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Hugo W. Nilson

J. M. Lemon

UNITED STATES DEPARTMENT OF THE INTERIOR
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By
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ABSTRACT

ALGIN, a salt of alginic acid extracted from kelp, and gelatin, produced from animal bones and skins, are used as stabilizing agents in the manufacture of ice cream and in certain other food products. Studies were conducted with growing male rats over a period of 10 weeks to determine the nutritive effects of the vegetable gum of algin and the protein of gelatin.

The apparent digestibility of algin varied directly with the level fed. Algin was utilized efficiently after absorption. Gelatin was highly digested irrespective of the level fed, but more food was required per unit increase in live weight. No characteristic symptoms were noted that could be attributed to acute or chronic toxicity. Both algin and gelatin were found to be wholesome foods.

METABOLISM STUDIES WITH ALGIN AND GELATIN

By HUGO W. NILSON, *Pharmacologist*, and J. M. LEMON, *Technologist, Division of Fishery Industries, Fish and Wildlife Service*

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INTRODUCTION

Algin, a salt of alginic acid extracted from kelp, and gelatin, produced from animal bones and skins, have been used for some time as stabilizing agents in the manufacture of ice cream and in similar food products. In ice cream they function through certain of their colloidal properties by affecting the size and nature of ice-crystal formation during the freezing and hardening process and storage; thereby producing a smoother product. Algin also is used to a considerable extent in stabilizing chocolate milk. Members of industries utilizing marine plants are particularly interested in determining some of the metabolic constants of these products.

This investigation was begun in March 1939 in the technological laboratories of the Fish and Wildlife Service. The algin and two grades of gelatin used were purchased from ice cream manufacturers. One grade was a bone gelatin of high gel strength, while the other was manufactured from skins. Gelatin manufactured from skins is used to a greater extent as a stabilizer in ice cream.

EXPERIMENTAL DATA

The algin tested was a sodium alginate product extracted from *Macrocystis pyrifera*, the giant kelp that grows off the coast of California. The product was standardized to uniform colloidal strength with sugar and dextrin and treated with phosphate to make it soluble in milk. Goodman (1935)[1] reported that about 0.15 to 0.25 percent of sodium alginate produced satisfactory stabilization of ice cream. Sommer (1938) pp. 428–433, found that 0.25 percent of sodium alginate was equal or slightly superior to 0.4 percent 225 Bloom gelatin for this purpose. Anderson, Dowd, and Helmboldt (1937) reported an improvement in the sodium alginate mix over a gelatin mix, and that algin

[1] Publications referred to parenthetically by date are listed in the literature cited, p. 9.

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as a stabilizer produced an ice cream with a smoother and creamier melt down.

Chemically, sodium alginate consists of the sodium salt of polymerized d-mannuronic acid in which the carboxyl groups are free and all of the aldehyde groups are shielded by linkages. About 71 percent of algin with sugar and dextrin may be classed as carbohydrate, and it was incorporated into diets in replacement of part of the sugar and dextrin (tables 1 and 2).

TABLE 1.—*Composition of diets used*

Ingredients	Percent by weight								
	Diet No. 1 (control)	Diet No. 2	Diet No. 3	Diet No. 4	Diet No. 5	Diet No. 6	Diet No. 7	Diet No. 8	Diet No. 9
Algin	-------	5.0	10.0	20.0	30.0	-------	-------	-------	-------
Gelatin	-------	-------	-------	-------	-------	5.0	10.0	20.0	30.0
Sucrose	27.0	25.1	23.4	19.1	15.8	26.0	25.0	23.0	20.4
Dextrin, tapioca	25.0	23.1	21.5	17.7	14.4	23.6	23.2	20.8	19.0
Lard	15.0								
Casein, technical	15.0								
Lactalbumin, technical	5.0	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Brewer's yeast, dried	5.0								
Wheat germ	2.0								
Cod liver oil	2.0								
Salt mixture, regular	4.0	-------	-------	-------	-------	4.0	4.0	4.0	4.0
Salt mixture, No. 1	-------	3.3	.5	-------	-------	-------	-------	-------	-------
Salt mixture, No. 2	-------	-------	2.0	2.0	-------	-------	-------	-------	-------
Supplement value:									
Protein equivalent	-------	-------	-------	-------	-------	2.0	5.0	10.0	15.0
Carbohydrate equivalent	-------	3.8	7.1	15.2	21.8	2.4	3.8	8.2	12.6
Mineral equivalent	-------	.5	1.5	2.0	3.0	-------	-------	-------	-------
Excess moisture	-------	.7	1.4	2.8	4.2	.6	1.2	1.8	2.4

[1] Same in all diets.

TABLE 2.—*The composition of the algin, gelatin, and the salt mixtures used*

Ingredients	Percent by weight					
	Algin with sugar and dextrin	Gelatin		Salt mixture		
		Skin	Bone	Regular	No. 1	No. 2
Moisture	14.0	12.0	12.0	-------	-------	-------
Mannuronic units	37.0	-------	-------	-------	-------	-------
Gelatin	-------	86.8	87.8	-------	-------	-------
Ash	15.0	1.6	.2	-------	-------	-------
Sucrose	17.0	-------	-------	-------	-------	-------
Dextrin	17.0	-------	-------	-------	-------	-------
Calcium lactate	-------	-------	-------	31.0	55.0	65.0
Dicalcium phosphate	-------	-------	-------	11.0	-------	-------
Dipotassium phosphate	-------	-------	-------	22.0	-------	-------
Potassium chloride	-------	-------	-------	12.5	15.0	18.0
Sodium chloride	-------	-------	-------	8.0	-------	-------
Calcium carbonate	-------	-------	-------	7.0	15.0	-------
Magnesium carbonate	-------	-------	-------	3.0	8.0	8.0
Magnesium sulfate	-------	-------	-------	3.7	1.5	-------
Ferric citrate	-------	-------	-------	1.3	4.0	6.5
Copper sulfate	-------	-------	-------	.4	1.0	1.5
Manganese sulfate	-------	-------	-------	.1	.5	1.0
Potassium iodide	-------	-------	-------	.01	.01	0.2

Gelatin, a byproduct of the meat-packing industry, is a protein derived from white fibers contained in pig and calf skins, and ossein obtained from bones. Two grades were used in the experimental studies. Since gelatin is a protein, it was incorporated into the experimental diets in partial replacement of the casein and lactalbumin which supplied the protein of the basal diet. Gelatin is an incomplete protein, so enough casein and lactalbumin was included in all diets to at least approximately balance the gelatin protein. In no instance did a diet contain more than 35 percent protein. This higher level of protein has been found satisfactory for normal growth of rats in experiments in which casein was fed (Morris, Palmer, and Kennedy 1933).

ANIMAL-FEEDING EXPERIMENTS

Male albino rats, 4 weeks old, were allotted into nine groups of six animals each, with an average initial live weight per group of 53 to 54 g. The maximum range in individual live weight was 48 to 58 g. The animals were individually housed in screen cages that were fitted with screen floors. Food and water were fed freely and the intake of each animal was recorded. The food intake of the various groups was calculated to the equivalent dry-matter content of the control diet, and the excess was added to the water-intake records.

Groups were allotted to be fed the control diet and diets containing approximately 5, 10, 20, and 30 percent algin with sugar and dextrin, and 5, 10, 20, and 30 percent gelatin (tables 1 and 2). Three rats of each gelatin-fed group received bone gelatin, while the other three received skin gelatin. The data show no significant differences in response, so the results from the subgroups were summarized together in the final computation. The quantities of algin or gelatin fed were chosen arbitrarily, but the smaller amount represents about 20 times the quantity usually incorporated in ice cream.

Only one 9-week test was originally contemplated. In order to obtain accurate apparent digestibility coefficients representing the entire period, paper towels were spread under the screen floors and the feces collected every 2 or 3 days. Feces samples from individual rats were kept in a cool place and later dried to determine the dry-matter content. Preliminary tests soon showed that the algin could not be readily extracted from the dried feces. Consequently another sample was collected during the tenth week for the determination of algin, total nitrogen, and dry-matter content. Data for gain in weight, and food and water requirements cover the 10-week period, while data on the apparent digestibility of algin and the total protein are limited to the tenth week.

The algin content of the feces collected during the tenth week was determined for individual rats by digesting a 3-g. sample with 10 ml. of

0.5N/NaOH and 20 ml. water. The suspension was filtered after the addition of 10 ml. of saturated $(NH_4)_2SO_4$ and the undigested matter was washed with an additional 10 ml. of $(NH_4)_2SO_4$. Twenty-five ml. of a 40 percent solution of $CaCl_2$ and 25 ml. of water were added to the filtrate to precipitate the algin. Filter aid was added and the suspension was filtered through a Buchner filter. The filter cake was washed thoroughly with a saturated $(NH_4)_2SO_4$ solution. The amount of algin was calculated from the difference in weight after drying and then igniting the filter cake. Some difficulty was experienced with occlusion of protein. This procedure probably does not give an absolutely true answer, but it was considered to be the best approximation that could be obtained under the circumstances. The total crude protein content was determined by the modified Kjeldahl method of the A.O.A.C. on samples of feces collected during the tenth week from individual rats fed the control and gelatin diets.

In general, the rats grew very well and at the close of the 10-week period the mean live weight per rat ranged from 332 g. for the group fed the control diet to 123 g. for the group fed the diet containing 30 percent gelatin. The comparative mean live weights were 314, 312, 243, and 208 g. for the algin-fed rats, and 286, 241, 209 and 123 g. for the gelatin-fed rats for the 5, 10, 20, and 30 percent levels, respectively.

Two rats died in each of the groups that were fed algin. These rats sickened and died from 9 to 17 days after being fed the experimental diet. Since these animals died within such a short period, it was considered probable that the bacterial flora of the intestinal tract may have been an important factor. The rats that died may not have become properly infected with a type of bacteria that could grow satisfactorily in the algin media. The problem of physical impaction did not appear to be a factor even with rats fed the high levels of algin.

In order to test the significance of this interpretation, certain rats which had been fed similar diets containing 20 and 30 percent agar during the 10-week period were fed a diet containing 20 percent algin. They were killed at about 4-day intervals and 1-g. samples of the contents of the small intestine and cecum were diluted and plated on nutrient agar.

The data in table 3 show that there was a marked decrease in the bacterial numbers about the twelfth day. The rats that lived through this period showed a subsequent higher bacterial count of the intestinal contents, while those that died showed evidence that the food was not properly digested. Complete differential studies were not made, but it appeared that the grayish-blue colonies were gram negative rods and the small brownish colonies were gram positive cocci. The data are very incomplete, but apparently the bacterial flora of the intestinal tract was an important factor in the efficient utilization of food and in the health of the animals.

No characteristic lesions were found during necropsy of the rats that died when fed the regular experimental diet. The mean total fecal excretion of dry matter per rat during the 10-week period was 26 g. for the control group; 35, 38, 33, and 29 g. for the groups fed algin; and 19, 24, 16, and 12 g. for the groups fed gelatin at the 5, 10, 20, and 30 percent levels. The dry-matter content of the feces varied from 57 to 78 percent.

The data in table 4 show that the rats of all groups except those fed the 30 percent level of gelatin grew rather uniformly, although, only the rats fed the 5 and 10 percent levels of algin grew as well as the control group.

TABLE 3.—*A study of the bacterial flora in the contents of the intestinal tracts of rats fed a diet that contained 20 percent agar prior to June 7*

Rat number and diet fed	Date	Bacterial count in millions per gram		Remarks
		Small intestine	Cecum	
20 percent agar:				
121	June 23	3.3	10	The intestines were fairly well filled with food. The cecum was small and compact. Grayish-blue colonies.
117	July 5	1.5	9	Do.
20 percent algin:				
125	June 11	2.1	628	All grayish-blue colonies.
124	June 15	4.4	21	About 3 times as much food in intestines as for rat No. 125. Grayish-blue and brown colonies.
123	June 19	.2	3	The intestines contained very little food, but the cecum contained considerable solid food. Grayish-blue and brown colonies.
118	June 23	3.0	14	The intestines were filled with what appeared to be algin. The cecum was quite large and well filled with a greenish material. Grayish-blue and brown colonies.
120	do			Killed because he was obviously sick. He did not have any food in the cecum or intestines.
126	June 26			Died No food was found in the cecum or intestines.
122	June 27	16.0	14	The intestines were filled with an algin-like substance. Grayish-blue, brown, and a few white colonies.

TABLE 4.—*Daily gain in weight of rats for the 10-week period*

Group designation	Number of survivors	Mean daily gain	Coefficient of variation	Mean difference from control	Percent level of t
		Grams	*Percent*	*Grams*	
Control	6	3.99	13		
Algin:					
5 percent	4	3.72	20	0.27	(1)
10 percent	4	3.53	9	.46	(1)
20 percent	4	2.98	9	1.01	4
30 percent	4	2.20	21	1.79	1
Gelatin:					
5 percent	6	3.31	5	.68	5
10 percent	6	3.10	11	.89	2
20 percent	6	2.22	10	1.77	<1
30 percent	6	.99	31	3.00	<1

[1] Nonsignificant difference.

The data in table 5 are interesting since they show that none of the rats fed algin required more food per gram gain in weight than the control rats. The greater variability of food requirement for the rats fed 30 percent algin (coefficient of variation equals 17 percent), and the smaller number of animals, made the difference from control of 0.85 g. insignificant. This finding need not be emphasized, since what happens when the lower levels are fed is of greater importance from the standpoint of stabilizers in foods. The groups fed gelatin all required more food per gram gain than did the control rats. There was no effect of increasing levels on the apparent digestibility of the dry matter of the diet, and the diets were digested surprisingly well in all instances.

The water requirement per gram gain in weight varies as may be expected (table 6). It is interesting to note that the rats fed the 5 and 10 percent levels of both algin and gelatin required about the same amount of water per gram gain in weight as the control group. Those fed the greater levels of either material showed a higher requirement which may be explained, at least in part, by the decreased growth rate. The mean, free-choice water consumption for the 10-week period was roughly 1,400 g. for the control group; 1,525, 1,450, 1,750, and 2,000 g. for the algin-fed rats; and 1,300, 1,275, 1,300, and 950 g. for the gelatin-fed rats.

TABLE 5.—*Food required per gram gain in weight and the apparent digestibility of dry matter for the 10-week period*

Group designation	Mean food required	Coefficient of variation	Mean difference from control	Percent level of *t*	Mean apparent digestibility of dry matter
	Grams	*Percent*	*Grams*		*Percent*
Control	3.00	6			96.6
Algin:					
5 percent	3.30	11	0.30	[1]	94.6
10 percent	3.22	8	.22	[1]	94.8
20 percent	3.34	8	.34	[1]	94.8
30 percent	3.85	17	.85	[1]	94.4
Gelatin:					
5 percent	3.31	4	.31	3	97.2
10 percent	3.54	9	.54	2	96.5
20 percent	4.17	16	1.17	1	97.1
30 percent	6.71	17	3.71	<1	97.0

[1] Non-significant difference.

TABLE 6.—*Water required per gram gain in weight for the 10-week period*

Group designation	Mean water required	Coefficient of variation	Mean difference from control	Percent level of *t*
	Grams	*Percent*	*Grams*	
Control	4.91	18		
Algin:				
5 percent	6.22	26	1.31	[1]
10 percent	5.94	8	1.03	[1]
20 percent	8.64	25	3.73	5
30 percent	13.24	8	8.33	<1
Gelatin:				
5 percent	5.67	17	.76	[1]
10 percent	5.98	9	1.07	[1]
20 percent	8.29	10	3.38	<1
30 percent	14.33	21	9.42	<1

[1] Nonsignificant difference.

The tenth week served as a feces-collection period in order to determine the digestibility of the algin and the total crude protein of the control diet and those containing gelatin. Some variation in apparent digestibility may be due to the fact that the food-intake data were taken from the period in which the feces were collected, and day-to-day fluctuations in food intake and feces excretion would have a greater effect during a period of 1 week than for a longer period. This factor may be responsible for part of the rather large coefficients of variation of 79 and 27 percent for the groups fed 5 and 10 percent algin diets (table 7).

The data in table 7 show an increasing apparent digestibility of the algin with increasing level in the diet. There was a statistically significant difference in every case. Either there was an adaptation of the intestinal tract to use a greater amount of the algin in the higher levels in order to conserve food supply, or the micro-organisms responsible for digestion grew better in the intestinal contents containing the higher levels of algin. The algin after digestion was apparently well utilized by the animal, since the data in table 5 show no significantly greater amount of food required per gram gain in weight as the algin content of the diet increased from 0 to 30 percent. Apparently the algin has considerable nutritive value and can be efficiently utilized when properly digested.

TABLE 7.—*Apparent digestibility of algin for a 1-week period*

Group designation	Mean apparent digestibility	Coefficient of variation	Mean difference within series	Percent level of t
Algin:	*Percent*	*Percent*	*Percent*	
5 percent	3.0	79		
10 percent	35.9	27	32.9	<1
20 percent	63.4	7	60.4	<1
30 percent	88.3	3	85.3	<1
10 percent				
20 percent			27.5	2
30 percent			52.4	<1
20 percent				
30 percent			24.9	<1

The data in table 8 show a very uniform apparent digestibility of the total protein of the control diet and the diets containing increasing levels of gelatin. The coefficients of variation were very small, indicating a high degree of uniformity. Apparently the gelatin was very digestible irrespective of the level fed, but the data in table 5 indicate a significantly higher quantity of food required per gram gain in weight when the gelatin was added to the diet and when the level of gelatin in the diet was increased.

TABLE 8.—*Apparent digestibility of the total protein of the diet for a 1-week period*

Group designation	Mean apparent digestibility	Coefficient of variation	Mean difference	Percent level of *t*
	Percent	*Percent*	*Percent*	
Control	89.2	1.4		
Gelatin:				
5 percent	88.5	1.8	0.7	[1]
10 percent	87.5	1.6	1.7	[1]
20 percent	89.8	1.4	.6	[1]
30 percent	92.1	1.3	2.9	[1]

[1] Nonsignificant difference.

DISCUSSION

These studies are concerned only with the nutritive values of the vegetable gum of algin and the protein of gelatin. In the final analysis, however, both products are used only in very small amounts as stabilizers in food products, because of their physical effects upon them. Although the nutritive values of the products are of secondary importance, both are wholesome. Two rats in each algin-fed group died soon after being fed the diets, but only negative lesions were found on necropsy. The survivors made reasonably satisfactory gains in weight. The gelatin-fed rats grew at a significantly lower rate than the control rats and required more food per gram gain in weight, but all lived. The group fed the control diet, and the combined groups which had previously been fed the 5 and 10 percent levels of algin or gelatin were fed the 5 percent levels of algin and gelatin and these groups have been continued on experiment for over 2 years. Deaths were finally due to pneumonia and malignant tumors, except 1 rat fed algin died of coronary embolism and another of possible digestive disturbance or intestinal infection.

SUMMARY

Male albino rats with an average initial live weight of 53 to 54 g. were fed individually for one 10-week period on a control diet supplemented with 5, 10, 20, and 30 percent of algin with sugar and dextrin, and 5, 10, 20, and 30 percent of gelatin. The apparent digestibility of the algin ranged from 3 to 88 percent, depending upon the level fed. The apparent digestibility of the total protein of the diets containing gelatin varied from about 88 to 92 percent. Both algin and gelatin were found to be wholesome foods.

Ten male rats of similar initial weight, but from another colony, were later fed the basal diet (salt replaced the salt mixture) plus 10 percent of algin with sugar and dextrin. None of the rats died during the 10-week period. The average daily gain was 3.20 g., and 3.32 g. of food and 8.04 g. of water were required for each gram gain in weight.

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