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Certain physiological and pathological effects of feeding various levels of fluorine and alleviators to lambs

J. M. Griffith

To the Graduate Council:

I am submitting herewith a thesis written by J. M. Griffith entitled "Certain physiological and pathological effects of feeding various levels of fluorine and alleviators to lambs." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Charles S. Hobbs, Major Professor

We have read this thesis and recommend its acceptance:

Sam L. Hansard, Harold J. Smith

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

December 3, 1953

To the Graduate Council:

I am submitting herewith a thesis written by J. M. Griffith entitled "Certain Physiological and Pathological Effects of Feeding Various Levels of Fluorine and Alleviators to Lambs." 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.

Charles S. Hobbs
Major Professor

We have read this thesis
and recommend its acceptance:

Sam L. Hansard

Harold J. Smith

Accepted for the Council:

E. A. Waters
Dean of the Graduate School

CERTAIN PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF FEEDING VARIOUS LEVELS OF FLUORINE AND ALLEVIATORS TO LAMBS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

J. M. Griffith

December 1953

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J. M. Griffith

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CHAPTER I

INTRODUCTION

The fluorine problem in livestock feeding is one of increasing importance. Fluorosis has been commonly defined as "chronic poisoning with fluorine" (American Illustrated Medical Dictionary). To some, the word may mean the condition that results when the abnormal ingestion of fluorine is sufficient to harm the animal (i.e., as the term is defined medically). To others, the word may mean the condition resulting from any ingestion of fluorine above normal levels.

Fluorosis in livestock has been encountered in several ways, one of which is the use of mineral supplements containing fluorine. These minerals are the different varieties of phosphate rock which contain 3 to 4 percent fluorine, and phosphatic limestones which contain fluorine in proportion to the amount of phosphorus present. In the manufacture of superphosphate from phosphate rock, approximately one-fourth to two-thirds of the fluorine is driven off by volatilization as hydrogen fluoride or as silicon tetrafluoride, as reported by Mitchell and Edman (1951).

Chronic fluorosis in farm animals has been reported from many countries in areas adjacent to industrial plants emitting fluorine-containing gases and dusts. The production of acid phosphate and defluorinated phosphate, the electrolytic production of aluminum, the manufacture of bricks from fluorine-bearing clays, the calcimining of ironstone, and certain enamelling processes have been chiefly involved. However, even the fumes from coal-burning furnaces may contribute to the industrial hazard, since coal and shales associated with coal contain from forty to several hundred parts per million fluorine, according to Churchill et al. (1948). Since fluorine is very active chemically and does not occur in a free state, the fluorine-bearing fumes from factories processing fluorine-containing ores may be largely hydrofluoric acid, and the fluorine-bearing dusts may consist of fluorides, such as sodium fluoride or cryolite, which have been volatilized and then condensed in the cooler surrounding air. The leaves of plants may absorb the gas and collect some of the dust on their surfaces, according to Mitchell and Edman (1952). The extent of contamination of the forage, therefore, will depend on several factors, such as the amount of fluorine-containing materials being emitted, weather conditions, prevailing winds, topography of surrounding terrain, and type of vegetation.

Drinking water can contribute significantly to the fluorine hazard if the fluorine content attains levels of several parts per million. Many of the conditions reported to be due to high fluorine water have been in Australia, India, Texas, Colorado and other western states.

Other fluorotic conditions have been reported that were caused by dusts and gases from volcanic eruptions. During eruption the fluorine-bearing gases and ashes flow out of the volcanic openings in tremendous quantities. Livestock may inhale the noxious gases and eat the ashes that have fallen on the plants.

A method for the determination of the amount of ingested fluorine that would be damaging or non-damaging over an extended period of time would, without a doubt, be of immeasurable value to the livestock producer and feeder. Also, some practical material, that could be fed or administered to livestock to alleviate part or all of the effects of fluorosis, would be of untold value to agriculture and industry.

Since there have been very few controlled fluorine feeding experiments conducted with sheep, it is considered of great importance to determine the effects of feeding various levels of fluorine to sheep and to study the effects of various alleviators. Results from this work may be of considerable economic value, not only to the sheep industry, but also to other livestock industries.

The purpose of this experiment was to study the several physiological and pathological changes associated with the feeding of various levels of fluorine to growing lambs. Also, a study was made of the alleviating effects of aluminum sulfate hydrate and aluminum chloride hydrate fed to growing lambs receiving 100 parts per million fluorine in their ration. The several phases of the experiment included:

1. The effects on efficiency of feed utilization.
2. The effects on weight gains.
3. The ability to digest crude fiber, crude protein, ether extract, and nitrogen-free extract.
4. The effects on fluorine, calcium, phosphorus and nitrogen balances.
5. The effects on blood levels of phosphorus, sodium, potassium, magnesium, and calcium as a function of time.
6. The effects on quantative fluorine storage in bone, heart, liver, kidney, and spleen.

7. Pathological changes in bone, heart, liver, kidney, and spleen.
8. Determination of the correlation of fluorine content of urine and dietary fluorine intake as a possible criterion for the diagnostic measurement of fluorosis in animals under field conditions.
9. The effects on certain micro-organisms in the rumen.

CHAPTER II

REVIEW OF LITERATURE

Many studies have been conducted to determine some of the effects of fluorine on man and animals; however, many of these studies have been made under field conditions and not from well controlled experiments. Excellent reviews of most of these studies have been presented by Mitchell and Edman (1952), Schmidt and Rand (1952), Roholm (1937), and McClure (1933).

Fluorine studies with animals have been made primarily with cattle, sheep, rats, and swine, with a few reports on dogs, rabbits, and horses. A limited number of controlled feeding experiments with sheep fed various levels of fluorine have been reported, and very few controlled studies on materials that would alleviate fluorosis in large animals.

Peirce (1938) reported on feeding young sheep for three years on control rations, plus Nauru rock phosphate supplying 60, 120, and 160 milligrams of fluorine daily; also, Florida rock phosphate supplying 170 milligrams of fluorine daily. His findings showed that sheep receiving 120 milligrams of fluorine per day or less grew normally, ate the same amount of feed, and remained in good health throughout the experiment. Those receiving larger doses of fluorine performed normally for about one year, after which their feed consumption, weight gain, and general health became greatly impaired. Sheep fed Florida rock phosphate were affected less than those on Nauru rock phosphate. The ingestion of these levels of fluorine did not significantly reduce the wool growth when compared with control animals. Doses of 120 milligrams or less of fluorine per day had no effect on the physical appearance of bones, but higher doses brought about the replacement of the normal yellowish color and luster by a white, chalky appearance. Walls of the bones became thickened, and in extreme cases, exostoses were produced on the long bones. The mandibles also showed exostoses and considerable increase in diameter. The sheep receiving 60 milligrams or more of fluorine per day showed changes in the teeth, roughly in proportion to the amount ingested. Ingestion of fluorine had no definite effect on the ash content of the bones, but it brought about a ten-fold or greater increase in the fluorine content of bones and teeth; however, other organs did not appear to be affected.

Hatfield et al. (1942) reported on western lambs that were fed a ration to which rock phosphate containing 3.85 percent fluorine was added to increase the daily intake to 1.5, 3.0, and 6.0 milligrams of fluorine per kilogram of body weight over a period of 170 days. At the end of this period the grain consumption was decreased and growth was depressed in the animals receiving 6.0 milligrams of fluorine per kilogram of body weight per day. As the fluorine intake increased, the breaking strength per gram of bone decreased proportionally and the percentage of fluorine in the bone increased. Also, the weight of the thyroid gland decreased as the amount of fluorine was increased in the ration.

Shrewsbury et al. (1944) reported on three experiments with growing lambs, and one experiment with breeding ewes carried through three reproduction periods. The maximum level of fluorine from rock phosphate safely tolerated by lambs, as indicated by their rate of gain, was 1.5 to 3.0 milligrams of fluorine per kilogram of body weight per day; however, at this level there was some reduction in growth. Fluorine from rock phosphate at a level of 1.5 milligrams per kilogram of body weight produced no visible detrimental effect on teeth except for slight discoloration; however, the 6.0 milligram level produced fractured and erroded teeth. Bone measurements, breaking strength, and bone ash were not significantly affected by any of the levels used, although there was a marked increase in the percentage of fluorine in the bones.

Shrewsbury's findings showed that fluorine from rock phosphate in rations of breeding ewes did not affect maintenance of body weight during the first year, but produced a deleterious effect during the second and third years, with the most pronounced effect resulting from the highest level of fluorine. The number of lambs born per ewe was not influenced by the level of fluorine in the ration, and the birth weights were affected only slightly, if at all, during the three-year period. The growth of the third-gestation lambs from ewes that received fluorine from rock phosphate for three years was not affected by the fluorine ingested by the ewes. Wool did not seem to be affected by the fluorine intake. The fluorine content of the rations did not bring about abnormal relationships in the blood calcium and phosphorus of ewes or lambs. Fluorine was concentrated in the bones of lambs in proportion to its level in the ration and length of time fed the ration. Ewe bones contained somewhat more fluorine than the lamb bones, but the content was not in proportion to the length of the experimental period.

Shrewsbury also indicated that the presence of adequate iodine in the ration did not counteract the deleterious effect of fluorine on the growth of lambs. However, fluorine increased the storage of iodine in the thyroid gland under conditions of both adequate and inadequate iodine feeding.

Peirce (1951) reported on sheep fed in pens for three and one-half years on a control ration and given rain water to which sodium fluoride was added to give a final concentration of 2.5, 5, 10, and 20 p.p.m. (parts per million) fluorine. The ingestion of fluorine appeared to have no adverse effects on general health, food consumption, or wool production in these sheep. At the end of two and one-half years, the weight of the sheep receiving 20 p.p.m. fluorine was significantly less, at the 5 percent level, than that of the control animals. The incisor and molar teeth of the sheep receiving water containing 5 p.p.m. fluorine were slightly mottled. Mottling was more evident in those receiving 10 p.p.m. fluorine, and marked in those receiving 20 p.p.m. fluorine. Fluorine ingestion caused the incisors to errupt at an earlier age, and brought about increases up to twenty-fold in the fluorine content of the bones and teeth.

Gaud et al. (1934) investigated edemic fluorosis in North Africa and found that jawbones of affected sheep contained from 1200 to 4400 p.p.m. fluorine. It was calculated that these animals had been ingesting about 6 milligrams of fluorine per kilogram of body weight per day when the sources of water, mud, vegetation, and dust were taken into account. The only symptom mentioned was worn and mottled teeth.

Slagsnold (1934) found that sheep, when fed hay containing 200 to 250 p.p.m. fluorine, began to lose their appetites within three months, and at the end of a year were anemic, stiff, and had developed exostoses.

Agate and co-workers (1949), in the Fort William area of Scotland, found marked chronic fluorosis in cattle and sheep, with symptoms of excessive tooth wear, lowered gain in weight, loss in milk production, and general poor health. The fluorine content of the mandibles of two sheep was 5,600 and 7,700 p.p.m. fluorine on a dry, fat-free basis. A herbage sample, which these animals had been consuming, contained 61 p.p.m. fluorine on a dry weight basis.

Chang, Phillips, and Hart (1934) reported on Holstein dairy cows that received 0.022, 0.044, and 0.088 percent fluorine, based on the total ration and supplied from rock phosphate, from four to six months of age until after the third calving. Their results showed the greatest quantity of fluorine accompanied calcium and phosphorus deposition; however, some fluorine occurred in all normal tissues. The bones and teeth contained large quantities of fluorine, while the more active organs such as the liver, kidney, heart muscle, and other tissues studied showed only small quantities of fluorine. The average fluorine content of the normal dentine and normal bones of these dairy cows was found to lie between 42 and 63 milligrams per 100 grams of dried tissue. The fluorine content of the liver, kidney, heart muscle, pancreas, thyroid, tendon, hair, and hoof was found to be less than 1 milligram per 100 grams of dried normal tissue. The inclusion of 0.088 percent fluorine, based on the total ration, resulted in an increased storage of fluorine in the bones and teeth, which amounted to 16 to 25 times that found in normal osseous structures; and the fluorine content of the internal organs was doubled.

Hobbs et al. (1951) reported that teeth of cows fed rations for 40 months containing approximately 15 to 30 p.p.m. of fluorine added to the control ration showed some staining and markings; 30 to 40 p.p.m. of added fluorine showed erosion, mottling, and pitting. At higher levels of fluorine feeding, all the effects were pronounced. Also, metabolism trials conducted on animals receiving added fluorine in the amounts of 10, 20, 30, 40, 50, 70, and 100 p.p.m. indicated that fluorine had little, if any, effect on the digestibility of the protein, fat, fiber, and N.F.E. in these experiments, but did result in a lower feed consumption at higher levels.

In the conclusion of a review of literature, Mitchell and Edman (1952) reported that the level of fluorine from rock phosphate which is on the borderline of toxicity for cattle, sheep, and pigs is about 100 p.p.m. of the total dry ration. They reported that the toxicity of inorganic fluorine compounds depends to some extent on their solubility in water above certain minimum levels of intake. Sodium fluoride and sodium fluosilicate are the most toxic and calcium fluoride is the least toxic among the compounds studied, mainly with the laboratory rat. As nearly as they could judge from the evidence available, the fluorine of sodium fluoride, up to levels that will affect the appetite and growth of animals, is about twice as toxic as the fluorine of cryolite, and presumably of rock phosphate.

Maynard (1947) stated that at first fluorine merely accumulates in the bones and teeth without evident harm, and considerable time elapses before structural injury becomes evident. The avidity of the bones and teeth for fluorine tends to protect the soft tissues against excessive concentrations; however, after the bones become saturated, the greater part of the absorbed fluorine is free to produce its general toxic effects on the organs and soft tissues.

Hobbs _et_ _al_. (1951) reported that the fluorine analysis of urine gives a fair method for the division between cows consuming rations containing less than 20 to 30 p.p.m. fluorine in the dry ration and those above this level. Also, studies showed there was considerable variation in the fluorine content of urine samples taken from the same cow at different times or from different cows eating rations containing the same fluorine level in the dry ration.

According to Blakemore _et_ _al_. (1948), urinary analysis was found to be a convenient way of diagnosing fluorosis, especially in sub-clinical cases where no dental signs or obvious skeletal changes were present. They concluded that a high urinary value may reflect either a high current consumption of fluorine, or the skeletal storage of fluorine in the past; and in any given outbreak of disease, close correlation can not be expected between clinical symptoms, urinary output of fluorine, and changing levels of pasture contamination.

Sharpless (1936) reported on various substances that would limit the toxic action of fluorine fed to rats. Salts of lanthanum, cerium, thorium, and boron, which form insoluble or complex fluorides, were found to give either no protection or, as in the case of lanthonum, only slight protection. Aluminum chloride was found to inhibit, to a large extent, the toxic effects of fluorine.

In these studies with rats, Sharpless used levels of 0.025 percent and 0.1 percent sodium fluoride and 0.5 percent and 2.0 percent aluminum chloride in the diet. Rats fed 0.1 percent sodium fluoride grew at approximately two-thirds the normal rate, but by adding 0.056 percent aluminum chloride to the diet, normal growth was obtained. Also, rats fed the diet containing 0.1 percent sodium fluoride had incisors with a rough and often pitted surface, and sections of the enamel were often chipped. However, rats fed this same diet with 0.5 percent aluminum chloride had incisors with a smooth surface, but they were bleached and the lowers wore down rapidly. It was assumed that aluminum is not absorbed by the rat; therefore the protective action must take place in the intestinal tract, probably by the formation of an aluminum and fluorine-containing compound which is very slightly dissociated.

Sharpless also reported that 1.0 percent calcium carbonate gave some protection, and 2.0 percent caused normal growth in rats receiving 0.1 percent sodium fluoride. Two percent calcium carbonate gave no significant protection to the incisors. The addition of 1.0 percent calcium chloride caused a lower rate of growth than that obtained from sodium fluoride alone.

Venkataramanan and Krishnasuramy (1948) confirmed the ameliorating effect of aluminum salts in fluorosis induced in albino rats, and have shown that the skeletal storage of fluorine is appreciably reduced by the presence of aluminum in the diet.

Majumdar and Ray (1946) reported the addition of calcium or phosphorus salts, in amounts adequate to bring the quantity of and the ratio between the two minerals to optimum levels, helped in protecting the animals for long periods against fluorine intoxication. Ingestion of aluminum sulphate was very effective in preventing fluorosis. A large positive balance of fluorine was observed in all animals given high quantities of the element. The estimation of net retention of fluorine gave no indication of the condition of the animal. However, the poorer the condition of an animal, the greater the quantity of fluorine excreted in the urine. They concluded that urinary output of fluorine was a good index of the intensity of fluorine intoxication. Serum calcium and blood inorganic phosphorus in adult animals were not affected by fluorine feeding.

Marcovitch and Stanley (1942) reported that aluminum sulphate and hydrated lime are capable of saving animals from a lethal dose of sodium fluoride. Boric acid also had some value as an antidote through the formation of a fluoborate. The aluminum sulfate forms cyrolite, which is found to be much less toxic.

Unpublished data at the University of Tennessee indicated that the amount of fluorine stored in the bone of the albino rat is proportional to the level of fluorine fed up to 400 p.p.m. of fluorine added, for a four and six week feeding period. This work indicated that $Al_2(SO_4)_3 \cdot 18\ H_2O$, sublimed $AlCl_3$, XH 1010 (aluminum compound containing 72.5 percent Al_2O_3), and $Al(OH)(C_2H_3O_2)_2$ reduce the absorption of fluorine, as measured by bone storage, when added to rations at levels of 0.5, 1.0, and 2.0 percent.

Once fluorine is deposited in the bone, $Al_2(SO_4)_3.18\ H_2O$, $AlCl_3$ (sublimed), or defluorophos did not increase removal of fluorine from the bone. The rate of fluorine turnover corresponds closely with calcium and phosphorus rate of turnover. It appeared that the amount of alleviation apparently is related to the amount of aluminum ions present in the ration. The lack of solubility of $Al_2O_3.XH_2O$ and amberlite and the solubility of the other aluminum salts suggest the possibility that the alleviation effect may conceivably be tied up with the solubility of the aluminum compounds and the resultant aluminum ion concentration.

CHAPTER III

EXPERIMENTAL PROCEDURE

Seventy-five crossbred feeder wether lambs, eight to twelve months of age and averaging approximately eighty-eight pounds in weight, were used in this study. These animals were fed for approximately a two-month pre-experimental period in an effort to rid them of internal parasites, get them on feed, and accustom them to individual feeding facilities. All animals were docked and treated routinely with phenothiazine. During this period, the daily ration of chopped lespedeza hay and a concentrate mixture (80 percent ground yellow corn and 20 percent wheat bran) was gradually increased until all animals were consuming approximately one pound of hay and one pound of concentrate per head per day. They had free access to salt and water. Individual feed records were initiated on all animals during the last four weeks of this period. The animals were fed in the early morning and late evening, feeding one-half pound of concentrate at each feeding.

Twelve of these lambs, selected at random, were placed in metabolism stalls for a three-week preliminary training period. During this three-week period, final changes and adjustments were made in the metabolism stalls to standardize the balance procedure which was used throughout the experiment.

Following this preliminary period, seven-day collections were made on twelve lambs to determine the digestibility of feeds and mineral balances on representative animals to be used as the control. Representative samples of hay, concentrate, urine, and feces were taken daily from each animal, from which an individual composite sample for the seven-day collection period was analyzed for fluorine, calcium, phosphorus, crude protein, crude fiber, ether extract, and nitrogen-free extract.

Three animals, selected at random from the seventy-five lambs, were sacrificed one day before the experiment started. Tissue samples were taken for fluorine analysis and pathological studies. Results from these studies were used as base controls for all lots.

The remaining seventy-two feeder wether lambs were weighed, paint-branded, and divided into six lots of ten each and three lots of four each, using the criteria of weights, previous feed consumption, and general appearance. The six lots (lots I through VI) of ten animals each were assigned treatments at random and the three lots (lots VII through IX) of four animals each were assigned treatments at random as shown in Table I.

Each animal was assigned an individual stanchion, which was given a number corresponding to the number on the animal. Each lot of animals had a separate loafing pen with free access to salt and clean, fresh water.

The concentrate mixture, consisting of 80 percent ground yellow corn and 20 percent wheat bran, was mixed every two weeks. Fluorine in the form of sodium fluoride was added in p.p.m. (parts per million) to the concentrate mixture, as shown in Table I. The sodium fluoride added to each batch of feed was pre-mixed with a small amount of concentrate previous to mixing with the bulk of the concentrate. After mixing each batch of feed, the mixer was thoroughly cleaned to prevent any contamination.

TABLE I

EXPERIMENTAL DESIGN OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviators Percent	Total F in Ration p.p.m.	Number of Animals	Average Initial Weight (lbs.)	Initial Weight Range (lbs.)
I	0	(Control)	6	10	88.4	81- 99
II	25		31	10	88.3	75- 96
III	50		56	10	88.1	75-113
IV	75		81	10	88.2	76- 96
V	100		106	10	88.3	77-104
VI	200		206	10	88.3	76-106
VII	100	+ .1% $Al_2(SO_4)_3$ [a]	106	4	88.0	78- 95
VIII	100	+ .5% $Al_2(SO_4)_3$ [a]	106	4	88.2	76-102
IX	100	+ .1% $AlCl_3$ [b]	106	4	88.0	81- 97

[a] $Al_2(SO_4)_3$ has 18 H_2O attached.

[b] $AlCl_3$ has 6 H_2O attached.

Aluminum sulfate hydrate ($Al_2(SO_4)_3 . 18\ H_2O$) and aluminum chloride hydrate ($AlCl_3 . 6\ H_2O$) were added in the amounts as shown in Table I, the percentage based on the total ration, and mixed with the concentrate as described for sodium fluoride. The concentrate mixture for each lot was then stored in separate galvanized containers.

Each animal was fed the concentrate mixture, and approximately one hour later the portion not consumed was removed from the feed box and weighed. After removing any concentrate which was refused, one-half pound of good quality chopped lespedeza hay was placed before each animal for approximately one hour, and then the hay weighback was taken. The animals were fed the same amount of concentrate and chopped lespedeza hay in the early morning and late evening from the beginning of the experiment until they were sacrificed. Every fourteen days the animals were individually weighed after the morning feeding, but before they had access to drinking water.

Blood samples were taken periodically for the determination of calcium, phosphorus, potassium, sodium, and magnesium. After the blood samples were drawn, they were allowed to clot at room temperature, centrifuged, and serum removed. The Clark-Collip modification of the Kramer-Tisdall method, as described by Hawk _et al_. (1949), was used for the determination of serum calcium. Phosphorus was determined by a slight modification of the Fiske and SubbaRow method as given by Hawk _et al_. (1949). Sodium and potassium in blood serum was determined by using the flame photometer according to the method described by Perkin-Elmar Instruction Manual (1949). The determination for serum magnesium was according to a combination of methods described by Simorsen et al. (1947) and the modified Denis method as described by Hawk et al. (1949).

Every twenty-eight days, two animals from each lot were placed on metabolism trials for seven days to obtain quantative digestibility and mineral balance data. There was a three-day preliminary period before each metabolism collection to accustom the animals to the metabolism units. During the metabolism trials, the feces were weighed and the urine measured each morning.

Representative samples of concentrate, hay, feed weighback, urine, and feces were taken daily from each animal to make up individual composite samples which were analyzed for fluorine, calcium, phosphorus, nitrogen, crude fiber, ether extract, and nitrogen-free extract.

The method used for the determination of fluorine in the various samples was a slight modification of the perchloric acid distillation and thorium nitrate titration method as described by Willard and Winter (1933). These modifications varied with the samples, since the materials included feeds, urine, feces, and bones for analysis. The fluorine content of feeds and feces was reported in p.p.m. fluorine on an air-dry basis. Urine and soft tissues were reported in p.p.m. fluorine as the samples were normally collected. Bones were reported in p.p.m. fluorine on an ash basis.

Calcium and phosphorus determinations on feeds, feces, and urine were made according to the Association of Official Agricultural Chemists (1950) standard methods or slight modification of these methods.

Proximate analysis of feeds and feces were determined by Association of Official Agricultural Chemists (1950) standard methods or slight modification of these methods. These analyses included ash, moisture, crude protein, ether extract, crude fiber, and nitrogen-free extract, which provided a basis for determining the digestibility of crude protein, crude fiber, ether extract, and nitrogen-free extract.

Every twenty-eight days, two animals each, selected at random, from lots I through VI and one animal each, except for the first period, from lots VII through IX were sacrificed. A gross autopsy was made on each animal. Bone, heart, liver, spleen, kidney, and lung samples were taken for pathological studies and fluorine analyses.

Because of hot weather, all animals that had not been sacrificed were sheared between the 84-day and 112-day periods.

During the third experimental period, rumen samples were taken, via stomach tube, from three animals each of lots I through VI and two animals each of lots VII through IX. Gram stains were prepared of the original material and examined carefully for the morphological types of organisms present with special emphasis on the presence of the coccoid-type organism and the tiny gram positive curved rod. Also, the culturability and concentration of organisms were determined on samples from each animal.

CHAPTER IV

RESULTS AND DISCUSSION

Feed Consumption

At the time the experiment was initiated the animals were consuming one pound of concentrate (80 percent ground yellow corn and 20 percent wheat bran) and approximately one pound of chopped lespedeza hay daily. The hay averaged 6 p.p.m. fluorine (range 4 to 8 p.p.m. F), and the grain for the control ration averaged 6 p.p.m. fluorine (range 4 to 10 p.p.m. F) throughout the experiment. Table II shows the average individual daily feed consumption for each lot per 28-day period throughout the experiment.

There was a slight decrease in the consumption of hay during the first 28-day period in the lots receiving the higher amounts of fluorine; however, lot I (control) consumed slightly less than lot II (25 p.p.m. F added) or lot III (50 p.p.m. F added). Lot VI (200 p.p.m. F added) and lot VII (100 p.p.m. F plus 0.1 percent aluminum sulfate hydrate) consumed less concentrate, especially during the first week, than the lots on lower levels of fluorine. Lot IX (100 p.p.m. F plus 0.1 percent aluminum chloride hydrate) had a good feed consumption record as compared to the other lots during this period.

Lot II (25 p.p.m. F added) consumed slightly more hay and concentrate throughout the experiment than did lot I (control) as shown in Table II. Lot VI (200 p.p.m. F added) consumed less hay and concentrate than the other lots. There was considerable variation in the amount of hay and

TABLE II

AVERAGE DAILY FEED CONSUMPTION OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot	Treatment		Feed Consumed Per 28-Day Period - Grams									
Lot	F Added	Alleviator	1		2		3		4		5	
No.	p.p.m.	Percent	Hay	Grain	Hay	Grain	Hay	Grain	Hay	Grain	Hay	Grain
I	0	(Control)	395	453	421	452	407	444	423	452	437	453
II	25		421	452	434	453	434	453	442	454	452	454
III	50		397	439	416	447	434	454	440	454	430	451
IV	75		379	426	408	449	408	444	420	453	397	434
V	100		379	431	408	452	418	453	435	454	445	452
VI	200		365	324	388	372	400	357	422	344	436	348
VII	100 ≠	.1% $Al_2(SO_4)_3$	387	377	382	425	425	446	442	447	448	450
VIII	100 ≠	.5% $Al_2(SO_4)_3$	402	414	406	422	431	438	440	448	430	447
IX	100 ≠	.1% $AlCl_3$	425	445	421	444	442	452	434	454	441	454

concentrate consumed within each lot throughout the experiment as noted throughout Table II.

During the 140-day feeding trial with lambs, there was no significant decrease in feed consumption in lots I through V (0 to 100 p.p.m. F added); however, there was a significant decrease in lot VI (200 p.p.m. F added). In general, there was no significant decrease in feed consumption of animals in lots VII, VIII, and IX (100 p.p.m. F plus 0.1 percent and 0.5 percent aluminum sulfate hydrate for lots VII and VIII, respectively, and 100 p.p.m. F plus 0.1 percent aluminum chloride hydrate for lot IX). This would indicate that aluminum sulfate hydrate or aluminum chloride hydrate has little or no effect on feed consumption of lambs at this level for a period of 140 days.

Weight Gains

The average beginning weight of the individual lots ranged from 88.0 to 88.4 pounds; however, there was considerable variation within each lot. Table III shows the average beginning weight, the average gain for the different periods, and the total average gain for the entire experiment.

During the first 28-day period, lots III, IV, and VII lost from 1 to 1.4 pounds and lot VI lost 5.2 pounds, which corresponds very closely to feed consumption. All lots gained weight in the second period. During the third period, all lots gained weight with the exception of lot VI. In general, this corresponds to the increase in feed consumption during the second and third periods. All animals were sheared during the fourth

TABLE III

AVERAGE BEGINNING WEIGHT AND GAIN OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Average Beginning Weight (lbs.)	Average Gain Per 28-Day Period (lbs.): 1	2	3	4[a]	5	Total Average Gain (lbs.)
I	0	(Control)	88.4	.8	7.0	5.0	- 9.0	3.5	7.3
II	25		88.3	.6	6.6	4.0	- 8.4	3.5	6.3
III	50		88.1	- 1.4	7.9	2.8	- 6.3	2.0	5.0
IV	75		88.2	- 1.2	6.6	1.5	- 9.8	2.5	- .4
V	100		88.3	0.0	6.1	2.5	- 7.8	4.5	5.3
VI	200		88.3	- 5.2	4.2	- .2	- 7.4	1.0	-7.6
VII	100 +	.1% $Al_2(SO_4)_3$	88.0	- 1.0	7.8	2.9	-12.0	6.0	3.7
VIII	100 +	.5% $Al_2(SO_4)_3$	88.2	1.0	4.2	4.0	-12.5	5.0	1.7
IX	100 +	.1% $AlCl_3$	88.0	3.0	5.8	2.0	-13.0	7.0	4.8

[a]All animals were sheared between the 3rd and 4th periods, which accounts for some of the loss in weight and some of the variation.

period, which partially accounts for the large loss in weight of all lots. All lots gained during the fifth period; however, the alleviator groups gained more.

During the 140-day feeding trial, the lambs in lots I and II (control and 25 p.p.m. F added) gained slightly more than those in the other lots. The total average gains in Table III indicate there is considerable variation between lots and that 200 p.p.m. fluorine added to the ration decreases the weight gains. Also, 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate fed to lambs receiving 100 p.p.m. F for 140 days apparently did not materially affect weight gains.

Digestibility

A summary of the average percentage apparent digestibility for crude proteins, crude fiber, ether extract, and nitrogen-free extract for sheep on various levels of fluorine is shown in Table IV. Column 3 in Table IV gives the number of animals from each lot that were on 7-day metabolism trials. All hay, concentrate, weighback, feces, and urine samples analyzed were a composite of 7-day collections.

The average percentage apparent digestibility of the various fractions is shown periodically in Table XIII. Due to a limited number of metabolism stalls, the animals in lots VII, VIII, and IX were on metabolism trials at a different time, but within the same 28-day period as those of lots I through VI.

TABLE IV

AVERAGE PERCENTAGE APPARENT DIGESTIBILITY OF VARIOUS NUTRIENTS FED TO LAMBS ON KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Number of Animals[a]	Percentage Digestibility: Crude Protein	Crude Fiber	Ether Extract	Nitrogen Free Extract
0	0	(Pre-experimental)	12	59.5	47.4	70.3	80.2
I	0	(Control)	8	61.8	44.1	68.1	77.6
II	25		9	54.2	39.3	59.2	75.9
III	50		8	61.2	50.6	72.0	79.1
IV	75		8	59.6	49.4	70.6	78.5
V	100		7	61.9	51.2	68.0	80.4
VI	200		8	56.6	49.3	70.4	78.0
VII	100 +	.1% $Al_2(SO_4)_3$	7	56.6	47.4	69.0	75.6
VIII	100 +	.5% $Al_2(SO_4)_3$	7	59.6	49.0	69.4	76.7
IX	100 +	.1% $AlCl_3$	7	58.6	46.2	64.0	70.4

[a]Each animal was on trial for 7 days.

There were some variations in the digestibility of the ration nutrients; however, these differences apparently were not associated with the fluorine content of the rations or with the length of time the animals had been consuming the various rations. Similar digestibility results have been obtained on cattle at the University of Tennessee which substantiate these findings. The addition of 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate to the ration of sheep receiving 100 p.p.m. fluorine did not cause any appreciable differences in the digestibility of the various nutrients.

Balance Studies

The fluorine, calcium, phosphorus, and nitrogen balances for sheep on various levels of fluorine are shown in Tables V, VI, VII, and VIII, respectively, and for the convenience of presentation will be discussed separately. Column 3 of these tables gives the number of animals from each lot that were on 7-day metabolism trials.

Tables XIV, XV, XVI, and XVII show the fluorine, calcium, phosphorus, and nitrogen balances, respectively, for sheep on various levels of fluorine for each 28-day period. This data was determined from the samples collected during the metabolism trials, each collection being for a 7-day period. The feeds and feeding practices for animals on metabolism trials were the same as those for the remaining part of the experiment. Water was not considered in these balance studies because the same source of low fluorine water was used by animals in all lots.

Fluorine Balance

The average daily milligrams of ingested, urinary, fecal, and retained fluorine for animals on various levels of fluorine are shown in Table V. Generally, the milligrams of ingested fluorine increases with the amount of fluorine added to the ration; however, in working with an element where micro amounts are present, the total amount of feed consumed, sampling errors, and chemical determination errors may cause some variation.

Collections made during the first and second periods (Table XIV) indicate the fluorine intake of animals from lots I through V (0 to 100 p.p.m. F added) corresponds to the amount added to the ration, but the fluorine intake of animals from lot VI (200 p.p.m. F added) was less than that of animals from lot V (100 p.p.m. F added). This can partially be explained by the lower feed consumption of animals from lot VI during the first and second experimental periods. Ingested fluorine from animals of lots I through VI increased with the fluorine content of rations for the third, fourth, and fifth periods, with the exception of lots III and IV during the fourth period and lot IV during the fifth period (Table XIV). The fluorine intake of lots VII, VIII, and IX corresponds very closely with that of lot V, indicating that 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate has very little effect on feed consumption of animals receiving 100 p.p.m. fluorine added to their rations.

The average urinary fluorine increased as the fluorine content of the rations increased for lots I through V (0 to 100 p.p.m. F added); however, some of the individual analyses were not consistent. This is in

TABLE V

AVERAGE DAILY FLUORINE BALANCE OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Number of Animals[a]	Fluorine - Milligrams: Intake	Urine	Feces	Retained	Retained Percent
0	0	(Pre-experimental)	12	3.6	.6	1.4	1.6	44.4
I	0	(Control)	10	6.1	3.8	2.3	0.0	0.0
II	25		10	27.4	9.1	3.6	14.7	53.6
III	50		9	40.6	10.4	5.9	24.3	59.8
IV	75		10	74.9	11.8	5.9	57.2	76.4
V	100		9	86.1	22.1	7.6	56.4	65.5
VI	200		10	107.4	18.6	11.3	77.5	72.2
VII	100 ≁	.1% $Al_2(SO_4)_3$	7	95.0	10.9	20.7	63.4	66.7
VIII	100 ≁	.5% $Al_2(SO_4)_3$	7	101.8	11.3	27.5	63.0	61.9
IX	100 ≁	.1% $AlCl_3$	7	92.5	11.3	33.3	47.9	51.8

[a]Each animal was on trial for 7 days.

concordance with work on cattle, reported by Hobbs et al. (1951), indicating that the urinary fluorine may assist in determining the level of fluorine being consumed at a given time, provided the level is low and a large number of animals are sampled. Urinary fluorine of animals from lot VI (200 p.p.m. F added) was less than that of animals from lot V (100 p.p.m. F added). This indicates that total urinary fluorine of animals on high fluorine intake (200 p.p.m. F added) may not be a very reliable estimate of fluorine consumption; however, it is granted that these ten 7-day collections are a limited amount of data.

Urine collections were taken from animals in lots VII, VIII, and IX during all periods except the first, and the analyses showed that urinary fluorine was lower for each period than that from animals in lot V (Table XIV). The overall summary (Table V) shows that animals from lots VII, VIII, and IX excreted approximately one-half as much urinary fluorine as did animals from lot V. This indicates that 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate significantly decreases the urinary fluorine of animals receiving 100 p.p.m. fluorine added to the ration.

The total fecal fluorine of animals from lots I through VI, generally, increased as the fluorine content of the ration increased (Table V); however, there was considerable variation by different animals and for different periods. This is in partial concordance with theories that total fecal fluorine may be used as an aid in determining levels of currently ingested fluorine, provided large numbers of animals are sampled.

Average daily fecal fluorine of animals from lots VII, VIII, and IX was approximately three to four times that of animals from lot V (Table V). This indicates that 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate tie up ingested fluorine in the gastro-intestinal tract with the aluminum chloride showing some advantage over the aluminum sulfate. This is in concordance with the decreased urinary fluorine of animals from these lots.

In general, the total retained fluorine increased as the fluorine content of the rations increased, although there was a great amount of variation between animals and periods of time. Part of this variation may result because the retained fluorine is determined by subtracting the urinary and fecal fluorine from the total ingested fluorine, thereby increasing the possibility of determination error. The addition of 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate to the ration of sheep receiving 100 p.p.m. fluorine did not indicate any significant effect upon fluorine retention. This is not in concordance with the 20 to 30 percent reduction in the fluorine content of bones from animals receiving the aluminum compounds as compared to those receiving the same amount of fluorine with no alleviators (Table X). However, it should be kept in mind that a balance trial is over a very short period of time and, at best, under some abnormal conditions. Mineral studies with sheep, cattle, and swine should be conducted over a good part of an animal's productive life for most useful results to the livestock industry.

Calcium Balance

Table VI gives the average daily grams of ingested, urinary, fecal, and retained calcium for sheep fed various levels of fluorine and alleviators. There were no significant differences in the average daily ingested, urinary, fecal, or retained calcium that could be associated with the level of fluorine intake, except that the ingested and retained calcium for lot VI was lower than that of the other lots. This can partially be explained by the lower feed consumption of animals in lot VI. The calcium balance did not appear to be affected by the use of dietary aluminum sulfate hydrate or aluminum chloride hydrate.

Phosphorus Balance

The average daily grams of ingested, urinary, fecal, and retained phosphorus is shown in Table VII. The phosphorus content of the samples collected from animals of lot I through lot VI during the third metabolism trial are not included in this table because of an unavoidable accident after the samples were collected; however, the samples that were analyzed for this period are shown in Table XVI.

The ingested phosphorus of animals from lot VI (200 p.p.m. F added) was lower than that of other lots, mainly because of a lower feed consumption. Table VII shows that the animals from lots VII, VIII, and IX had slightly more phosphorus in the feces, less retained, and a lower percent retained than that of animals from lot V. This may partially be explained by the lower phosphorus intake; however, it may have been caused by the addition of aluminum sulfate or aluminum chloride to the ration.

TABLE VI

AVERAGE DAILY CALCIUM BALANCE OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Number of Animals[a]	Calcium - Grams: Intake	Calcium - Grams: Urine	Calcium - Grams: Feces	Calcium - Grams: Retained	Retained Percent
0	0	(Pre-experimental)	12	4.45	.08	3.74	.63	14.2
I	0	(Control)	10	4.51	.08	3.28	1.15	25.5
II	25		10	4.41	.06	3.34	1.01	22.9
III	50		9	4.50	.06	3.12	1.32	29.3
IV	75		10	4.29	.03	2.92	1.34	31.2
V	100		9	4.35	.02	3.04	1.29	29.7
VI	200		10	3.67	.02	2.99	.66	18.0
VII	100 +	.1% $Al_2(SO_4)_3$	7	4.85	.05	3.18	1.62	33.4
VIII	100 +	.5% $Al_2(SO_4)_3$	7	4.86	.09	3.28	1.49	30.7
IX	100 +	.1% $AlCl_3$	7	4.79	.04	3.13	1.62	33.8

[a]Each animal was on trial for 7 days.

TABLE VII

AVERAGE DAILY PHOSPHORUS BALANCE OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Number of Animals[a]	Phosphorus - Grams: Intake	Phosphorus - Grams: Urine	Phosphorus - Grams: Feces	Phosphorus - Grams: Retained	Retained Percent
0	0	(Pre-experimental)	12	3.24	.01	2.55	.68	21.0
I	0	(Control)	8	3.74	.01	2.70	1.03	27.5
II	25		8	3.78	.01	2.74	1.03	27.2
III	50		7	3.74	.01	2.39	1.34	35.8
IV	75		8	3.58	.02	2.18	1.38	38.5
V	100		7	3.80	.03	2.18	1.59	41.8
VI	200		8	2.92	.03	1.76	1.13	38.7
VII	100 ≁	.1% $Al_2(SO_4)_3$	7	3.13	.01	2.73	.39	12.5
VIII	100 ≁	.5% $Al_2(SO_4)_3$	7	3.11	.01	2.59	.51	16.4
IX	100 ≁	.1% $AlCl_3$	7	3.12	.02	2.52	.58	18.6

[a]Each animal was on trial for 7 days.

Nitrogen Balance

The differences in nitrogen intake, excretion, and retention (Table VIII) were apparently not associated with the fluorine content of rations consumed, except animals in lot VI had a lower nitrogen intake, excretion, and retention than those on lower levels of fluorine. This may be caused by a lower feed consumption.

The fecal nitrogen was slightly greater and the urinary and retained nitrogen slightly less for animals in lot V (100 p.p.m. F added) than that of animals in lots VII, VIII, and IX (100 p.p.m. F added plus 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate, respectively). However, this is a small difference and is not in concordance with the digestibility of crude proteins.

Blood Studies

Blood samples were taken from all animals in each lot at various intervals throughout the experiment. The samples were allowed to clot, centrifuged, serum removed, and analyzed for phosphorus, sodium,potassium, magnesium, and calcium. Table IX shows the average analyses of the entire experiment for each lot. Column 3 in Table IX gives the total number of samples analyzed from each lot. It is noted that all analyses fall within the normal range for sheep at this age according to Dukes (1947).

Differences are noted in these analyses; however, these differences cannot be associated with the fluorine content of the rations. It is noted

TABLE VIII

AVERAGE DAILY NITROGEN BALANCE OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Number of Animals[a]	Nitrogen - Grams: Intake	Nitrogen - Grams: Feces	Nitrogen - Grams: Urine	Nitrogen - Grams: Retained	Retained Percent
0	0	(Pre-experimental)	12	15.23	6.16	4.33	4.74	31.1
I	0	(Control)	10	14.50	5.57	3.96	4.97	34.3
II	25		10	14.50	6.44	4.53	3.53	24.3
III	50		9	14.54	5.67	4.65	4.22	29.0
IV	75		10	13.94	5.64	4.11	4.19	30.1
V	100		9	14.28	5.63	4.82	3.83	26.8
VI	200		10	11.78	5.08	3.72	2.98	25.3
VII	100 ≠	.1% $Al_2(SO_4)_3$	7	14.65	4.81	5.38	4.46	30.4
VIII	100 ≠	.5% $Al_2(SO_4)_3$	7	14.27	4.90	5.08	4.29	30.1
IX	100 ≠	.1% $AlCl_3$	7	15.46	4.68	5.73	5.05	32.7

[a]Each animal was on trial for 7 days.

TABLE IX

PHOSPHORUS, SODIUM, POTASSIUM, MAGNESIUM AND CALCIUM CONTENT OF BLOOD FROM LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. of Samples Taken	Phosphorus mg. P/100 ml. Serum	Sodium mg. Na/100 ml. Serum	Potassium mg. K/100 ml. Serum	Magnesium mg. Mg/100 ml. Serum	Calcium mg. Ca/100 ml. Serum
I	0	(Control)	52	7.42	344.8	24.11	2.10	12.19
II	25		52	7.85	339.6	23.44	2.07	11.71
III	50		48	7.69	342.5	23.43	2.05	11.68
IV	75		52	8.35	339.4	22.47	2.12	11.81
V	100		52	9.21	343.3	24.89	2.36	11.48
VI	200		52	8.98	342.2	24.07	2.09	11.60
VII	100 ≁	.1% $Al_2(SO_4)_3$	24	8.20	343.4	28.01	2.14	12.10
VIII	100 ≁	.5% $Al_2(SO_4)_3$	24	7.89	340.6	24.36	2.18	12.71
IX	100 ≁	.1% $AlCl_3$	24	8.37	341.1	23.54	2.20	12.13

in Table IX that blood serum, from animals receiving the higher levels of fluorine, had a slightly higher phosphorus content than that of animals on a control ration.

Tables XVIII through XXII show that analyses of blood serum vary from one period to another. It is noted that the calcium content of blood serum at the 111-day period for all lots was, in general, higher than that for other periods. These variations at different periods apparently did not follow any trends that could be associated with the levels of fluorine in the rations used in this experiment.

These analyses indicated that the fluorine content of feed, up to 200 p.p.m. fluorine, did not affect the phosphorus, sodium, potassium, magnesium, or calcium level of blood serum from lambs on these treatments over a period of 140 days. Also, 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, or 0.1 percent aluminum chloride hydrate, in the ration, apparently did not show any consistent differences in animals receiving 100 p.p.m. fluorine for a period of 140 days.

Fluorine Content of Bones

Table X shows the fluorine content of a sagittal section of the metacarpals and the angle of the mandible bones from lambs sacrificed at the end of each experimental period. These results are reported in p.p.m. fluorine on an ash basis. To convert this value to a fat-free basis, decrease it by approximately one-third. Column 3 in Table X gives the number of animals sacrificed at the end of each period except as noted.

TABLE X

FLUORINE CONTENT OF METACARPALS AND MANDIBLES OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Number Killed Each Period	Fluorine Content of Bones[a] Per 28-Day Period: 1 Leg	1 Jaw	2 Leg	2 Jaw	3 Leg	3 Jaw	4 Leg	4 Jaw	5 Leg	5 Jaw
I	0	(Control)[b]	2	285	345	440	530	465	745	605	915	675	940
II	25		2	835	1025	1100	1450	1150	1800	1500	2000	1500	2350
III	50		2	895	1350	1633[c]	2133[c]	2200	2800	1800	3350	1900[d]	4000[d]
IV	75		2	1210	1550	2750	3250	3300	4500	3200	4600	3800	5850
V	100		2	1050	2200	3000	3900	3450	5300	3500[d]	6350	4950	8050
VI	200		2	1650	3400	3150	5550	3100	6850	4900	8300	6500	9500
VII	100 ≠	.1% $Al_2(SO_4)_3$	1	----	----	2600	3700	3600	5000	3400	5800	4200	6400
VIII	100 ≠	.5% $Al_2(SO_4)_3$	1	----	----	2700	3400	2400	3700	3000	4900	3200	5200
IX	100 ≠	.1% $AlCl_3$	1	----	----	2800	3600	2800	4000	3000	4800	3400	5900

[a]Reported in p.p.m. on ash basis.
[b]Pre-experimental lambs' legs averaged 470 p.p.m. F and jaw averaged 497 p.p.m. F.
[c]Average of three animals, but one animal was killed 45 days after experiment started.
[d]One animal.

No animals were killed from lots VII, VIII, and IX at the end of the first period, because only four animals were used in each of these lots, and it was assumed more valuable results could be obtained from the alleviator groups by holding them until the later periods.

The fluorine content of the mandibles was higher than the fluorine content of metacarpals of all animals, which is in concordance with other work on storage of fluorine in bones of animals that have been receiving rations containing large amounts of fluorine. Isotope studies with various minerals have substantiated these bone differences and effects of time on a given ration.

The base fluorine content of the bones increased as a function of intake and time on a given ration at each of the five experimental periods. Also, the fluorine content of bones increased with the length of time on a given ration, with the exception of lot IV during the third period, and this value was exceptionally high because the fluorine concentration in bones from one animal was very high.

The mandibles and metacarpals of animals for lots VII, VIII, and IX (100 p.p.m. F added plus alleviators) had a lower fluorine concentration for all periods than those of animals in lot V (100 p.p.m. F added). At the end of the second period this decrease in fluorine concentration was small for the alleviator groups; however, at the end of the fifth period it was approximately 15 percent for animals receiving 0.1 percent aluminum sulfate hydrate, approximately 35 percent for the animals receiving 0.5 percent aluminum sulfate hydrate, and approximately 30 percent for the animals receiving 0.1 percent aluminum chloride hydrate.

This shows that storage of fluorine in bone can be lowered by the addition of either aluminum sulfate hydrate or aluminum chloride hydrate to the ration of lambs ingesting increased levels of fluorine.

Histological sections of the jaw and leg bones did not show any significant differences that could be associated with the various treatments.

Fluorine Content of Soft Tissues

The average fluorine content of livers, hearts, kidneys, lungs, and spleens of animals sacrificed during the pre-experimental period and at the end of the first and fourth periods is shown in Table XI in p.p.m. fluorine on a wet basis. Fluorine determinations were made on tissues from one animal each of lots VII, VIII, and IX, and these animals were sacrificed at the end of the fourth period, consequently, providing a limited amount of data on these lots.

Differences were noted in the fluorine content of the various tissues; however, these differences apparently were not associated with fluorine intake. The kidneys from animals in lot VI, sacrificed at the end of the fourth period, were higher than those of animals in other lots, which may be due to urine in the kidney that was high in fluorine. The livers, lungs, and spleens from animals of lots VII, VIII, and IX that were sacrificed at the end of the fourth period, were lower in fluorine concentration than the tissues of corresponding animals of lot V; however, this data is too limited to assume the difference was due to the alleviators.

TABLE XI

FLUORINE CONTENT OF TISSUES OF LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Fluorine Content of Tissues Per 28-Day Period - (p.p.m.): 1 Liver	1 Heart	1 Kidney	1 Lung	1 Spleen	4 Liver	4 Heart	4 Kidney	4 Lung	4 Spleen
I	0	(Control)[a]	2	.23	.36	1.04	.54	.43	.30	.34	1.32	.59	.62
II	25		2	.63	.92	1.11[b]	.48	.71	1.64	.54	1.16	.48	.44
III	50		2	.33[b]	.14[b]	1.27[b]	.40[b]	.65[b]	.21	.49	1.22[b]	1.53[b]	.76
IV	75		2	.34	.21	.86[b]	.28	.58	.34	.40	1.68	.38	.53
V	100		2	.16	.11	.43	.27	.05	.62	.66	1.29	.80	1.08
VI	200		2	.47	.47[b]	1.42	.05	.50	.42	.48	2.73	.81	.44
VII	100 +	.1% $Al_2(SO_4)_3$	1	—	—	—	—	—	.55	.92	1.57	.75	.54
VIII	100 +	.5% $Al_2(SO_4)_3$	1	—	—	—	—	—	.31	.97	1.20	.56	.50
IX	100 +	.1% $AlCl_3$	1	—	—	—	—	—	.50	.42	.85	.38	.49

[a]Two pre-experimental animals' tissues averaged as follows: Liver, 0.89 p.p.m. F; heart, 1.5 p.p.m. F; kidney, 3.1 p.p.m. F; lung, 0.92 p.p.m. F; spleen, 0.92 p.p.m. F.

[b]One animal.

Histological sections of livers, hearts, kidneys, lungs, and spleens from lambs on the various treatments were made. These sections did not show any significant differences that could be associated with the various treatments under the conditions of this experiment.

Fluorine Content of Urine

Many workers have considered urinary excretion of fluorine by an animal as a method of obtaining an indication of fluorine ingestion. Blakemore _et al_. (1948) reported that the urinary level at any given time is influenced not only by the current rate of absorption from food, but also by the rate of excretion from the skeleton of fluorine stored in the past. Also, it is very important to consider the fluorine content of the present and past rations, length of time animals have been on a particular ration, age of the animal, and age of the animal when put on the ration in question.

Table XII shows the average p.p.m. fluorine in urine, overall average fluorine content, and the average daily milligrams of fluorine in urine from lambs fed known levels of fluorine and alleviators for different intervals of time. Considerable variation in the fluorine content of urine from one period to another is noted. In general, the fluorine content of urine during the last period is higher than that of the other periods.

In Figure 1 the p.p.m. fluorine in the ration and the p.p.m. fluorine in urine are plotted in broken lines and the milligrams of fluorine consumed and milligrams of urinary fluorine are plotted in solid

TABLE XII

FLUORINE CONTENT OF URINE FROM LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS FOR DIFFERENT TIME INTERVALS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Number of Animals[a]	Fluorine Content Periodically - (p.p.m.): 21 Days	54 Days	83 Days	112 Days	143 Days	Average F in Urine (p.p.m.)	Average F in Urine Per Day (mgs.)
I	0	(Control)[b]	2	3.0	11.0	7.0	3.5	7.5	6.4	3.8
II	25		2	36.5	22.5	17.0	11.5	34.0	24.3	9.1
III	50		2	43.0	34.0	35.0	10.0	49.0	32.6	10.4
IV	75		2	68.5	58.0	58.0	24.5	60.0	53.8	11.8
V	100		2	94.5	77.5	62.0	39.5	41.0[g]	65.3	22.1
VI	200		2	91.5	53.0	48.5	37.0	72.0	60.4	18.6
VII	100 +	.1% $Al_2(SO_4)_3$	2	----	65.5[c]	6.7[d]	33.5[e]	50.0[f,g]	37.3	10.9
VIII	100 +	.5% $Al_2(SO_4)_3$	2	----	66.5[c]	11.7[d]	37.0[e]	65.0[f,g]	42.2	11.3
IX	100 +	.1% $AlCl_3$	2	----	56.5[c]	10.5[d]	29.5[e]	54.0[f,g]	35.3	11.3

[a]Each animal on trial for 7 days during each period.
[b]Urine from twelve pre-experimental animals averaged 4.4 p.p.m. F.
[c]41 days from beginning of experiment.
[d]69 days from beginning of experiment.
[e]103 days from beginning of experiment.
[f]133 days from beginning of experiment.
[g]One animal.

CORRELATION OF FLUORINE IN RATIONS TO URINARY FLUORINE IN LAMBS

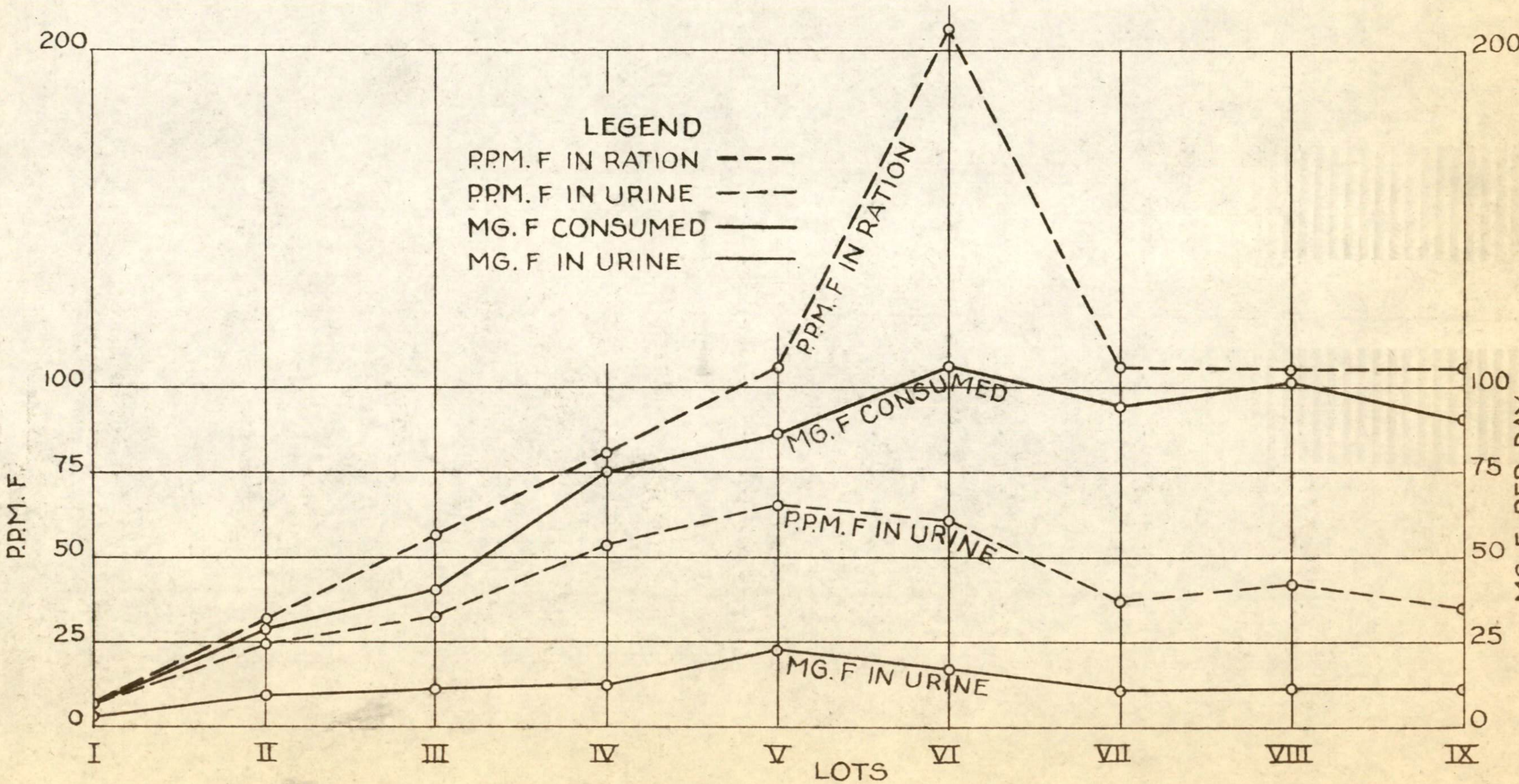

FIGURE 1.

lines for the entire experiment. The correlation of the plotted factors are as follows:

	Lots I - IX	Lots I - VI
p.p.m. F in urine vs p.p.m. F in current ration	.778[a]	.816[b]
p.p.m. F in urine vs mgs. F in urine	.927[a]	.933[b]
p.p.m. F in urine vs mgs. F consumed	.778[a]	.965[b]
mg. F in urine vs p.p.m. F in current ration	.741[a]	.798[b]

It was necessary to determine the correlations of these factors for lots I through VI and also for lots I through IX because alleviators were used in the rations of animals in lots VII through IX. In using these correlations, it is very important to keep in mind the alleviators used and also the fluorine content of bones as given in Table X.

This statistical analysis shows that the fluorine content of rations and urinary fluorine are correlated at either the .01 or .05 level. However, it should be emphasized that the fluorine content of urine alone should not be used to determine the approximate amount of fluorine consumed at a given time. The urinary fluorine of an animal

---

[a] Significant at .01 level.

[b] Significant at .05 level.

may also be influenced by the rate of excretion from the skeleton of fluorine stored in the past, amount and rate of fluorine in rations previously consumed, length of time on a given ration, age of the animal, and age of the animal when put on a ration containing increased amounts of fluorine.

Rumen Bacteriological Studies

Bacteriological studies were conducted on three animals each from lots I through VI and two animals each from lots VII through IX. The rumen samples were taken approximately sixty days after the animals were put on various levels of fluorine and alleviators. Gram stains were examined in the usual manner and no significant decrease appeared in the concentration of the coccoid-type organism or in the tiny gram positive curved rods. The degree of parasitism of fibers appeared to be uniformly heavy for all lots.

Bacteriological slide counts, which averaged about sixty billion per gram, were uniformly higher for the control animals than for those receiving fluorine. Animals receiving up to 200 p.p.m. fluorine maintained a count of approximately thirty-five to forty billion per gram, but animals receiving 200 p.p.m. fluorine showed about twenty-five billion per gram. Animals receiving 100 p.p.m. fluorine plus alleviators averaged about thirty billion per gram. Since the animals had been receiving the fluorine for only a short period of time, this may be only a temporary effect and the rumen flora may adjust itself to the conditions imposed by the presence of fluorine.

There appeared to be no apparent difference between lots in the height of cultural growth or the distribution of the various types of organisms isolated. Animals receiving 100 p.p.m. fluorine with and without aluminum compounds gave a similar bacteriological picture in respect to their rumen contents.

CHAPTER V

SUMMARY

The effects of fluorine and alleviators were studied periodically on feeder lambs over a period of 140 days. The basal ration consisted of one pound of concentrate (80 percent ground yellow corn and 20 percent wheat bran) and one pound of chopped lespedeza hay per head per day.

Sodium fluoride was added to the concentrate mixture of the first six lots (I through VI) in the following amounts: 0, 25, 50, 75, 100, and 200 p.p.m. F, respectively. Lots VII through IX received 100 p.p.m. F added as sodium fluoride plus 0.1 percent aluminum sulfate hydrate, 0.5 percent aluminum sulfate hydrate, and 0.1 percent aluminum chloride hydrate, respectively. The basal hay and concentrate averaged 6 p.p.m. fluorine.

Under the conditions of the experiment the lambs receiving 200 p.p.m. fluorine consumed slightly less feed and gained slightly less weight than animals in the other lots. The apparent digestibility values of crude protein, crude fiber, ether extract, and nitrogen-free extract did not show any significant differences that could be attributed to the various treatments.

In general, as the level of fluorine intake increased, the retained fluorine increased as measured by bone storage and balance studies. Calcium and nitrogen balance studies did not show any significant differences that could be attributed to the intake of fluorine or alleviators used in this study. Differences were noted in the phosphorus balance studies; however, these differences apparently were not associated with the fluorine intake, but there was an indication that the alleviators used in this study may decrease the phosphorus retention.

Sodium, potassium, magnesium, and calcium levels of blood from these lambs apparently did not show any significant differences that can be associated with the various levels of fluorine and alleviators.

The fluorine content of mandibles and metacarpals increased as the ingested fluorine of lambs increased. Bones from lambs receiving 100 p.p.m. fluorine with alleviators had a lower fluorine concentration than those of lambs fed similar rations without alleviators. The concentration of fluorine in bones of all lambs increased as a function of time.

Various levels of fluorine and alleviators fed to lambs, under the conditions of this experiment, were not measurably associated with the fluorine concentration of the livers, hearts, kidneys, lungs, and spleens of these lambs.

In general, the urinary fluorine increased as the fluorine intake increased, and the alleviators used in this study decreased the urinary fluorine as compared to urine from lambs receiving a similar treatment without alleviators.

The pathological and bacteriological studies did not show any consistent differences that could be significantly correlated with levels of fluorine intake or alleviators; however, there may be some indication of a decrease in the concentration of rumen micro-organisms in lambs receiving the higher levels of fluorine.

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APPENDIX

TABLE XIII

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE PERCENTAGE APPARENT DIGESTIBILITY OF VARIOUS NUTRIENTS FED TO LAMBS ON KNOWN FLUORINE LEVELS

				Crude Protein					Crude Fiber				
	Treatment			Digestibility Periodically Percent					Digestibility Periodically Percent				
Lot No.	F Added p.p.m.	Alleviator Percent	No. Animals	21 Days	54 Days	83 Days	112 Days	143 Days	21 Days	54 Days	83 Days	112 Days	143 Days
I	0	(Control)	2	53.8	60.7	----	72.4	60.3	34.0	50.7	----	47.2	44.6
II	25		2	60.2	55.0	40.0[e]	62.2	53.4	45.9	43.8	20.3[e]	44.3	42.2
III	50		2	60.0	60.6	54.6[e]	61.7	69.2[e]	49.5	50.2	46.5[e]	47.4	59.5[e]
IV	75		2	57.2	61.0	----	59.4	60.6	49.5	47.9	----	49.7	50.3
V	100		2	58.8	56.8	----	60.7	71.2[e]	46.0	46.0	----	49.7	63.2[e]
VI	200		2	54.4	54.4	----	60.6	56.8	47.6	50.4	----	49.3	49.8
VII	100 ≠	.1% $Al_2(SO_4)_3$	2	----	51.6[a]	55.9[b]	60.1[c]	58.8[d,e]	----	47.4[a]	52.2[b]	46.5[c]	43.7[d,e]
VIII	100 ≠	.5% $Al_2(SO_4)_3$	2	----	59.4[a]	56.3[b]	60.7[c]	62.1[d,e]	----	53.0[a]	47.3[b]	52.2[c]	43.7[d,e]
IX	100 ≠	.1% $AlCl_3$	2	----	55.3[a]	55.4[b]	66.2[c]	57.6[d,e]	----	49.6[a]	48.7[b]	49.6[c]	37.0[d,e]

TABLE XIII

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE PERCENTAGE APPARENT DIGESTIBILITY OF VARIOUS NUTRIENTS FED TO LAMBS ON KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Ether Extract: Digestibility Periodically Percent: 21 Days	54 Days	83 Days	112 Days	143 Days	Nitrogen Free Extract: Digestibility Periodically Percent: 21 Days	54 Days	83 Days	112 Days	143 Days
I	0	(Control)	2	68.8	70.7	——	66.2	66.8	76.4	78.6	——	77.2	78.3
II	25		2	68.5	67.0	42.3[e]	61.3	56.8	79.9	77.6	66.6[e]	79.5	75.9
III	50		2	74.2	73.8	69.7[e]	69.4	72.7[e]	80.4	79.7	76.0[e]	79.8	79.7[e]
IV	75		2	71.0	73.7	——	68.3	69.5	77.0	78.9	——	78.8	79.2
V	100		2	66.5	70.9	——	64.4	70.0[e]	79.8	79.4	——	79.4	83.0[e]
VI	200		2	69.6	77.4	——	69.4	65.2	75.4	77.3	——	80.8	78.6
VII	100 ≠	.1% $Al_2(SO_4)_3$	2	——	70.9[a]	76.5[b]	60.5[c]	68.1[d,e]	——	74.6[a]	77.0[b]	75.0[c]	75.7[d,e]
VIII	100 ≠	.5% $Al_2(SO_4)_3$	2	——	70.2[a]	73.2[b]	66.4[c]	67.9[d,e]	——	79.8[a]	76.3[b]	75.4[c]	75.2[d,e]
IX	100 ≠	.1% $AlCl_3$	2	——	63.8[a]	70.1[b]	62.2[c]	59.8[d,e]	——	77.6[a]	78.3[b]	——	75.2[d,e]

[a]41 days.
[b]69 days.
[c]103 days.
[d]133 days.
[e]One animal.

TABLE XIV

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY FLUORINE BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Fluorine Balance Periodically - Milligrams: Pre-experimental Intake	Pre-experimental Feces	Pre-experimental Urine	Pre-experimental Retained	21 Days Intake	21 Days Feces	21 Days Urine	21 Days Retained
0	0	(Pre-experimental)	12	3.6	1.4	.6	1.6	----	----	----	----
I	0	(Control)	2	----	----	----	----	4.5	2.6	1.0	.9
II	25		2	----	----	----	----	25.7	2.4	8.4	14.9
III	50		2	----	----	----	----	57.2	4.9	12.5	39.8
IV	75		2	----	----	----	----	79.9	6.2	7.1	66.6
V	100		2	----	----	----	----	100.4	6.8	19.6	74.0
VI	200		2	----	----	----	----	93.1	7.8	16.2	69.1
VII	100 ≠	.1% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
VIII	100 ≠	.5% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
IX	100 ≠	.1% $AlCl_3$	2	----	----	----	----	----	----	----	----

TABLE XIV

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY FLUORINE BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Fluorine Balance Periodically - Milligrams: 54 Days Intake	54 Days Feces	54 Days Urine	54 Days Retained	83 Days Intake	83 Days Feces	83 Days Urine	83 Days Retained
0	0	(Pre-experimental)	12	——	——	——	——	——	——	——	——
I	0	(Control)	2	7.7	1.8	4.7	1.2	6.2	2.3	7.1	- 3.2
II	25		2	32.6	3.5	9.3	19.8	10.9	4.1	10.2	- 3.4
III	50		2	41.2	4.2	12.8	24.2	31.7	9.0	12.5	10.2
IV	75		2	77.8	5.7	14.4	57.7	72.9	4.9	14.2	53.8
V	100		2	102.7	8.0	20.6	74.1	76.0	6.2	24.9	44.9
VI	200		2	82.4	8.2	16.6	57.6	106.5	11.8	18.2	76.5
VII	100 ≁	.1% $Al_2(SO_4)_3$	2	117.0[a]	12.2[a]	15.4[a]	89.4[a]	98.9[b]	47.8[b]	2.5[b]	48.6[b]
VIII	100 ≁	.5% $Al_2(SO_4)_3$	2	134.2[a]	16.2[a]	16.7[a]	101.3[a]	92.3[b]	55.8[b]	2.0[b]	34.5[b]
IX	100 ≁	.1% $AlCl_3$	2	111.0[a]	23.2[a]	14.4[a]	73.4[a]	106.4[b]	56.8[b]	3.3[b]	46.3[b]

TABLE XIV

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY FLUORINE BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Fluorine Balance Periodically - Milligrams: 112 Days Intake	112 Days Feces	112 Days Urine	112 Days Retained	143 Days Intake	143 Days Feces	143 Days Urine	143 Days Retained
0	0	(Pre-experimental)	12	----	----	----	----	----	----	----	----
I	0	(Control)	2	6.4	1.6	4.4	.4	5.8	3.2	2.0	.6
II	25		2	30.9	2.6	9.6	18.7	36.7	5.3	8.1	23.3
III	50		2	24.0	6.4	3.4	14.2	57.5[e]	4.3[e]	10.8[e]	42.4[e]
IV	75		2	100.2	6.8	7.0	86.4	43.9	6.1	16.3	21.5
V	100		2	55.2	8.2	19.4	27.6	106.7[e]	10.0[e]	29.9[e]	66.8[e]
VI	200		2	57.9	7.9	18.2	31.8	197.1	21.0	23.8	152.3
VII	100 ≁	.1% $Al_2(SO_4)_3$	2	68.0[c]	5.9[c]	10.8[c]	51.3[c]	96.9[d,e]	12.8[d,e]	18.9[d,e]	65.2[d,e]
VIII	100 ≁	.5% $Al_2(SO_4)_3$	2	75.7[c]	16.4[c]	9.9[c]	49.4[c]	108.4[d,e]	15.7[d,e]	22.0[d,e]	70.7[d,e]
IX	100 ≁	.1% $AlCl_3$	2	60.3[c]	25.9[c]	12.6[c]	21.8[c]	92.2[d,e]	21.6[d,e]	18.6[d,e]	52.0[d,e]

[a]41 days.
[b]69 days.
[c]103 days.
[d]133 days.
[e]One animal.

TABLE XV

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY CALCIUM BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Calcium Balance Periodically - Grams: Pre-experimental Intake	Pre-experimental Feces	Pre-experimental Urine	Pre-experimental Retained	21 Days Intake	21 Days Feces	21 Days Urine	21 Days Retained
0	0	(Pre-experimental)	12	4.45	3.74	.08	.63	----	----	----	----
I	0	(Control)	2	----	----	----	----	4.00	4.21	.14	- .35
II	25		2	----	----	----	----	3.95	3.74	.14	.07
III	50		2	----	----	----	----	4.06	3.93	.15	- .02
IV	75		2	----	----	----	----	3.87	3.83	.04	.00
V	100		2	----	----	----	----	4.04	3.75	.02	.27
VI	200		2	----	----	----	----	3.54	3.76	.01	- .23
VII	100 +	.1% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
VIII	100 +	.5% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
IX	100 +	.1% $AlCl_3$	2	----	----	----	----	----	----	----	----

TABLE XV

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY CALCIUM BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment		No. Animals	Calcium Balance Periodically - Grams							
	F Added p.p.m.	Alleviator Percent		54 Days				83 Days			
				Intake	Feces	Urine	Retained	Intake	Feces	Urine	Retained
0	0	(Pre-experimental)	12	——	——	——	——	——	——	——	——
I	0	(Control)	2	4.54	2.76	.03	1.75	4.08[e]	3.02[e]	.14[e]	.92[e]
II	25		2	5.19	3.18	.03	1.98	3.85	3.67	.04	.14
III	50		2	4.99	2.83	.05	2.11	4.31	2.96	.04	1.31
IV	75		2	4.98	2.54	.02	2.42	——	2.76	.02	——
V	100		2	4.73	3.04	.02	1.67	——	3.85	.03	——
VI	200		2	3.51	2.76	.02	.73	——	2.99	.02	——
VII	100 ≠	.1% $Al_2(SO_4)_3$	2	3.71[a]	3.40[a]	.06[a]	.25[a]	6.25[b]	2.96[b]	.06[b]	3.23[b]
VIII	100 ≠	.5% $Al_2(SO_4)_3$	2	4.03[a]	3.23[a]	.19[a]	.61[a]	6.20[b]	3.49[b]	.07[b]	2.64[b]
IX	100 ≠	.1% $AlCl_3$	2	3.93[a]	2.96[a]	.05[a]	.92[a]	6.32[b]	3.15[b]	.05[b]	3.12[b]

TABLE XV

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY CALCIUM BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Calcium Balance Periodically - Grams: 112 Days Intake	112 Days Feces	112 Days Urine	112 Days Retained	143 Days Intake	143 Days Feces	143 Days Urine	143 Days Retained
0	0	(Pre-experimental)	12	----	----	----	----	----	----	----	----
I	0	(Control)	2	4.77	3.35	.07	1.35	4.94	2.91	.03	2.00
II	25		2	4.54	3.10	.06	1.38	4.52	3.03	.04	1.45
III	50		2	4.77	3.15	.02	1.60	4.28[e]	2.37[e]	.02[e]	1.89[e]
IV	75		2	4.50	2.89	.01	1.60	3.80	2.42	.04	1.34
V	100		2	4.29	2.87	.02	1.40	4.31[e]	1.99[e]	.03[e]	2.29[e]
VI	200		2	3.74	2.62	.02	1.10	3.88	2.81	.03	1.04
VII	100 +	.1% $Al_2(SO_4)_3$	2	4.29[c]	3.03[c]	.02[c]	1.24[c]	5.44[d,e]	3.50[d,e]	.07[d,e]	1.87[d,e]
VIII	100 +	.5% $Al_2(SO_4)_3$	2	4.31[c]	3.18[c]	.04[c]	1.09[c]	4.97[d,e]	3.14[d,e]	.06[d,e]	1.77[d,e]
IX	100 +	.1% $AlCl_3$	2	4.08[c]	3.00[c]	.03[c]	1.05[c]	4.90[d,e]	3.66[d,e]	.03[d,e]	1.21[d,e]

[a]41 days.
[b]69 days.
[c]103 days.
[d]133 days.
[e]One animal.

TABLE XVI

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY PHOSPHORUS BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Phosphorus Balance Periodically - Grams: Pre-experimental Intake	Pre-experimental Feces	Pre-experimental Urine	Pre-experimental Retained	21 Days Intake	21 Days Feces	21 Days Urine	21 Days Retained
0	0	(Pre-experimental)	12	3.24	2.55	.01	.68	----	----	----	----
I	0	(Control)	2	----	----	----	----	3.47	3.02	.01	.44
II	25		2	----	----	----	----	3.41	2.52	.01	.88
III	50		2	----	----	----	----	3.42	2.45	.01	.96
IV	75		2	----	----	----	----	3.44	2.34	.01	1.09
V	100		2	----	----	----	----	3.46	2.46	.01	.99
VI	200		2	----	----	----	----	2.26	1.69	.01	.56
VII	100 ≁	.1% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
VIII	100 ≁	.5% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
IX	100 ≁	.1% $AlCl_3$	2	----	----	----	----	----	----	----	----

TABLE XVI

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY PHOSPHORUS BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	54 Days: Intake	54 Days: Feces	54 Days: Urine	54 Days: Retained	83 Days: Intake	83 Days: Feces	83 Days: Urine	83 Days: Retained
				Phosphorus Balance Periodically - Grams							
0	0	(Pre-experimental)	12	----	----	----	----	----	----	----	----
I	0	(Control)	2	3.59	2.58	.01	1.00	----	----	----	----
II	25		2	3.62	2.85	.01	.76	----	----	----	----
III	50		2	3.48	2.59	.02	.87	----	----	----	----
IV	75		2	3.37	2.03	.02	1.32	----	----	----	----
V	100		2	3.66	2.61	.01	1.04	----	----	----	----
VI	200		2	3.17	1.85	.01	1.31	----	----	----	----
VII	100 ≠	.1% $Al_2(SO_4)_3$	2	3.60[a]	2.98[a]	.01[a]	.61[a]	3.39[b]	2.67[b]	.01[b]	.71[b]
VIII	100 ≠	.5% $Al_2(SO_4)_3$	2	3.68[a]	2.77[a]	.01[a]	.90[a]	3.49[b]	2.75[b]	.01[b]	.73[b]
IX	100 ≠	.1% $AlCl_3$	2	3.54[a]	2.83[a]	.02[a]	.69[a]	3.22[b]	2.53[b]	.02[b]	.67[b]

TABLE XVI

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY PHOSPHORUS BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Phosphorus Balance Periodically - Grams: 112 Days Intake	112 Days Feces	112 Days Urine	112 Days Retained	143 Days Intake	143 Days Feces	143 Days Urine	143 Days Retained
0	0	(Pre-experimental)	12	----	----	----	----	----	----	----	----
I	0	(Control)	2	4.52	2.50	.02	2.00	3.39	2.69	.01	.69
II	25		2	4.76	2.61	.01	2.14	3.32	2.99	.01	.32
III	50		2	4.56	2.36	.01	2.19	3.28[e]	1.94[e]	.01[e]	1.33[e]
IV	75		2	4.73	2.16	.02	2.55	2.78	2.21	.01	.56
V	100		2	4.51	1.95	.03	2.53	3.37[e]	1.25[e]	.08[e]	2.04[e]
VI	200		2	3.13	1.31	.06	1.76	3.14	2.21	.03	.90
VII	100 ≁	.1% $Al_2(SO_4)_3$	2	2.36[c]	2.84[c]	.01[c]	- .49[c]	3.18[d,e]	2.12[d,e]	.01[d,e]	1.05[d,e]
VIII	100 ≁	.5% $Al_2(SO_4)_3$	2	2.06[c]	2.44[c]	.01[c]	- .39[c]	3.30[d,e]	2.18[d,e]	.01[d,e]	1.11[d,e]
IX	100 ≁	.1% $AlCl_3$	2	2.55[c]	2.28[c]	.02[c]	.25[c]	3.25[d,e]	2.37[d,e]	.01[d,e]	.87[d,e]

[a]41 days.
[b]69 days.
[c]103 days.
[d]133 days.
[e]One animal.

TABLE XVII

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY NITROGEN BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Nitrogen Balance Periodically - Grams: Pre-experimental Intake	Pre-experimental Feces	Pre-experimental Urine	Pre-experimental Retained	21 Days Intake	21 Days Feces	21 Days Urine	21 Days Retained
0	0	(Pre-experimental)	12	15.23	6.16	4.33	4.74	----	----	----	----
I	0	(Control)	2	----	----	----	----	15.15	7.01	4.90	3.24
II	25		2	----	----	----	----	14.99	5.96	5.96	3.07
III	50		2	----	----	----	----	15.10	6.05	5.53	3.52
IV	75		2	----	----	----	----	14.70	6.31	3.24	5.15
V	100		2	----	----	----	----	14.72	6.07	4.47	4.18
VI	200		2	----	----	----	----	10.72	4.81	3.86	2.05
VII	100 ≁	.1% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
VIII	100 ≁	.5% $Al_2(SO_4)_3$	2	----	----	----	----	----	----	----	----
IX	100 ≁	.1% $AlCl_3$	2	----	----	----	----	----	----	----	----

TABLE XVII

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY NITROGEN BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	No. Animals	Nitrogen Balance Periodically - Grams: 54 Days Intake	54 Days Feces	54 Days Urine	54 Days Retained	83 Days Intake	83 Days Feces	83 Days Urine	83 Days Retained
0	0	(Pre-experimental)	12	——	——	——	——	——	——	——	——
I	0	(Control)	2	14.07	5.52	4.46	4.09	15.00[e]	——	2.98[e]	——
II	25		2	14.35	6.46	4.71	3.18	14.68[e]	8.87[e]	4.71[e]	1.10[e]
III	50		2	14.41	5.68	5.51	3.22	14.78[e]	6.71[e]	4.36[e]	3.71[e]
IV	75		2	14.02	5.47	4.53	4.02	——	5.48[e]	3.81	——
V	100		2	14.12	6.10	4.56	3.46	——	7.39	4.47	——
VI	200		2	11.53	5.26	3.84	2.43	——	5.66[e]	7.46	——
VII	100 +	.1% $Al_2(SO_4)_3$	2	13.58[a]	4.04[a]	6.58[a]	2.96[a]	14.97[b]	6.60[b]	3.08[b]	5.29[b]
VIII	100 +	.5% $Al_2(SO_4)_3$	2	13.45[a]	5.12[a]	5.46[a]	2.87[a]	14.05[b]	6.14[b]	3.62[b]	4.29[b]
IX	100 +	.1% $AlCl_3$	2	13.53[a]	4.11[a]	6.06[a]	3.36[a]	14.38[b]	6.42[b]	4.51[b]	3.45[b]

TABLE XVII

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE DAILY NITROGEN BALANCE OF LAMBS FED KNOWN FLUORINE LEVELS (continued)

Lot No.	Treatment F Added p.p.m.	Alleviator Percent	No. Animals	Nitrogen Balance Periodically - Grams 112 Days Intake	Feces	Urine	Retained	143 Days Intake	Feces	Urine	Retained
0	0	(Pre-experimental)	12	——	——	——	——	——	——	——	——
I	0	(Control)	2	14.11	3.90	2.51	7.70	14.69	5.84	3.98	4.87
II	25		2	13.94	5.28	4.39	4.27	14.65	6.84	2.98	4.83
III	50		2	13.80	5.28	3.75	4.77	14.91[e]	4.60[e]	3.24[e]	7.07[e]
IV	75		2	13.81	5.61	3.89	4.31	13.22	5.18	4.76	3.28
V	100		2	13.80	5.43	4.52	3.85	14.71[e]	4.24[e]	6.63[e]	3.84[e]
VI	200		2	11.61	4.55	3.08	3.98	13.24	5.68	4.10	3.46
VII	100 ≁	.1% $Al_2(SO_4)_3$	2	15.45[c]	3.66[c]	6.16[c]	5.63[c]	14.58[d,e]	5.10[d,e]	6.01[d,e]	3.47[d,e]
VIII	100 ≁	.5% $Al_2(SO_4)_3$	2	14.97[c]	3.24[c]	5.88[c]	5.85[c]	14.93[d,e]	5.30[d,e]	5.64[d,e]	3.99[d,e]
IX	100 ≁	.1% $AlCl_3$	2	18.81[c]	3.39[c]	6.35[c]	9.07[c]	14.80[d,e]	4.96[d,e]	6.28[d,e]	3.56[d,e]

[a]41 days.
[b]69 days.
[c]103 days.
[d]133 days.
[e]One animal.

TABLE XVIII

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE PHOSPHORUS CONTENT OF BLOOD FROM LAMBS FED FLUORINE AT VARIOUS LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Phosphorus Per 100 ml. of Serum Periodically - Milligrams: 11 Days	27 Days	41 Days	55 Days	69 Days	83 Days	111 Days	Number Samples Taken	Average
I	0	(Control)	7.24	8.06	6.80	7.60	7.48	6.92	7.86	52	7.42
II	25		8.68	7.67	7.77	8.18	6.76	7.38	8.08	52	7.85
III	50		8.61	8.00	7.30	7.85	6.90	6.47	7.23	48	7.69
IV	75		8.56	8.28	8.43	8.92	7.93	7.91	7.98	52	8.35
V	100		9.06	9.58	8.78	9.91	9.57	7.97	9.38	52	9.21
VI	200		9.80	8.76	8.76	9.30	8.57	8.07	9.18	52	8.98
VII	100 +	.1% $Al_2(SO_4)_3$	8.36	8.75	7.76	8.91	7.48	7.87	7.84	24	8.20
VIII	100 +	.5% $Al_2(SO_4)_3$	8.50	7.76	7.88	8.55	6.97	7.78	7.18	24	7.89
IX	100 +	.1% $AlCl_3$	9.22	8.08	8.42	9.08	6.83	8.08	8.44	24	8.37

TABLE XIX

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE SODIUM CONTENT OF BLOOD FROM LAMBS FED FLUORINE AT VARIOUS LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Sodium Per 100 ml. of Serum Periodically - Milligrams: 11 Days	27 Days	41 Days	55 Days	69 Days	83 Days	111 Days	Number Samples Taken	Average
I	0 (Control)		341.1	339.1	349.9	341.1	365.5	342.3	340.0	52	344.8
II	25		333.2	338.1	341.2	344.5	339.8	342.5	342.0	52	339.6
III	50		339.8	338.6	346.0	344.7	345.2	348.4	335.7	48	342.5
IV	75		333.2	334.0	346.8	341.1	340.7	342.2	342.5	52	339.4
V	100		340.8	338.6	343.8	344.1	350.5	346.0	343.0	52	343.3
VI	200		340.4	339.7	343.0	346.0	345.0	341.3	340.5	52	342.2
VII	100 ≠	.1% $Al_2(SO_4)_3$	341.6	344.4	341.5	346.0	340.3	346.3	345.5	24	343.4
VIII	100 ≠	.5% $Al_2(SO_4)_3$	338.6	335.2	341.5	340.0	341.3	351.3	337.5	24	340.6
IX	100 ≠	.1% $AlCl_3$	343.4	334.9	346.3	337.0	344.0	345.0	334.5	24	341.1

TABLE XX

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE POTASSIUM CONTENT OF BLOOD FROM LAMBS FED FLUORINE AT VARIOUS LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Potassium Per 100 ml. of Serum Periodically - Milligrams: 11 Days	27 Days	41 Days	55 Days	69 Days	83 Days	111 Days	Number Samples Taken	Average
I	0	(Control)	21.93	26.20	21.92	26.56	25.31	22.60	24.25	52	24.11
II	25		21.12	24.65	21.79	25.28	25.26	22.76	24.68	52	23.44
III	50		22.61	24.96	21.76	25.36	22.50	23.00	23.86	48	23.43
IV	75		21.80	22.05	21.25	25.62	22.18	22.97	26.48	52	22.47
V	100		23.29	27.22	22.27	26.03	26.09	23.75	25.79	52	24.89
VI	200		22.66	25.08	23.17	27.21	24.32	22.87	22.86	52	24.07
VII	100 +	.1% $Al_2(SO_4)_3$	30.16	24.85	23.67	34.58	32.92	25.77	23.29	24	28.01
VIII	100 +	.5% $Al_2(SO_4)_3$	24.22	23.91	23.20	26.95	23.75	22.29	26.75	24	24.36
IX	100 +	.1% $AlCl_3$	22.81	22.81	24.22	25.95	23.75	23.12	22.98	24	23.54

TABLE XXI

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE MAGNESIUM CONTENT OF BLOOD FROM LAMBS FED FLUORINE AT VARIOUS LEVELS

Lot No.	Treatment: F Added p.p.m.	Treatment: Alleviator Percent	Magnesium Per 100 ml. of Serum Periodically - Milligrams: 11 Days	27 Days	41 Days	55 Days	69 Days	83 Days	111 Days	Number Samples Taken	Average
I	0	(Control)	2.30	2.23	1.07	2.16	2.91	2.26	1.32	52	2.10
II	25		2.33	2.55	1.38	1.98	2.39	2.26	1.00	52	2.07
III	50		2.59	2.11	1.42	1.93	2.34	2.16	1.26	48	2.05
IV	75		2.23	2.56	1.35	2.19	2.35	2.35	1.12	52	2.12
V	100		2.70	2.52	1.41	2.37	2.77	2.57	1.64	52	2.36
VI	200		2.35	2.40	1.22	2.12	2.68	2.40	1.21	52	2.09
VII	100 ≠	.1% $Al_2(SO_4)_3$	2.19	2.04	2.22	2.53	2.32	2.38	1.33	24	2.14
VIII	100 ≠	.5% $Al_2(SO_4)_3$	2.89	2.19	1.48	1.88	2.16	2.46	1.71	24	2.18
IX	100 ≠	.1% $AlCl_3$	2.70	1.98	2.53	1.42	2.31	2.15	1.16	24	2.20

TABLE XXII

THE EFFECTS OF FLUORINE INTAKE, ALLEVIATORS AND TIME UPON THE AVERAGE CALCIUM CONTENT OF BLOOD FROM LAMBS FED FLUORINE AT VARIOUS LEVELS

Lot No.	Treatment		Calcium Per 100 ml. of Serum Periodically - Milligrams							Number Samples Taken	Average
	F Added p.p.m.	Alleviator Percent	11 Days	27 Days	41 Days	55 Days	69 Days	83 Days	111 Days		
I	0	(Control)	11.81	11.49	12.04	13.07	12.36	12.18	13.30	52	12.19
II	25		10.78	10.62	11.94	12.48	12.83	11.77	13.20	52	11.71
III	50		10.89	10.59	11.32	11.88	13.18	12.60	15.17	48	11.68
IV	75		11.59	11.28	11.52	12.16	13.35	11.18	12.90	52	11.81
V	100		10.98	10.49	12.48	11.86	11.25	11.42	12.48	52	11.48
VI	200		10.41	10.76	12.42	11.81	11.92	11.73	13.90	52	11.60
VII	100 +	.1% $Al_2(SO_4)_3$	11.23	11.25	11.80	12.23	12.47	12.23	15.25	24	12.10
VIII	100 +	.5% $Al_2(SO_4)_3$	12.47	11.85	12.05	11.82	14.00	12.37	16.50	24	12.71
IX	100 +	.1% $AlCl_3$	11.85	11.75	12.10	11.32	13.13	12.33	13.35	24	12.13