes producing a percentage error of around 6.5% in segment usage time when compared with a five-minute fix interval. By regressing the GPS fix interval against proportional percent error, a quadratic equation resulted that adequately describes the data ( $R^2 = 0.85$ ):  $y = -0.0035 x^2 + 0.3219 x$ . The assumption is made that there is an intercept of zero. As the fix interval approaches zero, the exact movements of the animals are monitored and the error in our estimates of pasture utilization also approach zero. Using the equation presented above, the pasture utilization error introduced when using the smallest possible collar fix interval of five minutes is estimated at 1.5%.

The above error estimates are derived from averaging the errors observed in each of the collar records, and are errors that can be expected when estimating individual animal use of the pasture. To estimate total pasture use by a herd, the same analysis is performed on the sum of the collar records for each segment. Somewhat lower errors can be expected as the individual errors from each collar tend to cancel each other out. Table 4 shows smaller errors, ranging from about 1.5% for the 10-minute sampling interval to about 4% for the 30-minute sampling interval.

The errors shown are relatively small, and accepting the 6.5% error associated with sampling cow positions every 30 minutes in return for a longer study period and less redundant data may be reasonable, especially in more extensive grazing applications. However, the above analysis only considers continuous activity such as grazing; it does not consider discrete events such as watering. To characterize the possibility of missing discrete

Table 1. Percentage of time spent by each animal in each segment of pasture—November 1997.

Collar Number	Segment Number															
	A1	B1	C1	D1	A2	B2	C2	D2	A3	B3	C3	D3	A4	B4	C4	D4
19	6.5	22.8	17.0	1.2	2.2	6.0	6.4	1.2	3.9	2.8	2.1	0.1	21.5	1.3	3.5	1.4
17	4.9	24.7	15.4	0.5	9.8	2.7	2.6	1.2	8.7	2.0	3.1	0.4	18.3	1.9	3.5	0.4
9	3.8	19.0	13.2	0.4	3.4	17.2	3.6	0.2	14.8	4.6	2.4	0.4	7.6	3.6	5.1	0.7
4	4.7	15.1	23.6	1.3	3.0	3.9	4.3	0.5	6.9	3.6	1.4	0.6	13.8	10	6.7	0.3

Table 2. Percentage of time spent by each animal in each segment of pasture—May 1998.

Collar Number	Segment Number															
	A1	B1	C1	D1	A2	B2	C2	D2	A3	B3	C3	D3	A4	B4	C4	D4
19	4.9	0.7	2.0	0.5	3.6	10.3	12.7	5.3	9.6	3.2	17.2	4.4	11.8	7.7	5.3	1
11	3.2	0.3	1.1	0.0	2.3	11.8	5.7	0.6	14.5	10.0	21.3	1.6	13.0	10.5	2.6	1.4
18	2.5	0.0	8.3	4.2	3.3	11.5	8.3	4.2	10.1	7.6	11.8	3.8	12.1	8.7	12.8	2.2

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events, the number of possible drinking events was investigated for each cow over the two study periods. Table 5 shows the number of times the animal was within the vicinity of the water for the November and May data sets. The “vicinity” was defined as a 32-foot radius for the November data set and was increased to a 48-foot radius for the May data set because there were so many more occurrences. During the very hot period in May, the cows spent more occasions at the waterer, and there were periods of up to seven hours within the vicinity of the waterer. However, short periods of watering activity were observed in the cooler ambient air temperatures during the November data set. With a significant number of events occurring for short periods of time, the researcher must recognize that these events may be missed if the GPS sampling interval is increased.

**Table 3.** Percentage of error for single animal/pasture segment use, comparing increasing GPS fix intervals.

	GPS Fix Interval (min)				
	5 <sup>a</sup>	10	15	20	30
November 1997	0	2.93	4.48	5.73	7.30
May 1998	0	2.37	3.43	5.03	5.56
Average	0	2.65	3.96	5.38	6.43

<sup>a</sup>5-minute intervals basis for error determination.

**Table 4.** Percentage of error for multiple animal/pasture segment use, comparing increasing GPS fix intervals.

	GPS Fix Interval (min)				
	5 <sup>a</sup>	10	15	20	30
November 1997	0	1.4	2.3	2.8	3.8
May 1998	0	1.7	1.7	2.5	4.3
Average	0	1.55	2.00	2.65	4.05

<sup>a</sup>5-minute intervals basis for error determination.

**Table 5.** Number of discrete events of different duration around watering point.

	Length of Time around Watering Point (min)					
	0-5	5-10	10-15	15-20	20-30	>30
<b>November 1997<sup>a</sup></b>						
Collar 9	3	0	3	0	1	0
Collar 4	7	2	1	0	1	0
Collar 19	6	4	1	1	0	0
Collar 17	1	0	2	0	2	1
<b>May 1998<sup>b</sup></b>						
Collar 19	4	2	3	2	1	13
Collar 11	7	2	2	1	6	11
Collar 18	5	1	1	0	3	11

<sup>a</sup>Four-day period.

<sup>b</sup>Seven-day period.