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To the Graduate Council:

I am submitting herewith a thesis written by Joseph John Mascola entitled "The Use of Esophageal-Fistulated Steers to Determine Fluorine Intake of Cattle Grazing Fluorine-Contaminated Forage." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

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THE USE OF ESOPHAGEAL-FISTULATED STEERS TO DETERMINE FLUORINE
INTAKE OF CATTLE GRAZING FLUORINE-CONTAMINATED FORAGE

A Thesis
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Joseph John Mascola
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ABSTRACT

During the grazing seasons of three consecutive years, esophageal-fistulated steers were used to determine fluorine intake of cattle grazing fluorine-contaminated pastures. Forage samples obtained by the fistulated steers were adjusted for losses of fluorine due to saliva leaching. The adjusted fluorine content of this forage (selected forage) was compared with that of samples of available forage. The effects of season, soil fluorine level, plant height, steer and year on the fluorine content of selected forage were studied.

Significant differences between the available forage and the selected forage was observed. These differences indicated that esophageal-fistulated animals more accurately determined the total fluorine intake of grazing animals than did samples of the available forage.

Variations due to steer and year were not found to significantly ($P > .05$) affect fluorine level of selected forage. Season exerted a very highly significant effect ($P < .001$) on the fluorine content of selected forage. Forage grown in the winter and spring seasons had considerably higher fluorine content than forages grown in the summer and autumn. Soil fluorine level had a very highly significant effect ($P < .001$) on the fluorine content of selected forage. Fluorine content of forage increased approximately 3 ppm for each 100 ppm increase in soil fluorine. Plant height exerted a highly significant ($P < .01$) effect on the fluorine content of the forage. Within the range of plant heights studied (0.75 to 6.0 inches), fluorine content of the forage decreased about 19 ppm for each one-inch increase in plant height.

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CHAPTER I

INTRODUCTION

Over a period of many years, research has shown that consumption of excessive amounts of fluorine for a prolonged period of time causes abnormal and usually harmful changes in body chemistry of domestic animals. Fluorine appears naturally in high phosphate soils and unnaturally as a result of contamination of forage crops and water from industrial sources. It is important, then, that we have a reliable method for measuring the amount of fluorine actually ingested by animals grazing pastures where fluorine contamination is suspected.

The traditional method for the determination of ingested fluoride levels has been to randomly clip samples of the available forage throughout the pasture. These samples are pooled and well mixed, then chemical analysis for fluorine content is performed.

There is reason to believe, however, that the available forage does not give a true estimate of the amount of fluorine actually ingested by grazing animals. Selectivity exhibited by grazing animals resulted in differences between the composition of available forage and the composition of the diet actually consumed by the animal.

When a sample is hand-clipped care is taken to get a representative sample of the forage, however, grazing animals are known to consume many things besides the forage they are grazing. Among these abnormal constituents in their diet, soil is perhaps the most important. It is a well established fact that most of the fluorine ingested with forages is

not bound within the plant, but instead occurs in the form of soil which is deposited on the plant in some manner. For this reason, more fluorine is ingested from eating fluorine contaminated soil than from eating the forages grown on this soil.

The esophageal-fistula was developed as a means of more accurately determining the diet of grazing animals. This method more accurately measures the quality of selected forages than hand clipping of samples. While quite satisfactory in many respects, it also has its shortcomings. The sample is subject to chemical changes due to salivary contamination and leaching as well as changes caused by sample preparation.

This experiment was designed to determine if significant differences are obtained between the fluorine content of available forage and that of the forage selected by grazing animals. Esophageal-fistula samples were adjusted to compensate for losses of fluorine that occur due to the leaching effect of saliva flowing through the forage in the collection bag. A difference between the two methods, after fistula effects were accounted for, would indicate that selectivity and the ingestion of materials other than forage resulted in consumption of different amounts of fluorine than was indicated by the fluorine content of the available forage. Such an occurrence would demonstrate the superiority of the using esophageal-fistulated animals to measure fluorine intake of grazing animals. The effects of soil fluorine level, plant height, season, year and steer on the fluorine content of available and selected forage was also studied.

CHAPTER II

REVIEW OF LITERATURE

I. FLUORINE

Fluorine as a Required Nutrient

Fluorine has long been known to play an important role in the prevention of dental caries in humans and certain laboratory animals (McKay, 1942; Hodge and Sognnaes, 1946). McClure and Zipkin (1958) recommend fluoridation of water (at a rate of 1 ppm) as a means of partial prevention of dental caries. The mechanism by which fluorine prevents dental caries has not definitely been established. However, Bibby and Brudevold (1954) indicated in their review, that research substantiated the theory that fluoride incorporated in the structure of the tooth results in reduced solubility of the enamel and allows it to resist the action of acids responsible for tooth decay.

There has been much controversy over the essentiality of fluorine as a nutrient. An essential nutrient has been considered as one necessary for the function of at least one biological process involved in normal development or maintenance of health. Many nutritionists believe that the role played by fluorine in the prevention of dental caries makes it an essential nutrient (Maurer and Day, 1957).

Fluorine, however, has not definitely been shown to be essential for normal growth or health in animals. Maurer and Day (1957) prepared a diet which was nutritionally adequate for the feeding of experimental

rats through three generations and into the fourth, without supplying more than 0.007 ppm of utilizable fluorine. They found no differences in tooth soundness between rats fed this diet and rats receiving the same diet but with 2 ppm fluorine in the drinking water. Wuthier and Phillips (1959) fed caries-susceptible rats on diets varying from 0 to 7.2 ppm fluorine. They found fluorine fed at these levels did not inhibit the occurrence of dental caries but did not cause fluorosis.

The levels of fluorine required by livestock, if indeed it is required, have not accurately been determined. The National Research Council does not list fluorine requirements for horses, sheep, swine, beef cattle or dairy cattle, but instead discusses only the toxicosis caused by excess dietary fluorine (NRC, 1966; NRC, 1968a; NRC, 1968b; NRC, 1970; NRC, 1971). In discussing beef cattle, they state that specific evidence is lacking to show an essential function of fluorine in growth, health or skeletal development. In the case of swine, fluorine is not mentioned. Maynard and Loosli (1962) sum up the essentiality of fluorine by stating that "fluorine may have some beneficial action in the body other than lessening of dental caries, but from the standpoint of animal feeding, its harmful effect is the one of practical importance."

Ingestion of Excess Fluorine

It has long been known that ingestion of excessive fluorine produces toxic effects in livestock (Hobbs et al., 1954). It is important therefore to understand the factors responsible for the occurrence of fluorine in feedstuffs. High phosphate soils and industrial emissions are the two principle sources of fluorine contamination.

High phosphate soils are high in fluorine. Hobbs et al. (1954) demonstrated the fluorine-bearing capacity of such soils and their relationship to the occurrence of fluorosis in cattle in Middle Tennessee. MacIntire, Hardin and Buehler (1958) found the fluorine content of the soils in this area to be as high as 4815 ppm. Maynard and Loosli (1962) stated that raw rock phosphate contained about four percent fluorine, unless it had been defluorinated.

Industrial emissions have been a prime source of fluorine pollution of soils, vegetation and water. MacIntire, Hardin and Buehler (1958) indicated that gaseous or solid fluoride materials were emitted into the air from phosphate mining or processing operations. Merriman, Moorman and Hobbs (1956) found that the fluorine content of the vegetation around an aluminum smelting plant varied with the prevailing wind direction and distance from the smelting plant. In England, Burns and Allcroft (1964) found fluorosis in cattle to be largely the result of certain industrial emissions while Shupe et al. (1962) in the United States reported emissions from a steel plant to be as toxic to cattle as sodium fluoride.

Air-borne fluorine as such was probably not harmful to livestock, and MacIntire, Hardin and Buehler (1958) found such fluorides to be of little harm to vegetation. The real threat was the contamination of pastures by fluorides which had settled on the plants from the air. For this reason, Suttie (1969a) considered the problem of air-borne fluorides to be so serious that he proposed air quality standards which would limit the amount of fluorides an industry would be allowed to discharge into the air.

MacIntire, Hardin and Buehler (1958) found rainwater to contain appreciable amounts of fluorine which it acquired from the atmosphere. However, clear waters from ponds, streams and springs were found to be largely free from harmful levels of fluorine. They observed that fluorine in water was the result of fluorine-contaminated soil particles, which were suspended in the water.

Ingested soils and vegetation contaminated by high levels of fluorine are the most serious causes of fluorosis. Plants incorporate fluorine from the soil into their cell structures; however, MacIntire, Hardin and Buehler (1958) have shown that this uptake was insignificant unless the soil was deficient in calcium. Soil particles which were contaminated by fluorine, were deposited on the vegetation by means of wind or raindrop splash and this was the principle source of fluorine ingested by grazing livestock (Hobbs et al., 1954; Merriman, Moorman and Hobbs, 1956; MacIntire, Hardin and Buehler, 1958; Hobbs and Merriman, 1962; Hobbs et al., 1962; and Merriman and Hobbs, 1962).

Suttie (1969b) found that dairy feeds contained an average of 21 ppm fluorine, ranging from 3 to 296 ppm. Alfalfa hays, which were thought to be free from industrial pollution, ranged from 0.8 to 36.5 ppm fluorine with a mean of 3.6 ppm. Compton, Remmert and Mellenthin (1965) found that plant samples taken in July averaged 31 ppm fluorine while samples taken in the same area in October averaged 92 ppm fluorine. Merriman, Moorman and Hobbs (1956), Merriman and Hobbs (1962) and Hobbs and Merriman (1962) found that fluorine contents of pastures were higher in winter than in summer. They attributed this seasonal difference to

the accumulation of fluorine from raindrop splash when the forage grew slowly or was dormant.

Grazing livestock not only ingest soil that has accumulated on the leaves of forage, but they also ingest soil directly. This becomes a serious problem when pastures are short. They may ingest high amounts of soil while attempting to ingest the forage. If this soil contains fluorine in sufficient amounts, it will contribute to the fluorosis problem. Healy, McCabe and Wilson (1970) demonstrated mineral absorption from ingested soil by sheep drenched with soil containing radioisotopes of cobalt, manganese, selenium and zinc. Healy and Wilson (1971) also observed mineral absorption by sheep from ingested soil. Healy and Ludwig (1965) found that soil was ingested in the greatest amounts during the cold, wet months, when pastures were short and grazing pressure was high. They found that sheep ingested as much as 12 kg of soil between July and October or about 1.5 kg/day at the peak of soil ingestion. Healy (1968) reported that dairy cattle eat as much as 200 to 350 kg of soil per year.

Fluorine Toxicosis

Excess fluorine intake produces fluorosis, a nutritional disease. Work has been done to determine at what levels of ingestion fluorine becomes toxic. Hobbs et al. (1954), Suttie, Miller and Phillips (1957a), Suttie, Miller and Phillips (1957b) and Suttie, Phillips and Miller (1958) have established 30 ppm fluorine in the feed on an as-fed basis to be a safe level, 40-50 ppm as a marginal zone in which some animals

were affected and others were not, and 50 ppm to be the toxic level at which fluorosis occurs. Hobbs et al. (1954), Merriman, Moorman and Hobbs (1956), Hobbs and Merriman (1962), Merriman and Hobbs (1962), Greenwood et al. (1964), Miller and Phillips (1966) and Shupe (1966) have studied factors governing the effect of fluorine on the structural and physiological processes in animals. These factors were the amount of fluorine ingested, duration of time of ingestion, type and solubility of fluoride ingested, age of the animal at the time of ingestion, level of nutrition of the animal, certain stress factors, and the individual biological response of the animal.

Ingestion of excess fluorine affects certain chemical reactions in the body. Chamberlain and Burroughs (1962) found that magnesium and manganese are somewhat interchangeable in the body, and fluorine will affect the function of either. They noted that fluorine interfered with enzymes in the Krebs cycle which are responsible for the conversion of citrate to isocitrate. Maplesden et al. (1960) reported fluorine to have no effect on the activity of liver alkaline phosphatase of rabbits or rats, while Greenwood et al. (1964) found that bone alkaline phosphatase activity increased with high fluorine ingestion by cattle. Sievert and Phillips (1959) found that mitochondrial fatty acid oxidase activity was strikingly decreased in the kidney of the fluorotic rat.

Ingestion of high levels of fluorine resulted in deposition of fluorine in the various tissues of the body. The blood levels of fluorine were found to increase with increased fluorine ingestion but when fluorine was removed from the diet, blood levels of fluorine

dropped rapidly (Carlsen et al., 1960; Carlsen, Armstrong and Singer, 1960; Bell, Merriman and Greenwood, 1961; and Suttie and Faltin, 1971). Hobbs et al. (1954), Suttie, Phillips and Miller (1958), Newell and Schmidt (1958), Suttie and Phillips (1959b), Hobbs and Merriman (1962), and Greenwood et al. (1964) demonstrated that there is little storage of fluorine in the blood, nor is the blood apparently harmed by the presence of fluorine. Suttie, Phillips and Miller (1958), Newell and Schmidt (1958) and Greenwood et al. (1964) observed that there was little or no storage of fluorine in the soft tissues, nor were these tissues adversely affected by the ingestion of excessive amounts of fluorine.

The skeletal system is the major storage site of fluorine in the body. Miller and Phillips (1956) and Suttie and Phillips (1959a) found that the rate and extent of skeletal fluorine retention was negatively correlated with animal age. The amount of fluorine retained by bone also varied with type. Newell and Schmidt (1958), Suttie, Phillips and Miller (1958), and Suttie (1967) observed that porous bone was uniformly higher in fluorine than compact bones. Within the bone itself, fluorine concentration was not evenly distributed. Suttie and Phillips (1962) found the fluoride concentration was higher at the ends of the shaft than at the center. It has been suggested (Suttie, 1967) that a bone biopsy should be performed to determine the extent of fluorosis. Hobbs et al. (1954), Suttie, Phillips and Miller (1958), Suttie, Gesterland and Phillips (1961), Hobbs and Merriman (1962), Merriman and Hobbs (1962) and Suttie (1964) reported that fluorine content of compact bone above 5500 ppm indicated the presence of fluorosis. They found that levels of

4500-5500 ppm were marginal where some animals developed fluorosis and others did not. Levels below 4500 ppm were considered harmless. Newell and Schmidt (1958) fed levels of fluorine as high as 2.5 milligrams per kilogram of body weight per day to dairy cattle for six years. They observed bone lesions consisting of roughening of the periosteal surfaces of the compact bones, and the presence of plaques of new bone in the adjacent periosteum of the metacarpal, metatarsal and rib bones.

Fluorine was found to increase in the urine as fluorine ingestion increased (Hobbs et al., 1954; Newell and Schmidt, 1958; Hobbs and Merriman, 1962; Merriman and Hobbs, 1962; Shupe et al., 1963; and Greenwood et al., 1964). However, as noted by Greenwood et al. (1964), it only varied with current intake, and was not a measure of the animals body status. Thus, as stated by Shupe et al. (1963), it could not effectively be used as a method of diagnosis for fluorosis.

Bell, Merriman and Greenwood (1961) observed changes in the fluorine level of saliva after dosing two heifers with about 80 mc each of F^{18} fluoride. They found that the salivary fluorine level reached its peak of 1.3×10^{-4} percent of dose per ml at five minutes after dosing.

There are compounds which tend to reduce the effect of excess fluorine. Suttie, Miller and Phillips (1957a) and Suttie, Miller and Phillips (1957b) found that the inclusion of calcium in the ration of cattle increased their tolerance to fluorine. In a similar manner, Greenwood et al. (1964) found that cows receiving aluminum sulfate deposited significantly less fluorine in their ribs.

Symptoms of Fluorosis

Fluorosis can usually be recognized by a number of symptoms. Burns and Allcroft (1964) stated that dental lesions are the most common clinical symptom of fluorosis. Dental fluorosis is not in itself a definite index of fluorine toxicosis (Suttie and Phillips, 1960) however, mottling, defective calcification and hypoplasia of the enamel are pathological conditions associated with ingestion of excess fluorine (Garlick, 1955). Dental lesions of the type described above were observed in experimental work by Neely and Harbaugh (1954), Newell and Schmidt (1958), Suttie and Phillips (1960), Greenwood et al. (1964), Suttie (1969a) and Suttie and Faltin (1971). Neely and Harbaugh (1954) noticed these lesions were more severe on the incisors being formed at the time the animal was consuming a diet high in fluorine. Suttie and Faltin (1971) observed that fluorosis occurred when crown formation was completed before exposure to fluoride and also when the fluoride exposure preceded any calcification of the crown.

Lameness and bone lesions are also symptoms of fluorosis. They do not usually appear until after a prolonged period of exposure to high fluorine diets. Burns and Allcroft (1964) attributed lameness to changes in the bone structure caused by excess fluorine. Feeding cattle at 49 and 93 ppm fluorine, Greenwood et al. (1964) observed intermittent lameness after 2-1/2 years in all cows on a 93 ppm diet, and after 4-1/2 years in some cows on the 49 ppm diet.

Suttie, Miller and Phillips (1957b) observed that after the early symptoms of increased fluorine ingestion no other symptoms appeared

except a steady rise in bone fluorine concentrations until after a considerable period of time elapsed. When further symptoms finally appeared, lameness was among them. Neely and Harbaugh (1954) studied cattle with a natural water supply, averaging four to five ppm fluorine for six years. They observed no sign of lameness or bone lesions at this level of fluorine ingestion.

Burns and Allcroft (1964) found a relation between severity of lameness and severity of dental lesions. This agrees with work by Greenwood et al. (1964), who found dental and bone lesions were correlated with amounts of fluorine ingested, amount of fluorine in the bone, age of the animal, duration of exposure and the body processes of the individual animal.

Neely and Harbaugh (1954), Suttie, Miller and Phillips (1957b) and Newell and Schmidt (1958) found no direct relationship between increased fluorine ingestion and diarrhea or elongated hooves in cattle. Newell and Schmidt (1958) did observe unthriftiness and roughened hair coats on cattle fed 2.0 to 2.5 milligrams of fluorine per kilogram of body weight per day.

Effect of Fluorosis on Productivity of Livestock

In general, fluorosis had little adverse effect on the productivity of livestock unless the fluorosis was extremely severe. Greenwood et al. (1964) observed no changes in feed efficiency in cattle receiving 93 ppm fluorine in their diet. It has been reported by Hobbs et al. (1954); Merriman and Hobbs (1962) and Hobbs and Merriman (1962) that feed consumption was not affected at low levels of fluorine ingestion (below

40 ppm in cattle and 100 ppm in sheep). They found that reduced feed consumption occurred more rapidly as the level of fluorine ingestion increased. Hobbs et al. (1954), Merriman and Hobbs (1962) and Hobbs and Merriman (1962) found that fluorine in neither soil nor water had a direct effect on weight gains, however, reduced feed consumption did result in reduced weight gains. Milk production was studied by Neely and Harbaugh (1954), Suttie, Miller and Phillips (1957a), Newell and Schmidt (1958), Suttie and Phillips (1959b) and Wuthier and Phillips (1959). They found that milk production was unaffected by ingested fluorine levels of up to 50 ppm in the feed. Greenwood et al. (1964) found a decrease in milk production of cattle fed 93 ppm fluorine in their feed after their second lactation. They attributed this reduction in milk yield to the osteofluorosis and intermittent lameness which usually resulted in reduced feed intake.

Reproductive efficiency and general health of offspring of animals ingesting excessive fluorine was studied by Hobbs et al. (1954), Neely and Harbaugh (1954), Suttie, Miller and Phillips (1957b), Newell and Schmidt (1958), Wuthier and Phillips (1959), Hobbs and Merriman (1962), Merriman and Hobbs (1962) and Greenwood et al. (1964). In all cases they found that reproductive efficiency and the health of the offspring in animals ingesting high levels of fluorine were not adversely affected. Placental transfer of fluorine was studied by Suttie, Miller and Phillips (1957b), Newell and Schmidt (1958) and Maplesden et al. (1960). Newell and Schmidt and Suttie et al. observed a measureable transfer of fluorine across the placenta of cattle, while Maplesden et al. found no appreciable transfer of fluorine across the placenta of rats or rabbits.

Thus far, there is no evidence to indicate that recovery from fluorosis is possible. Phillips, Suttie and Zebrowski (1963) experimented with cattle that had been on diets containing up to 2.0 mg F/kg of body weight for four years. These cattle were placed on a normal dairy ration for two years and their responses were observed. During the latter period, a portion of their skeletal fluoride was mobilized and excreted but the majority of their body fluoride remained. They observed that there was no apparent improvement in dental or bone lesions. Crampton and Harris (1969) stated that no defense had yet been found against the harmful effects of the ingestion of excess fluorine.

II. ESOPHAGEAL-FISTULATED ANIMALS

History of the Development of the Esophageal Fistula

In a review dealing with the development and use of the esophageal fistula, Van Dyne and Torell (1964) pointed out that the esophageal fistula was used by Magendie and Ryer in 1847, Claude Bernard in 1855 and Pavlov in 1889. They explained that it was only in the last decade that the technique has been used widely with ruminants. Prior to this, it was used almost exclusively in monogastric animals and more frequently for physiological and psychological experiments than for nutritional research. They stated that surgery of the esophagus is difficult, resulting in numerous losses of animals, and they explained why this difficulty exists: (1) the esophagus lacks a serosa, (2) in order to expose the esophagus in surgery it is necessary to open the mediastinal structures and fascial planes, (3) there is a very poor blood supply to

the esophagus, (4) the esophagus cannot be restricted from movement, and (5) there is an absence of the greater omentum.

Van Dyne and Torell (1964) recount a previously unpublished esophageal-fistulation procedure which overcame many of the problems encountered in earlier attempts. The following is a brief description of this procedure. The animal was adjusted to pelleted feed or green herbage for several weeks prior to surgery, but feed and water were withheld for 24 hours immediately prior to surgery. After the animal was anesthetized by means of a general anesthetic, it was placed in a right lateral recumbancy and the rear legs extended and tied. The forelegs were doubled back under the body and tied by means of a butterfly (or "tomfool") knot. The surgical area was disinfected and clipped. The head was slightly elevated and held by means of a halter rope. A steel rod with a hard rubber ball on the end was passed down the esophagus and manipulated to aid in making the incision and blunt dissection through the tissues. After the rod was passed, the supporting block was moved back under the neck so that the fluids drained from the nose and mouth. The fistula should be located as near as possible to the ventral midline of the neck, and about midway between the jaw and brisket. After removal of an oval-shaped piece of skin (the size of the fistula), the tissues and muscles were separated and the esophagus exposed. A short longitudinal incision was made in the esophagus and the sides of the incision are sutured. Sutures pass through the esophagus, submucosa, and inner layers of the skin. Incision and suturing was continued until the entire perimeter of the fistula was sutured. The cannula or plug

was then inserted and the area disinfected and treated with fly repellent. The animal was kept off coarse feed and water for 24 hours postoperative, or returned to grass. Antibiotics were given until the incision was healed. The sutures may be removed in seven to ten days. An experienced surgeon and two assistants can complete a fistula in about one hour.

Use of Esophageal-Fistulated Animals

In recent years, the use of esophageal-fistulated cattle and sheep is becoming widespread, primarily to study selective grazing. Selectivity is determined by comparing the chemical and botanical composition of forage samples that are available in the pasture with the chemical and botanical composition of the forage the animal actually eats. Van Dyne and Torell (1964), Bohman and Lesperance (1967), and Harris et al. (1967), in their reviews, stated that the major application of the esophageal fistula was to the problem of determining the diet selected by grazing livestock. Cook et al. (1958) and Weir and Torell (1959) used esophageal-fistulated sheep to determine selectivity. Lesperance et al. (1960), Lesperance, Bohman and Marble (1960), Bredon, Torell and Marshall (1967), Campbell et al. (1968), Adler (1969), Barth and Kazzal (1971) and Coleman et al. (1971) used esophageal-fistulated steers to study selectivity exhibited by cattle.

The basic theory behind the use of the esophageal fistula for determining selectivity was explained by Barth and Kazzal (1971) and Coleman et al. (1971). They explained that if sufficient forage was available, grazing animals preferred certain plant species to others.

They indicated that a forage sample clipped to give an estimate of the nutritive value of the total available forage could differ from the nutritive value of the forage selected by a grazing animal. Forage removed from an esophageal fistula is forage that is selected by the grazing animal and therefore should be representative of the diet actually eaten by grazing livestock.

Most researchers reported methods of handling and maintaining esophageal-fistulated animals that were similar in technique. The animals were usually maintained on pastures similar in composition to the forage being sampled (Barth and Kazzal, 1971; and Coleman et al., 1971). In some cases, the fistulated animals were maintained on the experimental pastures (Cook et al. 1958). To insure grazing during the sampling period, feed was withheld from 4-8 hours (Campbell et al., 1968) to periods lasting overnight (Bredon, Torell and Marshal, 1967; Barth et al., 1970; and Barth and Kazzal, 1971). Prior to sampling the closing devices were removed from the cannulas (Lesperance, Bohman and Marble, 1970; Bredon, Torell and Marshall, 1967; and Barth and Kazzal, 1971) and the animals were fitted with collection bags. Alder (1969) did not use a collection bag on the animals, but instead allowed the boluses of selected forage to drop to the pasture where they were immediately retrieved. The length of the sampling period was not the same in all cases. Torell (1954) indicated that the sampling periods should be short in order that the collected forage will remain free of regurgitated material. Barth and Kazzal (1971) and Campbell et al. (1968) used 20 minute sampling periods. Bredon, Torell and Marshall (1967), under

African conditions, used a sampling period of 45 minutes. Van Dyne and Torell (1964) in their review indicated a short sampling period to be most common, but under desert range conditions as much as two to four hours are needed to collect a sample. Torell (1954), Lesperance, Bohman and Marble (1960), and Barth et al. (1970) prevented the esophageal-fistulated animals from drinking during the sampling period. This practice prevented the massive leaching of nutrients caused by water running through the collected forage.

Adjustments of Esophageal-Fistula Data

Theoretically, the differences between clipped forage samples and those collected through an esophageal fistula are due to selectivity in grazing. However, Barth et al. (1970), Barth and Kazzal (1971) and Coleman et al. (1971) have shown that the percentages of some of the chemical constituents of forage obtained through an esophageal-fistula are altered due to fistula effects and sample preparation. Basically, crude fiber, ash, and N.F.E. percentages are artificially changed due to these factors.

Lesperance, Bohman and Marble (1960), Barth et al. (1970), and Barth and Kazzal (1971) reported the crude protein content of forage to be unaltered by fistula effects. It was reported (Lesperance, Bohman and Marble, 1960 and Barth and Kazzal, 1971) that crude fiber content was artificially increased by passage through an esophageal fistula. For this reason, crude protein values need not be adjusted, but crude fiber values of forage collected through an esophageal-fistula must be adjusted for these effects in order for a true estimate of the selected diet to be obtained.

Lesperance, Bohman and Marble (1960), Barth et al. (1970) and Barth and Kazzal (1971) reported an artificial decrease in the N.F.E. content of forage collected through an esophageal fistula. They explained that the reduction in N.F.E. content of this forage was due to the increase in crude fiber. As the percentage of one constituent increased, it was compensated by a decrease in another constituent.

Unrealistic values for certain nutrients selected by grazing animals have been reported because no adjustments were made to correct for fistula effects. For this reason, Coleman et al. (1971) used methods of adjusting A.D.F. and N.F.E. for fistula effects based on the findings of Barth et al. (1970) and Barth and Kazzal (1971).

Saliva appears to be a major factor involved in the alteration of forage samples when they pass through the esophageal fistula. Van Dyne and Torell (1964), Lesperance, Bohman and Marble (1960), Hoehne, Clanton and Streeter (1967), Guthrie, Rollins and Hawkins (1968), Barth et al. (1970) and Barth and Kazzal (1971) reported that the ash content of forage samples increased due to the effect of the esophageal fistula. Van Dyne and Torell (1964), in their review, and Guthrie, Rollins and Hawkins (1968), indicated saliva to be approximately 0.9 percent ash, on an 'as-secreted' basis. According to Lesperance, Bohman and Marble (1960) saliva was mostly ash, on a dry matter basis, and this accounted for the increased ash content of the forages passed through an esophageal fistula. However this increases in ash content caused by saliva contamination can be adjusted for by expressing the other chemical constituents on an 'organic-matter' basis.

While the increase in ash content appears to be the major effect of saliva on forage samples, it also has a leaching effect on the forage nutrients. Hoehne, Clanton and Streeter (1967) reported a decrease in the carbohydrate content of forages passed through an esophageal fistula. They attribute this decrease to the leaching effect of saliva and the possibility of chemical constituents in the saliva combining with the plant enzymes to digest some of the carbohydrate fraction of the forage.

Drying temperature plays a role in the chemical changes involved in the preparation of forage samples for analysis. Hoehne, Clanton and Streeter (1967) found higher chloride levels in forage samples dried at 50°C than in those dried at 100°C. They reported that Jones in 1962 found less reduction in carbohydrate content of forage samples dried at 80°C than those dried at 100°C. Barth et al. (1970) found an increase in acid-insoluble lignin in forage samples dried at 65°C as compared to samples dried at 45°C. This indicates that lower drying temperatures result in decreased alteration of the composition of forage samples collected through an esophageal fistula.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. PASTURES

The pastures of this study consisted of five lots, each approximately ten acres in size. They were established according to average fluorine measurements of the soil taken from 1955 to 1960. The pastures were maintained at either high (more than 3 inches) or low (less than 3 inches) plant height. Four experimental treatments based on fluorine measurement and plant height were established as follows:

Treatment 1 - Pasture 1 - low soil fluorine content (434 ppm), high plant height.

Treatment 2 - Pasture 2 - low soil fluorine content (594 ppm), low plant height.

Treatment 3 - Pasture 3 - high soil fluorine content (3744 ppm), high plant height.

Treatment 4 - Pastures 4 and 5 - high soil fluorine content (2118 ppm), low plant height.

Pasture composition was determined in the last year of the experiment. At this time, the major species comprising these pastures were lespedeza (*Lespedeza stipulacea*), 19%; blue grass (*Poa pratensis*), 18%; white clover (*Trifolium repens*), 14%; orchardgrass (*Dactylis glomerata*), 4%; crab grass (*Digitaria sanguinalis*), 23%; and miscellaneous species, 22%. Prior to seeding and throughout the experiment, the pastures were fertilized with P, K, and Ca based on soil analysis and agronomic recommendations.

The pastures employed were permanently grazed by a beef cow-calf herd involved in a lifetime study of the effects of soil-borne and water-borne fluorine on the general health, production, reproduction, and fluorine deposition in the tissues (Merriman and Hobbs, 1962).

II. FISTULATED STEERS

Four Hereford steers from the university herd were fitted with esophageal fistulas at approximately one year of age, using similar techniques and closing devices as those described by Van Dyne and Torell (1964). Post-operative care was provided as outlined by High (1966). At the start of the experiment, three of the steers were yearlings and one was 2 years old. Between forage sampling periods, the fistulas were closed and the steers were kept on pastures similar in species composition to those being sampled.

III. SAMPLING TECHNIQUE

Forage samples were collected over a 3-year period. Sampling of pastures 2 and 4 began during the first year and sampling of pastures 1, 3 and 5 during the second year of the experiment. A sample of the forage from each pasture was hand clipped once monthly, during times of sufficient forage for grazing. Forage was clipped at about every fifty paces in a predetermined pattern. These clipped samples were intended to represent the average botanical and chemical composition of the forage available in the pasture and will be referred to as "available forage."

Esophageal-fistulated steers were used to collect forage samples

representative of the chemical and botanical composition of the diet selected by grazing cattle on the same dates that available forage samples were obtained. These samples will be referred to as "fistula forage samples." Two to four distulated steers were used to sample each pasture. Prior to sampling, fistula plugs were removed and collection bags, made of lightweight canvas with a screen bottom to allow saliva drainage, were strapped to the animals' neck.

The steers were placed in the pastures to be sampled, and allowed to graze for about 45 minutes. Following the sample collection, bags and contents were removed, plugs were replaced, and the steers were returned to their regular pastures.

Several times during the final year of the experiment, saliva samples were obtained from the steers prior to sampling. These samples were analyzed for fluorine content, and provided an estimate of the amount of fluorine normally occurring in the saliva. Saliva which had drained through the forage sample in the collection bag was also sampled and analyzed for fluorine content. The results were used in determining the amount of fluorine removed from the 'fistula forage sample' by the saliva.

IV. ADJUSTMENT OF ESOPHAGEAL-FISTULA DATA

'Fistula forage samples' are not representative of the diet selected by the grazing steers, because the fluorine secreted in the saliva adds fluorine to the fistula forage sample, and the leaching action of saliva flowing through the forage in the collection bag removes

some of the fluorine from the sample. In order to calculate the amount of fluorine actually ingested with the forage, the concentration of fluorine in the 'fistula forage samples' was adjusted using the following steps:

- (1) $F \text{ in 'fistula sample' (mg) = weight of 'fistula sample' (kg) } \times F \text{ in 'fistula sample' (ppm)}$
- (2) $F \text{ in saliva that passed through 'fistula sample' (mg) = saliva secreted (l) } \times F \text{ in saliva that passed through 'fistula sample' (mg/l)}$
- (3) $F \text{ secreted in saliva (mg) = saliva secreted (l) } \times F \text{ secreted in saliva (mg/l)}$
- (4) $F \text{ removed by saliva (mg) = } F \text{ in saliva that passed through 'fistula sample' (mg) - } F \text{ secreted in saliva (mg)}$
- (5) $F \text{ in 'selected forage' (mg) = } F \text{ in 'fistula sample' (mg) + } F \text{ removed by saliva (mg)}$
- (6) $F \text{ in 'selected forage' (ppm) = } \frac{F \text{ in 'selected forage' (mg)}}{\text{Weight of 'fistula sample' (kg)}}$

Putnam, Lehman and Davis (1966) found cattle ingesting amounts of feed less than 0.8 percent of their body weight, secreted about 13.4 kg saliva for each kg of feed eaten. Guthrie, Rollins and Hawkins (1968) observed that cattle grazing coastal bermudagrass produced 11.3 kg saliva per kg of body weight. Based on these results, it was estimated that the esophageal-fistulated steers in this study secreted 12 liters of saliva for each kg of feed ingested. The average weight of the 'fistula forage samples' was approximately one kg.

An analysis of saliva samples, taken immediately before the steers began grazing, indicated that a mean fluorine content of 0.41 mg/l was present. After grazing, samples were taken of the saliva as it dripped

from the collection bags. These samples yielded an average fluorine content of 0.86 mg/l.

Using these data and the above mathematical relationships, the 'fistula forage samples' were adjusted to yield a new variable, which will be referred to as 'selected forage'.

V. ANALYSIS FOR FLUORINE CONTENT

The forage samples, both selected and available, were placed in containers and 6 g of calcium oxide was added. Calcium oxide combined with the fluorine and bound it in the form of CaF_2 , thus preventing the loss of fluorine from the sample. The sample was dried at 60°C and ground in a Wiley mill to pass through a 40-mesh screen. The method of Willard and Winter (1933) employing perchloric acid distillation and thorium nitrate titration was used to determine fluorine content. The fluorine concentration from this analysis was expressed on an 'air-dry' basis.

VI. STATISTICAL ANALYSIS

Student's paired "T" test (Remington and Schork, 1970) was used, pairing each 'fistula forage sample' and each sample of 'selected forage' with the corresponding sample of 'available forage'. Differences between sampling methods were determined by year and treatment in this manner.

Least squares analyses of variance according to the procedures outlined by Harvey (1960) were calculated to determine the effect of individual steer, year, season, soil fluorine level and plant height on

the fluorine content of 'available forage', 'fistula forage samples', and 'selected forage'.

CHAPTER IV

RESULTS AND DISCUSSION

I. COMPARISON OF THE SAMPLES

Fluorine Content of Available, Fistula and Selected Forage Samples

The raw means for the fluorine content of the available, fistula and selected forage samples are presented in Table 1. The available, fistula and selected forage samples in treatment 1 had mean fluorine contents of 25, 27 and 35 ppm, respectively. Similarly, the mean fluorine content of the forage samples in treatment 2 were 52, 59 and 67 ppm. Samples from these two low soil F treatments had considerably lower fluorine contents than those from treatment 3 which had mean fluorine contents of 91, 99 and 107 ppm or treatment 4 with mean fluorine contents of 138, 137 and 145 ppm for available, fistula and selected forage samples, respectively (the two high soil F treatments). These results agree with the work of Hobbs et al. (1954), Merriman, Moorman and Hobbs (1956), MacIntire, Hardin and Buehler (1958), Hobbs and Merriman (1962), Hobbs et al. (1962) and Merriman and Hobbs (1962) who found that fluorine content of forages was related to the fluorine content of the soil on which they were grown. The fluorine content of the available and fistula samples were similar, however, the selected forage were considerably higher in fluorine than the available forage. This is in agreement with the results of Barth et al. (1970), Barth and Kazzal (1971) and Coleman et al. (1971) who found selected forage differed significantly in chemical and botanical composition from samples of the available forage.

TABLE 1

RAW MEANS FOR FLUORINE CONTENT (ppm) OF 'AVAILABLE FORAGE',
'FISTULA FORAGE SAMPLES' AND 'SELECTED FORAGE'
(air-dry basis)

No.	Treatment		Number of Observations	Type of Forage Sample		
	Soil F (ppm)	Plant Height		Available	Fistula	Selected
1	434	high	35 (10) ^a	25	27	35
2	594	low	71 (29) ^a	52	59	67
3	3744	high	45 (14) ^a	91	99	107
4	2118	low	99 (36) ^a	138	137	145

^aSeveral fistula or selected samples were often paired with the same sample of available forage. The number of samples of available forage appears in parentheses.

The differences in fluorine content (Table 2) of available and selected forage in the high F treatments (3 and 4) were of similar magnitude as those of the low F treatments (1 and 2), yet the differences were significant ($P < .01$) for treatments 1 and 2 but not for treatments 3 and 4. This can be explained in part by the greater variation in fluorine content among forage samples collected from high fluorine soils as indicated by the standard errors presented in Table 3. In addition, fewer comparisons were made of samples collected from low fluorine soil than from high fluorine soil. A difference of 16 ppm in treatment 3 was less significant than the difference of 15 ppm in treatment 2 because this difference occurred for a pasture which had a fluorine content over six times that of treatment 2.

Variation Within Available and Selected Forage

The standard error and coefficients of variation for available and selected forage are reported in Table 3. The average of the percent variation for all treatments of available forage and selected forage showed that available forage had 92 percent variation from the mean fluorine content while selected forage varied 78 percent. The fluorine content of both the available and selected forage appeared to be extremely variable, however, the available forage was considerably more variable than the selected forage. These results indicated that although fluorine intake could be determined with fair accuracy, extreme variation would make more accurate determination of fluorine intake unlikely unless other factors affecting variation in fluorine content could be identified and their effect evaluated.

TABLE 2

COMPARISON OF DIFFERENCES BY PAIRED "T" WHERE THE FLUORINE CONTENT (ppm)
OF 'FISTULA FORAGE SAMPLES' AND 'SELECTED FORAGE' IS PAIRED
WITH THAT OF 'AVAILABLE FORAGE'

No.	Treatment		Number of Observations	Mean Differences	
	Soil F	Plant Height		Available minus Fistula	Available minus Selected
1	434	high	35 (10) ^a	-3	-10 ^b
2	594	low	71 (29) ^a	-7	-15 ^b
3	3744	high	45 (14) ^a	-9	-16
4	2188	low	99 (36) ^a	+1	-7

^aSeveral fistula or selected samples were often paired with the same sample of available forage. The number of samples of available forage appears in parentheses.

^bSignificantly different ($P < .01$)

TABLE 3

RAW MEANS, STANDARD ERRORS AND COEFFICIENTS OF
VARIATION FOR AVAILABLE AND SELECTED FORAGE

No.	Treatment		Number of Observations	Mean	Standard Error	C.V. (%)
	Soil F (ppm)	Plant Height				
1	434	high				
		Available	10	25	3.4	81
		Selected	35	35	3.4	58
2	594	low				
		Available	29	52	3.3	53
		Selected	71	67	5.5	69
3	3744	high				
		Available	14	91	17.8	132
		Selected	45	107	14.6	91
4	2118	low				
		Available	36	138	14.3	104
		Selected	99	145	13.7	94

II. FACTORS AFFECTING FLUORINE CONTENT OF FORAGE

Several factors were studied to determine the factors which significantly affect the fluorine content of forage selected by livestock. These factors were year, individual steer, season, soil fluorine level and plant height. Least squares means of fluorine content of selected forage are presented by year, steer and season in Table 4. The variations between years and steers were found to have no significant effect ($P > .05$) on the fluorine content of selected forage. The difference between seasons was very highly significant ($P < .001$).

Effect of Season on the Fluorine Content of Forage

The least squares means of the fluorine content of selected forage, as presented in Table 4, indicated that in the winter and spring seasons steers selected forage with higher average fluorine content than in the summer or autumn. These seasons were chosen to coincide as closely as possible with the growth patterns of the forage being grazed. Merriman, Moorman and Hobbs (1956), Merriman and Hobbs (1962) and Hobbs and Merriman (1962) found increased fluorine content in forages during the winter months. In the winter, the increase in fluorine content probably resulted from additional accumulation of soil on the surface area of the plant during times when the plants grew slowly or were dormant. Also, grazing animals are likely to ingest more soil in the winter because plants are shorter.

In the spring, fluorine content was also high as compared to summer and fall. This may have been the result of increased rainfall and

TABLE 4
LEAST SQUARES MEANS OF FLUORINE CONTENT (ppm)
OF SELECTED FORAGE

	Selected Forage
Year	
1966	155
1967	95
1968	107
Steer no.	
12	143
41	110
600	119
800	104
Season ^a	
Winter	192
Spring	122
Summer	86
Autumn	77

^a Winter = January, February and March;
Spring = April, May and June; Summer = July, August
and September; Autumn = October, November and
December

a concomitant amount of raindrop splash during this season. It has been shown that raindrop splash is the principle mechanism by which fluorine contaminated soil is deposited on plants (Hobbs et al., 1954; Merriman, Moorman and Hobbs, 1956; MacIntire, Hardin and Buehler, 1958; Hobbs and Merriman, 1962; Hobbs et al., 1962; and Merriman and Hobbs, 1962).

The amount of variation in fluorine content of forage explained by differences in season and the other variables studied are reported in Table 5. The R^2 value increased by 0.184 with the addition of season to the model, which indicated that approximately 18 percent of the variation in the fluorine content of the selected forage was due to the effect of season, when year is held constant.

Relationship of Soil Fluorine Level to the Fluorine Content of Forage

Soil fluorine level was found to have a very highly significant ($P < .001$) effect on the fluorine content of forage selected by grazing animals. As indicated in Table 5, approximately 10 percent of the variation in fluorine content of selected forage was due to differences in soil fluorine content, when variation due to year and season was removed. Least squares regression analysis indicated that forage fluorine content increased about 3 ppm with each 100 ppm increase in soil fluorine content. These results agreed with the research of MacIntire, Hardin and Buehler (1958), and Merriman and Hobbs (1962) who found that forage fluorine content increased with increased soil fluorine content. It is hypothesized that the principle form of fluorine contamination was that of soil deposited on the plants by raindrop splash. It was obvious that soil

TABLE 5

VARIATION EXPLAINED BY SEQUENTIALLY ADDING VARIABLES IN THE
REGRESSION ANALYSIS OF FLUORINE CONTENT OF SELECTED FORAGE

Variable	R^2	ΔR^2
Year	0.008	0.008
Season	0.192	0.184
Soil F	0.289	0.097
Plant height	0.310	0.021
Steer	0.332	0.022

which was high in fluorine contributed a larger amount of fluorine to the forage. This increase in fluorine may have been the result of upsplash.

Effect of Plant Height on Fluorine Content of Forage

The least squares analysis of variance indicated that plant height had a highly significant ($P < .01$) effect on the fluorine content of forage selected by grazing livestock, on a within year, season and soil fluorine level basis. Within the range of plant heights studied (0.75 - 6.0 inches) approximately two percent of the variation in fluorine content of selected forage was due to the effect of plant height (Table 5). The low R^2 value attributed to plant height in the model used was due to the high correlation between plant height and season. Based on the results of the regression analyses, the fluorine content of forage decreased about 19 ppm for each one inch increase in plant height. MacIntire, Hardin and Buehler (1958) indicated that the parts of the plant closest to the soil accumulated the greatest amount of soil as the result of raindrop splash. All forage of short plants is close to the soil. Therefore the average fluorine content of such plants was higher than that of tall plants, where the average fluorine content also includes parts of the plant higher above the soil which are less contaminated. Results of the present study are in agreement with those obtained by Merriman and Hobbs (1962) who found that short plants yielded diets higher in fluorine than those of taller plants. Another factor was evident in increasing the fluorine content of selected forage maintained at short plant heights, in addition to the higher fluorine content of the various forage samples due to short plant height as described above.

Animals grazing short pastures ingest greater amounts of soil (in addition to that on the forage) than animals grazing tall pastures (Healy and Ludwig, 1965). Animals grazing short pastures on fluorine-contaminated soils ingested more fluorine in this manner than did animals grazing tall pastures.

CHAPTER V

SUMMARY

During the grazing seasons of three consecutive years, esophageal-fistulated steers were used to determine fluorine intake of cattle grazing fluorine-contaminated pastures. Forage samples obtained by the fistulated steers were adjusted for losses of fluorine due to saliva leaching. The adjusted fluorine content of this forage (selected forage) was compared with that of samples of available forage. The effects of season, soil fluorine level, plant height, steer and year on the fluorine content of selected forage were studied.

Significant differences between the available forage and the selected forage were observed. These differences indicated that esophageal-fistulated animals more accurately determined the total fluorine intake of grazing animals than did samples of the available forage.

Variations due to steer and year were not found to significantly ($P > .05$) affect fluorine level of selected forage. Season exerted a very highly significant effect ($P < .001$) on the fluorine content of selected forage. Forage grown in the winter and spring seasons had considerably higher fluorine content than forages grown in the summer or autumn. Soil fluorine level had a very highly significant effect ($P < .001$) on the fluorine content of selected forage. Fluorine content of forage increased approximately 3 ppm for each 100 ppm increase in soil fluorine. Plant height exerted a highly significant ($P < .01$)

effect on the fluorine content of the forage. Within the range of plant heights studied (0.75 to 6.0 inches), fluorine content of the forage decreased about 19 ppm for each one-inch increase in plant height.

LITERATURE CITED

LITERATURE CITED

- Alder, F. E. 1969. The use of cattle with esophageal fistulae in grassland experiments. J. British Grassland Soc. 24:6.
- Barth, K. M., J. E. Chandler, M. E. Fryer and H. C. Wang. 1970. Effects of saliva and drying temperature on composition and digestibility of forage samples collected through esophageal fistulas. J. Anim. Sci. 31:794.
- Barth, K. M. and N. T. Kazzal. 1971. Separation of true selective grazing by cattle from effects of the esophageal fistula. J. Anim. Sci. 33:1124.
- Bell, M. C., G. M. Merriman and D. A. Greenwood. 1961. Distribution and excretion of F^{18} fluoride in beef cattle. J. Nutr. 73:379.
- Bibby, B. G. and F. Brudevold. 1954. Fluoridation as a Public Health Measure, edited by James H. Shaw. American Association for the Advancement of Science, Washington, D.C.
- Bohman, V. R. and A. L. Lesperance. 1967. Methodology research for range forage evaluation. J. Anim. Sci. 26:820.
- Bredon, R. M., D. T. Torell and B. Marshall. 1967. Measurement of selective grazing of tropical pastures using esophageal fistulated steers. J. Range Manage. 20:317.
- Burns, K. N. and R. Allcroft. 1964. Fluorosis in cattle 1. Occurrence and effects in industrial areas of England and Wales 1954-57. Ministry of Agr. Fisheries and Food, Animal Disease Surveys Rpt. 2., Part 1.
- Campbell, C. M., K. S. Eng, Jr., A. B. Nelson and L. S. Pope. 1968. Use of the esophageal fistula in diet sampling with beef cattle. J. Anim. Sci. 27:231.
- Carlson, C. H., W. D. Armstrong and L. Singer. 1960. Distribution and excretion of radiofluoride in the human. Proc. Soc. Exp. Biol. and Med. 104:235.
- Carlson, C. H., W. D. Armstrong, L. Singer and L. B. Hinshaw. 1960. Renal excretion of radiofluoride in the dog. Am. J. Physiol. 198:829.
- Chamberlain, C. C. and W. Burroughs. 1962. Effect of fluoride, magnesium, and manganese ions on in vitro cellulose digestion by rumen micro-organisms. J. Anim. Sci. 21:428.

- Coleman, S. W., K. M. Barth, J. B. McLaren, T. W. High, H. C. Wang, and C. S. Hobbs. 1971. Composition of diets selected by grazing beef cattle determined after adjusting for esophageal-fistula effects. Tenn. Agr. Exp. Sta. Bull. 483.
- Compton, O. C., L. F. Remmert and W. M. Mellenthin. 1965. Fluorine levels in crops of the Dallas area in 1962 and 1963. Oregon Agr. Exp. Sta. Spec. Rtp. 187.
- Cook, C. W., J. L. Thorne, J. T. Blake and J. Edlefsen. 1958. The use of esophageal fistula cannulas for collecting forage samples by grazing sheep. J. Anim. Sci. 17:189.
- Crampton, E. W. and L. E. Harris. 1969. Applied Animal Nutrition (2nd ed.). W. H. Freeman and Company, San Francisco.
- Garlick, N. L. 1955. The teeth of the ox in clinical diagnosis IV. Dental fluorosis. Am. J. Vet. Res. 16:38.
- Greenwood, D. A., J. L. Shupe, G. E. Stoddard, L. E. Harris, H. M. Nielsen and L. E. Olsen. 1964. Fluorosis in cattle. Utah Agr. Exp. Sta. Spec. Rtp. 17.
- Guthrie, L. D., G. H. Rollins and G. E. Hawkins. 1968. Evaluation of the esophageal-fistula cannula and hand-clip methods of sampling coastal bermudagrass pastures. J. Dairy Sci. 51:710.
- Harris, L. E., G. P. Lofgreen, C. J. Kercher, R. J. Raleigh and V. R. Bohman. 1967. Techniques of research in range livestock nutrition. Utah Agr. Exp. Sta. Bull. 471.
- Harvey, W. A. 1960. Least-squares analysis of data with unequal subclass numbers. U. S. D. A. Agr. Res. Ser. 20-8.
- Healy, W. B. 1968. Ingestion of soil by dairy cows. New Zealand J. Agric. Res. 11:187.
- Healy, W. B. and T. G. Ludwig. 1965. Wear of sheep's teeth I. The role of ingested soil. New Zealand J. Agric. Res. 8:737.
- Healy, W. B., W. J. McCabe and G. F. Wilson. 1970. Ingested soil as a source of microelements for grazing animals. New Zealand J. Agric. Res. 13:503.
- Healy, W. B. and G. F. Wilson. 1971. Deposits on rumen epithelium associated with ingestion of soil. New Zealand Agric. Res. 14:122.
- High, T. W. 1966. Dietary selectivity by steers grazing two types of pasture. Unpublished Doctoral dissertation, University of Tennessee, Knoxville.

- Hobbs, C. S. and G. M. Merriman. 1962. Fluorosis in beef cattle. Tenn. Agr. Exp. Sta. Bull. 351.
- Hobbs, C. S., R. P. Moorman, Jr., J. M. Griffith, J. L. West, G. M. Merriman, S. L. Hansard and C. C. Chamberlain. 1954. Fluorosis in cattle and sheep. Tenn. Agr. Exp. Sta. Bull. 235.
- Hodge, H. C. and R. F. Sognnaes. 1946. Experimental caries and a discussion of the mechanism of caries inhibition by fluorine. Dental Caries and Fluorine, F. R. Moulton, editor. American Association for the Advancement of Science, Washington, D. C.
- Hoehne, O. E., D. C. Clanton and C. L. Streeter. 1967. Chemical changes in esophageal fistula samples caused by salivary contamination and sample preparation. J. Anim. Sci. 26:628.
- Lesperance, A. L., V. R. Bohman and D. W. Marble. 1960. Development of techniques for evaluation of grazed forage. J. Dairy Sci. 43:682.
- Lesperance, A. L., E. H. Jensen, V. R. Bohman and R. A. Madsen. 1960. Measuring selective grazing with fistulated steers. J. Dairy Sci. 43:1615.
- MacIntire, W. H., L. J. Hardin, M. H. Buehler. 1958. Fluorine in Maury County, Tennessee. Tenn. Agr. Exp. Sta. Bull. 279.
- Maplesden, D. C., I. Motzok, W. T. Oliver and H. D. Branion. 1960. Placental transfer of fluorine to the fetus in rats and rabbits. J. Nutr. 71:70.
- Mason, R. L. and D. E. Williams. 1962. The density of some bones of cattle fed different levels of fluorine. Tenn. Farm and Home Sci. Progress Rpt. 44.
- Maurer, R. L. and H. G. Day. 1957. The non-essentiality of fluorine in nutrition. J. Nutr. 62:561.
- Maynard, L. A. and J. K. Loosli. 1962. Animal Nutrition (5th ed.). McGraw-Hill Book Co., New York.
- McClure, F. J. and I. Zipkin. 1958. Dental Clinics of North America. W. B. Saunders Co., Philadelphia.
- McKay, F. S. 1942. Fluorine and Dental Health, edited by F. R. Moulton. American Association for the Advancement of Science, Washington, D. C.
- Merriman, G. M. and C. S. Hobbs. 1962. Bovine fluorosis from soil and water sources. Tenn. Agr. Exp. Sta. Bull. 347.

- Merriman, F. M., R. P. Moorman, Jr. and C. S. Hobbs. 1956. Survey of the possible occurrence and extent of fluorosis in cattle on selected farms in Blount County. Tenn. Agr. Exp. Sta., Knoxville, Tenn.
- Miller, R. F. and P. H. Phillips. 1956. The effect of age on the level and metabolism of fluorine in the bones of the fluoridated rat. J. Nutr. 59:425.
- Neely, K. L. and F. G. Harbaugh. 1954. Effects of fluoride ingestion on a herd of dairy cattle in the Lubbock, Texas area. J. Am. Vet. Med. Assoc. 124:344.
- Newell, G. W. and H. J. Schmidt. 1958. The effects of feeding fluorine, as sodium fluoride, to dairy cattle - A six year study. Am. J. Vet. Res. 19:363.
- N.R.C. 1966. Nutrient requirements of horses. National Research Council, Washington, D.C.
- N.R.C. 1968a. Nutrient requirements of sheep. National Research Council, Washington, D.C.
- N.R.C. 1968b. Nutrient requirements of swine. National Research Council, Washington, D.C.
- N.R.C. 1970. Nutrient requirements of beef cattle. National Research Council, Washington, D.C.
- N.R.C. 1971. Nutrient requirements of dairy cattle. National Research Council, Washington, D.C.
- Phillips, P. H., J. W. Suttie and E. J. Zebrowski. 1963. Effects of dietary sodium fluoride on dairy cows. VII. Recovery from fluoride ingestion. J. Dairy Sci. 46:513.
- Putnam, P. A., R. Lehmann and R. E. Davis. 1966. Feed intake and salivary secretion by steers. J. Anim. Sci. 25:817.
- Remington, R. D. and M. A. Schork. 1970. Statistics With Applications to the Biological and Health Sciences. Prentice-Hall, Inc., New Jersey.
- Shupe, J. L. 1966. Diagnosis of fluorosis in cattle. Utah Exp. Sta. Journal Paper No. 564.
- Shupe, J. L., L. E. Harris, D. A. Greenwood, J. E. Butcher and H. M. Nielsen. 1963. The effect of fluorine on dairy cattle. V. Fluorine in the urine as an estimator of fluorine intake. Am. J. Vet. Res. 24:300.

- Shupe, J. L., M. L. Miner, L. E. Harris and D. A. Greenwood. 1962. Relative effects of feeding hay atmospherically contaminated by fluoride residue, normal hay plus calcium fluoride and normal hay plus sodium fluoride to dairy heifers. *Am. J. Vet. Res.* 23:777.
- Sievert, A. H. and P. H. Phillips. 1959. Metabolic studies on the sodium fluoride-fed rat. *J. Nutr.* 68:109.
- Suttie, J. W. 1964. Effects of inorganic fluorides on animals. *J. Air Pollut. Control Assoc.* 14:461.
- Suttie, J. W. 1967. Vertebral biopsies in the diagnosis of bovine fluoride toxicosis. *Am. J. Vet. Res.* 28:709.
- Suttie, J. W. 1969a. Air quality standards for the protection of farm animals from fluorides. *J. Air Pollut. Control Assoc.* 19:239.
- Suttie, J. W. 1969b. Fluorine content of commercial dairy concentrates and alfalfa forage. *Agric. and Feed Chem.* 17:1350.
- Suttie, J. W. and E. C. Faltin. 1971. Effect of a short period of fluoride ingestion on dental fluorosis in cattle. *Am. J. Vet. Res.* 32:217.
- Suttie, J. W., R. Gesteland and P. H. Phillips. 1961. Effects of dietary sodium fluoride on dairy cows. VI. In young heifers. *J. Dairy Sci.* 44:2250.
- Suttie, J. W., R. F. Miller and P. H. Phillips. 1957b. Studies on the effects of dietary NaF on dairy cows. I. The physiological effects and the developmental symptoms of fluorosis. *J. Nutr.* 63:211.
- Suttie, J. W., R. F. Miller and P. H. Phillips. 1957a. Effects of dietary NaF on dairy cows. II. Effects on milk production. *J. Dairy Sci.* 40:1485.
- Suttie, J. W. and P. H. Phillips. 1959a. The effect of age on the rate of fluorine deposition in the femur of the rat. *Archives of Biochem. and Biophysics* 83:355.
- Suttie, J. W. and P. H. Phillips. 1959b. Studies on the effects of dietary sodium fluoride on dairy cows. V. A three year study on mature animals. *J. Dairy Sci.* 42:1063.
- Suttie, J. W. and P. H. Phillips. 1960. Studies of the effects of dietary NaF on dairy cows. IV. Dental changes as the result of long-term ingestion. *J. Nutr.* 71:109.

- Suttie, J. W. and P. H. Phillips. 1962. Distribution of fluorine in the bovine metatarsus. *Am. J. Vet. Res.* 23:1107.
- Suttie, J. W., P. H. Phillips and R. F. Miller. 1958. Studies of the effects of dietary sodium fluoride on dairy cows. III. Skeletal and soft tissue fluorine deposition and fluorine toxicosis. *J. Nutr.* 65:293.
- Torell, D. T. 1954. An esophageal fistula for animal nutrition studies. *J. Anim. Sci.* 13:878.
- Van Dyne, G. M. and D. T. Torell. 1964. Development and use of the esophageal fistula: A review. *J. Range Manage.* 17:7.
- Weir, W. C. and D. T. Torell. 1959. Selective grazing by sheep as shown by a comparison of the chemical composition of range and pasture forage obtained by hand clipping and that collected by esophageal fistulated sheep. *J. Anim. Sci.* 18:641.
- Wuthier, R. E. and P. H. Phillips. 1959. The effects of long-time administration of small amounts of fluoride in food or water on caries-susceptible rats. *J. Nutr.* 67:581.

APPENDIX

APPENDIX

TABLE 6

FLUORINE CONCENTRATION (ppm) IN 'FISTULA FORAGE
SAMPLES' AND 'AVAILABLE FORAGE'

Date	Fistula				Available
	Steer No.				
	12	41	600	800	
Pasture 1					
March 29, 1967	65.6	63.5	57.3	59.1	62.5
June 1, 1967	11.5	16.8	15.1	22.4	14.5
June 19, 1967	25.7	12.3	90.2	58.8	8.5
July 20, 1967	15.6	7.7	8.2	12.7	7.0
Aug. 29, 1967	9.4	10.0	6.9	9.1	7.9
Oct. 23, 1967	16.8	19.1	----	22.2	16.6
Dec. 9, 1967	24.8	32.4	----	22.6	48.5
Feb. 6, 1968	23.2	24.8	----	15.2	39.2
March 19, 1968	34.2	43.2	----	29.8	38.8
April 10, 1968	29.6	19.0	----	18.7	9.4
Pasture 2					
June 9, 1966	----	69.4	----	----	79.8
June 10, 1966	----	94.4	----	108.8	55.2
July 28, 1966	106.3	----	----	----	115.2
July 29, 1966	27.2	----	25.5	----	37.6
Sept. 19, 1966	----	23.2	----	17.1	53.7
Sept. 20, 1966	----	15.0	----	16.7	41.2
Oct. 28, 1966	----	47.5	----	40.3	44.3
Oct. 28, 1966	----	33.1	----	30.6	
Nov. 22, 1966	----	159.1	52.8	----	77.6
Nov. 23, 1966	145.0	69.4	94.4	----	72.2
Jan. 11, 1967	83.1	----	----	----	75.4
Jan. 11, 1967	101.9	----	----	88.4	56.3
Feb. 14, 1967	92.1	----	----	144.7	108.9
Feb. 15, 1967	----	----	----	101.6	129.6
Feb. 15, 1967	----	----	131.4	----	117.0

TABLE 6 (Continued)

Date	Fistula				Available
	Steer No.				
	12	41	600	800	
Pasture 2 (continued)					
April 27, 1967	209.4	129.0	138.6	85.4	89.6
June 2, 1967	36.4	35.5	95.2	57.6	13.2
June 20, 1967	10.5	16.6	42.8	15.8	33.5
July 21, 1967	11.2	----	12.3	14.4	29.0
Aug. 28, 1967	21.6	16.0	11.1	17.0	41.6
Sept. 22, 1967	15.2	52.7	----	14.5	38.3
Oct. 24, 1967	30.8	31.1	----	76.1	29.2
Dec. 8, 1967	42.2	----	----	42.5	49.0
March 19, 1968	140.0	49.4	----	107.5	91.2
April 10, 1968	175.6	----	----	----	63.0
June 11, 1968	41.1	37.2	----	25.0	23.0
June 26, 1968	----	32.4	----	47.1	49.2
Sept. 10, 1968	32.0	28.5	----	38.6	42.8
Oct. 2, 1968	54.2	21.4	----	31.3	20.3
Oct. 30, 1968	35.0	33.8	----	60.4	42.5
Pasture 3					
April 27, 1967	142.8	154.3	114.3	143.8	40.8
June 2, 1967	87.2	74.6	71.7	51.1	23.8
June 20, 1967	36.9	15.7	53.0	34.4	99.6
July 21, 1967	54.0	24.7	65.4	34.0	21.9
Aug. 28, 1967	17.8	----	24.0	53.6	15.8
Sept. 22, 1967	52.9	42.0	----	82.3	164.8
Oct. 24, 1967	74.3	85.7	----	85.9	123.7
March 19, 1968	481.2	498.1	----	239.4	492.5
April 10, 1968	127.0	146.1	----	117.8	159.6
June 11, 1968	154.5	84.3	----	107.4	30.9
July 17, 1968	----	97.4	----	80.4	38.8
Sept. 10, 1968	159.0	76.1	----	85.0	56.5
Oct. 2, 1968	144.0	61.5	----	67.1	23.0
Oct. 30, 1968	32.7	26.2	----	8.3	20.0

TABLE 6 (Continued)

Date	Fistula				Available
	Steer No.				
	12	41	600	800	
Pasture 4					
April 25, 1966	----	247.1	----	----	275.0
April 26, 1966	----	159.4	----	----	139.6
June 9, 1966	----	----	----	106.3	126.3
July 28, 1966	----	----	122.0	----	112.8
July 29, 1966	115.8	----	82.6	----	170.8
Sept. 19, 1966	----	137.2	----	66.3	375.0
Oct. 28, 1966	----	125.4	----	145.8	143.5
Oct. 28, 1966	----	143.5	----	149.7	
Nov. 23, 1966	156.1	204.0	80.2	----	200.0
Jan. 11, 1967	----	310.2	----	210.8	145.0
Jan. 11, 1967	----	103.7	195.0	----	120.0
Feb. 14, 1967	----	108.9	162.1	----	115.4
Feb. 15, 1967	367.5	----	382.5	----	258.0
Feb. 15, 1967	----	----	----	225.2	270.2
March 28, 1967	725.0	----	445.4	354.4	649.8
April 27, 1967	619.4	311.0	389.9	269.7	420.5
June 2, 1967	----	127.3	180.6	74.8	85.5
June 20, 1967	95.0	27.8	68.0	36.3	42.4
July 21, 1967	106.0	36.0	47.2	34.8	32.0
Aug. 28, 1967	61.6	103.8	22.6	53.0	86.3
Oct. 24, 1967	36.4	58.8	----	45.2	68.0
Dec. 8, 1967	----	----	----	102.9	116.2
March 19, 1968	306.2	332.5	----	250.0	316.9
April 10, 1968	366.6	239.4	----	303.4	178.3
June 11, 1968	103.7	41.8	----	40.3	24.7
June 26, 1968	216.2	74.4	----	55.8	322.4
July 17, 1968	33.8	40.6	----	45.0	37.2
July 17, 1968	104.1	46.1	----	77.4	26.0
Sept. 10, 1968	572.2	174.6	----	----	123.4
Oct. 2, 1968	60.0	38.7	----	42.2	32.5
Oct. 10, 1968	47.8	33.2	----	146.5	75.1

TABLE 6 (Continued)

Date	Fistula				Available
	Steer No.				
	12	41	600	800	
	Pasture 5				
March 29, 1967	178.2	120.2	143.3	72.4	85.5
June 1, 1967	86.4	45.0	54.3	42.3	28.1
June 19, 1967	23.4	44.9	11.2	39.0	7.1
July 20, 1967	67.7	39.6	45.0	37.0	13.0
Aug. 29, 1967	25.8	26.2	23.4	19.5	47.2
Oct. 23, 1967	35.3	33.4	----	34.4	101.8

TABLE 7

COMPARISON OF FLUORINE CONTENT (mg/l) OF SALIVA SAMPLES
TAKEN PRIOR TO GRAZING AND THOSE TAKEN AFTER GRAZING¹

Date	Pasture	Fluorine content before grazing	Fluorine content after grazing
Sept. 10, 1968	2	----	2.65
Sept. 10, 1968	2	0.00	0.00
Sept. 10, 1968	3	0.55	----
Sept. 10, 1968	4	0.50	0.55
Sept. 10, 1968	4	0.55	1.60
Oct. 2, 1968	2	0.35	1.00
Oct. 2, 1968	2	0.55	1.00
Oct. 2, 1968	3	0.35	1.40
Oct. 2, 1968	3	0.55	0.85
Oct. 2, 1968	4	0.35	1.50
Oct. 2, 1968	4	0.55	1.60
Oct. 30, 1968	2	0.50	1.00
Oct. 30, 1968	2	0.20	0.40
Oct. 30, 1968	3	0.50	1.00
Oct. 30, 1968	3	0.20	1.00
Oct. 30, 1968	4	0.50	0.50
Oct. 30, 1968	4	0.20	1.10

¹Samples taken after grazing were collected as they drained from the collection bag after flowing through the forage sample.

TABLE 8

RAW MEANS AND DIFFERENCES WHERE 'FISTULA FORAGE SAMPLES'
AND 'SELECTED FORAGE' ARE PAIRED WITH 'AVAILABLE
FORAGE' TO DETERMINE VARIATION OF FLUORINE
CONTENT (ppm) AMONG YEARS

Year	Number of pairs	Means			Mean Differences	
		Available	Fistula	Selected	Available minus fistula	Available minus selected
1966	34	119	95	102	24	17
1967	143	80	85	93	-5	-13**
1968	73	93	107	115	-14	-22*

*Significantly different ($P < .05$)

**Significantly different ($P < .01$)

TABLE 9

RAW MEANS AND DIFFERENCES WHERE 'FISTULA FORAGE SAMPLES'
AND 'SELECTED FORAGE' ARE PAIRED WITH 'AVAILABLE
FORAGE' TO DETERMINE VARIATION OF FLUORINE
CONTENT (ppm) AMONG STEERS

Steer	Number of pairs	Means			Mean Differences	
		Available	Fistula	Selected	Available minus fistula	Available minus selected
12	67	89	115	123	-27*	-34**
41	73	87	84	92	3	-5
600	36	91	99	107	-8	-15
800	74	90	77	85	13	5

*Significantly different ($P < .01$)

**Significantly different ($P < .001$)

TABLE 10

AVERAGE PLANT HEIGHTS (inches)

Pasture	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
<u>1966</u>												
1	----	0.50	1.50	2.50	3.00	4.00	2.50	4.00	6.00	3.00	2.00	2.50
2	----	0.50	1.00	2.00	----	3.00	2.50	3.50	4.00	3.00	1.50	1.00
3	----	0.50	2.00	3.00	----	4.00	2.50	4.00	4.00	3.50	2.00	1.50
4	----	0.50	1.50	2.00	----	3.00	2.50	3.50	4.00	2.50	1.50	----
5	----	0.50	1.50	2.00	3.00	3.00	1.50	3.50	3.00	2.00	1.50	1.50
<u>1967</u>												
1	1.00	1.00	1.00	3.00	4.00	3.00	3.50	5.00	6.00	4.00	2.00	1.50
2	0.75	0.75	0.75	1.50	2.50	2.00	2.50	4.00	4.00	3.00	1.50	0.75
3	0.75	0.75	0.75	2.50	3.00	2.50	3.50	3.00	5.00	2.50	2.00	3.00
4	0.75	0.75	0.75	2.00	2.50	2.50	2.50	5.00	4.00	2.00	1.50	3.00
5	0.75	0.75	0.75	1.50	2.00	2.00	2.00	4.00	4.00	2.00	1.50	0.75
<u>1968</u>												
1	1.50	1.50	2.00	4.00	8.00	4.00	----	2.00	3.00	4.00	----	1.50
2	0.75	0.75	1.00	2.00	3.00	3.00	----	1.50	2.50	2.00	----	1.50
3	1.50	1.00	2.00	3.00	5.00	4.00	2.00	2.00	3.00	3.00	----	0.75
4	0.75	0.75	1.00	2.00	3.00	3.00	1.50	1.50	2.50	2.00	----	0.75
5	0.75	0.75	1.00	1.50	2.00	2.50	----	1.50	2.00	2.00	----	0.75

VITA

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