(19) Lorenz, W. (to Farbenfabriken Bayer A.-G.), U. S. Patent **2,701,225** (Feb. 1, 1955).
(20) Mattson, A. M., Spillane, J. T., Pearce, G. W., J. Agr. Food Chem. **3,** 319 (1955).
(21) Plapp, F. W., Casida, J. E., Department of Entomology, University of Wisconsin, Madison, Wis., 1957, unpublished results.
(22) Robbins, W. E., Hopkins, T. L., Eddy, G. W., *J. Econ. Entomol.* **49,** 801 (1956).
(23) Schrader, G., *Angew. Chem.* **66,** 265 (1954).
(24) Shell Development Co., 1956, personal communication.
(25) Siggia, S., "Quantitative Organic Analysis via Functional Groups," Wiley, New York, 1949.
(26) Umbreit, W. W., Burris, R. H., Stauffer, J. H., "Manometric Techniques and Tissue Metabolism," 2nd ed., Burgess Publ. Co., Minneapolis, Minn., 1949.

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FLUORINE TOLERANCE OF LAMBS

Effect of Various Levels and Sources of Fluorine in the Fattening Ration of Columbia, Rambouillet, and Targhee Lambs

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Fluorine is generally present in almost all feeds consumed by livestock. Low levels can be ingested for indefinite periods without economic loss, but it is toxic when ingested in amounts above the critical level—the degree of toxicity depending on amount and length of time over which it is consumed. Forages with high concentration of fluorine are often found in areas where certain industrial processes expel fluorides into the air. Hay containing up to 160 p.p.m. of fluorine on a dry basis, when mixed with 50% grain, to make a ration containing 112 p.p.m. of fluorine on a dry basis, can be fed to fattening lambs for 14 weeks without causing measurable bad effects upon feed lot performance.

Comprehensive reviews of the toxicity of fluorine by De Eds (*1*), McClure (*5*), Roholm (*13*), Peirce (*11*), Greenwood (*3*), Mitchell and Edman (*7*), and Phillips *et al.* (*12*) state that fluorides are widely distributed in soil, rocks, water, and plants, and, under certain conditions, the concentrations are high enough to affect animal and human nutrition adversely.

Most of the investigation on the toxicity of fluorine has been with man, cattle, rats, and swine—relatively little has been reported on fattening lambs. Studies reported by Slagsvold (*15*), Velu (*17*), and Roholm (*13*) established that fluorosis occurred in sheep when they consumed water or feed containing high levels of fluorine. Hobbs *et al.* (*4*) reported that fattening lambs, receiving 200 p.p.m. of fluorine in the diet, consumed slightly less feed and gained slightly less weight than animals receiving 100 p.p.m. or less of fluorine in their diet.

Peirce (*10*) reported a 3-year study in which feed consumption, growth, and health were not affected when 120 mg. or less of fluorine per day were consumed. Shrewsbury *et al.* (*14*) reported that sheep receiving 6.0 mg. of fluorine per kg. of body weight per day showed decreased grain consumption and depressed growth.

Experimental

Ninety weanling wether lambs of equal numbers of Columbia, Rambouillet, and Targhee breeds were used in the trial. After being weighed, the lambs were separated, according to breed and weight, into groups of 10. The nine groups of 10 lambs each were allotted at random to the treatments in Table I. The three lambs on each treatment were fed in a pen together. The average initial weight was 31.7, 32.5, and 32.7 kg., for Columbia, Rambouillet, and Targhee, respectively.

A pre-experimental ration consisting of alfalfa hay, water, salt, and a mineral mixture of salt and dicalcium phosphate (containing 80 p.p.m. of fluorine) was fed for a week prior to the trial. During this period, the lambs were treated with phenothiazine for internal parasites and vaccinated for entero-toxemia and contagious exanthema.

At the beginning of the trial, the wool on each lamb was sheared from about a 5-inch square area posterior to the shoulder. In the center of this area, the wool was clipped close with size 00 electric clippers. Two 3 × 3 cm. squares were tattooed in this area. At the end of the trial, the wool was clipped from the tattooed areas on each sheep and used to determine wool production during the trial.

The trial was conducted for 14 weeks. Throughout the trial the lambs were fed to maintain the designated fluorine levels based on hay consumption (Table I). Five levels ranging from that in normal hay to 160 p.p.m. of fluorine were fed. Two sources of fluorine were used: one from sodium fluoride added to normal hay and the other from hay containing a fluoride residue from the stacks of an industrial plant. Only hay with a 55-p.p.m. fluorine residue was obtainable; therefore, for the 80- and 160-p.p.m. levels it was necessary to add some sodium fluoride to the diet. During the first 4 weeks of the experiment the grain was gradually increased and the hay decreased. The different fluorine levels were maintained by using varying proportions of two-grain mixtures, one containing sodium fluoride and the other free of sodium fluoride; and by varying the amounts of hay with a normal fluorine content and hay containing a high fluorine residue.

At the start of the fifth week the lambs were fed a diet consisting of alfalfa hay, 50%; barley, 26.5%; wheat, 10%; dried beet pulp, 10%; omalass (dried cane molasses product manufactured by VyLactos Laboratories, Inc., Des Moines, Iowa), 2.5%; sodium chloride, 0.5%; and dicalcium phosphate 0.5%; and remained on it throughout the balance of the trial. Sufficient sodium fluoride replaced barley to give the fluorine levels outlined in Table I. Ninety-five per cent of the ration was fed in

Table I. Experimental Design and Amount of Fluorine Consumed Per Unit of Dry Matter and Per Kilogram of Body Weight

		Fluorine Levels Based on P.P.M. of Moisture-Free Hay[a]									
		Basal (12)		20		40		80		160	
Breed of Sheep	Source of Fluorine	Dry matter, p.p.m.	Kg. wt., mg.	Dry matter, p.p.m.	Kg. wt., mg.	Dry matter, p.p.m.	Kg. wt., mg.	Dry matter, p.p.m.	Kg. wt., mg.	Dry matter, p.p.m.	Kg. wt., mg.
Columbia	NaF	14[b]	0.51	16	0.60	28	1.09	53	2.09	108	3.65
	Fluoride residue[c]	14	0.48	21	0.72	36	1.11	62	2.20	117	4.01
	Average	14	0.50	18	0.66	32	1.10	58	2.15	112	3.83
Rambouillet	NaF	14	0.46	16	0.56	28	0.94	54	1.63	109	4.21
	Fluoride residue[c]	14	0.42	20	0.67	37	1.21	62	2.28	114	3.54
	Average	14	0.44	18	0.62	32	1.08	58	1.96	112	3.88
Targhee	NaF	14	0.46	16	0.58	28	0.84	53	2.08	109	3.56
	Fluoride residue[c]	14	0.53	21	0.62	36	1.39	64	2.04	114	4.41
	Average	14	0.50	18	0.60	32	1.12	58	2.06	112	3.98
Av.	NaF	14	0.48	16	0.58	28	0.96	53	1.93	109	3.80
	Fluoride residue[c]	14	0.48	21	0.67	36	1.24	63	2.17	115	3.99
Grand av.		14	0.48	18	0.62	32	1.10	58	2.05	112	3.90

[a] Variations due to sampling, mixing, and analysis caused some variation from the levels of fluorine planned; therefore parts per million of fluorine of each diet as actually fed are given. [b] Three lambs were fed together as a group in each pen. [c] Hay with only 55-p.p.m. fluorine residue was obtainable, therefore for the 80- and 160-p.p.m. levels some sodium fluoride had to be added to the diet.

Table II. The Average for Various Treatments and Breed Are Shown For Several Test Variables

Treatment	Number of Animals	Daily Feed Intake, Lb.	Daily Gain, Lb.	Grease Wool Weight, G.	Clean Wool Weight, G.	Fiber Diameter, γ	Market Grade Score[a]	Carcass Grade Score[a]	Dressing, %
Breed									
Columbia	30	2.83	0.33	1.66	1.07	27.4	2.7	2.2	49.6
Rambouillet	30	2.76	0.35	1.28	0.82	21.1	2.9	2.4	49.8
Targhee	30	2.96	0.34	1.41	0.88	26.1	2.6	2.1	50.9
Source of fluorine									
NaF	45	2.85	0.31	1.48	0.94	24.6	2.8	2.3	49.9
Fluoride residue	45	2.85	0.34	1.49	0.90	25.2	2.6	2.2	50.2
Level of fluorine (p.p.m.) in dry diet									
Basal (14)	18	2.98	0.34	1.26	1.78	24.2	2.7	2.3	51.1
18	18	2.74	0.32	1.53	0.91	24.1	2.7	2.1	49.4
32	18	2.90	0.33	1.58	1.07	25.6	2.7	2.3	49.8
58	18	2.90	0.33	1.52	0.94	24.9	2.6	2.2	50.3
112	18	2.73	0.31	1.51	0.90	25.5	2.7	2.1	49.7
Av.	90	2.85	0.33	1.48	0.92	24.9	2.7	2.2	50.0

[a] Prime was graded, 1; choice, 2; good, 3; utility, 4; and cull, 5.

0.5-inch round pellets and the other 5% as chopped alfalfa hay to ensure normal rumination.

The results were analyzed statistically by an analysis of variance (*16*). Differences between means were subjected to Duncan's (*2*) multiple range test. Differences were considered significant at $P < 0.05$ and highly significant at <0.01.

Table III. Method of Analysis Showing Mean Squares for Several Test Variables

		Mean Squares				
Source of Variation	Degrees of Freedom	Daily weight gain	Grease wool weight	Clean wool weight	Fiber diameter	Carcass grade
Replication (R)	2	25.47	0.033	0.042	2.08	0.045
Level of fluorine (F)	4	31.06	0.282[a]	0.188[a]	9.09	0.178
Breed of sheep (B)	2	68.38	0.088[a]	0.510[a]	333.02[a]	0.580[b]
Source of fluorine (S)	1	150.80	0.002	0.023	7.66	0.280
Interaction						
F × B	8	99.71	0.062	0.080	6.44	0.119
F × S	4	109.16	0.088	0.036	2.14	0.168
B × S	2	207.73	0.036	0.081	11.81	0.575
Error	65[c]	79.20	0.072	0.048	39.31	0.184
Total	89					

[a] p<0.01. [b] p<0.05. [c] One lamb died and average value of pen was used in the analysis with degrees of freedom for error reduced accordingly.

Results and Discussion

Feed Consumption. Individual daily feed consumption could not be obtained, as the lambs were fed in groups of three. Feed consumption per lamb, therefore, is based on the average consumed per lot. The average daily feed intake for the main effects is shown in Table II. As there were no significant interactions, the averages for these interrelationships are not reported. There were no significant differences among any of the treatments in feed intake (Table III). Hobbs *et al.* (*4*), showed similar effects, but did observe a decreased feed consumption when lambs received 200 p.p.m. of fluorine in the diet.

Gain in Weight. The average gain of all lambs was 0.33 pound per day (Table II). The source and level of fluorine and the breed of lambs did not significantly affect the gain (Table III).

In a previous trial conducted at this station, Nelson (*9*) also showed that there were no significant differences among Columbias, Targhees, and Rambouillets.

Market Grade. Live market grades were assigned a numerical score, corresponding to the following values: prime, 1; choice, 2; good, 3; utility, 4; and cull, 5. There were no significant differences among scores owing to treatment and breed (Tables II and III).

Dressing Percentage and Pelt Weights. The average dres.ing percentage for all lambs was 50%, with the pelts averaging 15.9 pounds per lamb. Treatment and breed effects were not significant for either of these factors (Tables II and III).

Carcass Weight and Grade. The average warm carcass weight for all lambs was 50.9 pounds (Table II). Differences among treatments and breeds were not significant (Table III). Numerical scores similar to those used for market grades were assigned to each carcass grade. The average carcass grade score was 2.2, which corresponds to a grade of low choice. Carcass grades were 2.2, 2.4, and 2.1 for the Columbia, Rambouillet, and Targhee breeds, respectively. These differences among breeds were significant at the 5% level of probability.

Clinical and Histological Observation. There were no clinical symptoms or gross abnormalities which could be attributed to the various levels or sources of fluorine intake. Histological evaluation of bone, liver, thyroid, kidney, and adrenal glands revealed no significant pathology.

Wool Weight. The average of the wool sampled from the two tattooed areas was used to compare wool production of the lambs in the various treatments and breeds.

The average grease wool weights for the fluorine treatments were 1.26, 1.53, 1.58, 1.52, and 1.51 grams, respectively, for the basal, 20, 40, 80, and 160 p.p.m. levels. Duncan's multiple range test (*2*) showed that the lambs receiving additional fluorine in the diet produced more wool than the control lambs (Table IV). No satisfactory explanation for the difference is apparent. A subsequent trial conducted at this station did not show that added fluorine in the diet increased wool growth.

Table IV. Least Significant Ranges and Results of Multiple Range Test for Comparing Wool Production with Levels of Fluorine in Diet

	Least Significant Ranges							
Number of means	2		3		4		5	
Probability levels	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01
	0.181	0.241	0.191	0.251	0.197	0.258	0.201	0.264

	Treatment Means Ranked in Order from Lowest to Highest[a]				
Treatment	Basal	180 p.p.m.	60 p.p.m.	20 p.p.m.	40 p.p.m.
Mean	1.26	1.51	1.52	1.53	1.58

[a] There was no significant difference between the means underlined but there was a significance at the 0.01 level of probability between each of the underlined means and the mean not underlined.

There have been no reports showing a stimulating effect from the feeding of fluorine. Moulton (*8*) reported that small amounts of fluorine in the drinking water exerted a favorable effect on dental health by reducing the incidence of dental decay. Excess quantities of fluorine in the diet have been shown to cause the development of exostosis and skeletal enlargement. These enlargements have not been reported as being associated with other increases in growth or production. This phase of work needs to be further investigated before a conclusion can be drawn. Clean wool weights followed the same pattern as grease weights.

The weights of the wool samples from the Columbia, Rambouillet, and Targhee lambs averaged 1.66, 1.28, and 1.41 grams, respectively (Table II).

Duncan's multiple range test (*2*) showed that the Columbia lambs produced more wool than the other two breeds and that Targhee lambs produced more wool than Rambouillet lambs. Nelson (*9*) reported the Columbias and Targhees produced more wool than the Rambouillet, but there was no difference between Columbia and Targhee.

Fiber and Staple Length. The average fiber lengths for Columbias, Rambouillets, and Targhees were 1.9, 1.3, and 1.6 inches, respectively. These differences were highly significant. Duncan's multiple range test (*2*) showed that Columbia lambs had longer wool than the Rambouillet lambs, but there was no difference between the Rambouillet and Targhee lambs. Staple length values followed the same pattern as fiber length. There were no differences in fiber or staple length with respect to level or source of fluorine.

Fiber Diameter. Fluorine levels or source had no effect on fiber diameter. The average fiber diameter was 27.4, 21.1, and 26.1 microns for the Columbia, Rambouillet, and Targhee breeds, respectively. Duncan's multiple range test (*2*) showed that Columbias and Targhees had a highly significant greater fiber diameter than Rambouillets.

Fluorine Consumption. The average fluorine consumption is shown in Table I. Based on hay consumption (dry basis) the amounts of fluorine were: basal (12), 20, 40, 80, and 160 p.p.m.; for the total ration (dry basis): 14, 18, 32, 58, and 112 p.p.m.; and per kilogram body weight the amounts were 0.48, 0.62, 1.10, 2.05, and 3.90 mg., respectively. The average weight of the lambs at the start of the trial was 32.3 kg. and at the end of the trial, 46.8 kg. These levels of fluorine did not adversely affect the performance of the lambs over a 12-week feeding period. Hence, lambs can be fattened on hay containing at least 160 p.p.m. of fluorine when it is fed with 50% grain, provided the total dry matter consumed does not contain more than 112 p.p.m. of fluorine. These results agree with those of Hobbs *et al.* (*4*).

With the adoption of proper feeding programs, many feeds containing fluorine concentrations toxic to growing and breeding classes of sheep may be utilized in short-term lamb fattening rations. The lambs used in this trial were raised in an area which, in so far as is known, does not contain above normal amounts of fluorine in the water and vegetation. Lambs which have been raised in areas where the fluorine residue is high may not respond in the same manner.

The Committee on Animal Nutrition of the National Research Council (*6*) felt that permissible levels of fluorine in commercial, general-purpose feed should assure more than borderline safety and recommended that the permissible levels of fluorine be 0.003% (30 p.p.m.) of the total dry ration for sheep. A more recent revision by a similar committee [Phillips *et al.* (*12*)] suggests a level of 70 to 100 p.p.m. for sheep. The higher levels in this trial exceeded the concentration recommended by the National Research Council. However, the higher levels would probably produce fluorosis if fed for extended periods of time. The results, however, show a need for establishing levels for breeding animals fed over a long period and for fattening animals fed for a short period for slaughter.

Literature Cited

(1) De Eds, F., *J. Am. Dental Assoc.* **19,** 861 (1932).
(2) Duncan, D. B., *Diometrics* **11,** 1–42 (1955).
(3) Greenwood, D. A., *Physiol. Revs.* **20,** 582 (1940).
(4) Hobbs, C. S., *et al.*, Bull. **235,** University of Tennessee, Knoxville, Tenn., 1954.
(5) McClure, F. J., *Physiol. Revs.* **13,** 277 (1933).
(6) Mitchell, H. H., *Natl. Research Council Circ.* **113** (1942).
(7) Mitchell, H. H., Edman, M., *Nutrition Abstr. & Revs.* **21,** 787 (1952).

(8) Moulton, F. R. (editor), *Pub. Am. Assoc. Advance. Sci.*, 1946.
(9) Nelson, E. A., M.S. thesis, Utah State University, Logan, Utah, 1953.
(10) Peirce, A. W., *Australia Council Sci. Ind. Research, Bull.* **121** (1938).
(11) Peirce, A. W., *Nutrition Abstr. & Revs.* **9**, 253 (1939).
(12) Phillips, P. H., Greenwood, D. A., Hobbs, C. J., Huffman, C. F., *Natl. Research Council Publ.* **381** (1955).
(13) Roholm, K. A. F., "Fluorine Intoxication," H. K. Lewis, London, 1937.
(14) Shrewsbury, C. L., Hatfield, J. D., Doyle, L. P., Andrews, F. N., *Ind. Agr. Expt. Sta., Bull.* **499** (1944).
(15) Slagsvold, L., *Norsk Vet.* **46,** 1 (1934).
(16) Snedecor, G. W., "Statistical Methods," 4th ed., Iowa State College Press, Ames, Iowa, 1946.
(17) Velu, H., *Acad. vet. France bull.* **4** (N.W.), 392 (1931).

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AMINO ACID SUPPLEMENTS

Lysine Supplementation of a Breakfast Cereal and Milk Combination

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Cereal proteins in such sources as wheat flour and bread have been improved considerably in protein quality with supplements of synthetic lysine. This investigation was made to determine whether a positive growth response would be obtained from the addition of 0.5% DL-lysine hydrochloride to a wheat flakes breakfast cereal, with and without the milk used in a customary serving. A 4-week rat feeding test indicated that the lysine supplement gave a growth response when wheat flakes were fed without milk—no significant increases in growth or protein efficiency were noted with milk.

WHEAT PROTEIN is deficient in lysine, which would be the limiting amino acid if wheat were the only source of protein in the diet. Rosenberg, Rohdenburg, and Baldini (*5*) found that the addition of 0.2% L-lysine to a diet containing 90% dried bread supplemented with salts, vitamins, and fat gave a significant increase in growth to weanling rats. Albanese *et al.* (*1*) showed that body weights and nitrogen balances of infants consuming milk were improved by a lysine supplement.

Many prepared breakfast cereals are composed of wheat, and whether supplementation of these foods with lysine would improve their nutritive value when fed with milk and sugar, as normally eaten, is of interest. This may be of special importance, as Baldwin, Lowry, and Thiessen (*3*) showed that much of the lysine content of proteins may be rendered unavailable to the body as a result of heat treatment of food, such as occurs in the manufacture of toasted cereals.

Materials and Methods

A mixture was made to give the ratio of 4 ounces of fresh whole milk and 8 grams of sugar (1 teaspoon) to 1 ounce of wheat flakes. Whole dry milk solids (Parlac) was used in place of fresh whole milk.

The wheat flakes cereal was pulverized in a Fitzpatrick mill before it was blended with the other dietary constituents. A similar mixture was made to which DL-lysine hydrochloride was added to give a concentration of 0.5% based on the cereal. These two mixtures, as well as the wheat flakes and the lysine-supplemented wheat flakes, were incorporated into diets for a 28-day rat feeding test.

A fifth diet was made with casein as the reference protein. Each kilogram of diet was supplemented with the following vitamins expressed as milligrams per kilogram of diet: choline chloride, 1000; *dl*-α-tocopherol diacetate, 100; pyridoxine hydrochloride, 20; calcium pantothenate, 50; riboflavin, 50; thiamine hydrochloride, 12; nicotinic acid, 90; biotin, 0.3; folic acid, 0.9; inositol, 200; *p*-aminobenzoic acid, 200; and 2-methyl-1,4-naphthoquinone diacetate, 2. This mix was diluted to 2 grams with cornstarch before blending into each diet. Corn oil was used to adjust the fat content of the wheat flakes diets to 6% and to give the casein diet a fat content equal to that of the cereal-milk diets (8%). Three drops of cod liver oil were given twice weekly to each rat to supply vitamins A and D. Salt mix, USPXIV, was added at a 4% level to all diets.

Each diet was fed to six weanling Wistar strain male albino rats obtained from Carworth Farms. The average weight of each group of animals was 59 to 60 grams at the beginning of the test. Food intakes were adjusted each day to equalize the protein intakes of the animals eating the two wheat flakes diets and those consuming the other three rations. Distilled water was given *ad libitum*. Each animal was housed in an individual raised-bottom cage in an air-conditioned room kept at 75° ± 2° F.

The protein content of the wheat flakes diet was 10% and that of the wheat flakes, milk, and sugar diet 12.5%. The casein diet contained 14% protein.

Table I. Four Weeks' Rat Growth from Lysine Supplementation of Wheat Flakes, with and without Milk and Sugar

Diet	Av. Body Weight Change, G.	Food[a] Efficiency	Protein[b] Efficiency
Wheat flakes	− 9 ± 0.9	Negative	Negative
Wheat flakes + 0.5% DL-lysine	− 1 ± 0.9	Negative	Negative
Wheat flakes, milk, and sugar	+87 ± 5.1	0.29	2.30 ± 0.07
Wheat flakes + 0.5% DL-lysine HCl, milk, and sugar	+83 ± 3.4	0.27	2.15 ± 0.06
Casein	+93 ± 3.5	0.33	2.37 ± 0.05

[a] Food efficiency = gram gain in body weight per gram food consumed.
[b] Protein efficiency = gram gain in body weight per gram protein consumed.