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Heat-Stable Toxic Principle in Tung Meal Requires Consideration

OILSEED PROCESSING

Detoxification and Toxicological Studies Of Tung Meal

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Use of tung meal as an animal feed is infeasible because of its toxic nature; hence experiments were performed to resolve some of the apparent contradictory statements on the detoxification of this material. Employing rats as test animals it was found that a tung meal, prepared by hexane extraction of tung kernels, could not be detoxified completely by a combination of autoclaving and extraction with ethyl alcohol. Tung press cake (obtained by a commercial expeller process involving exposure to high temperatures) was detoxified almost completely by extraction with ethyl alcohol. The application of heat alone does not appear to effect complete detoxification of tung meal. The occurrence of a heat-stable toxic principle seems to require consideration in any proposed commercial detoxification process.

THE FRUIT OF THE TUNG TREE (*Aleurites fordii*) has long been used as a source of the valuable tung or China wood oil of commerce. Introduced into the United States in 1902, this tree has been cultivated in the Gulf Coast area to give rise to an important southern industry. An exhaustive bibliography on the chemistry and technology of tung products has been published recently by Planck, Pack, and Skau (*19*).

To obtain commercial tung oil, the seeds (or nuts) from the mature fruit commonly are crushed in an expeller press; the cake of meal obtained in this process is sometimes solvent-extracted to remove the residual oil. Analyses of meals left after petroleum ether extraction of both seed kernels and whole seeds have indicated that both materials should be valuable as animal feedstuffs, as they are fairly rich in protein and carbohydrate (*7, 13*).

This valuable outlet for the oil-free tung meal, however, is closed because of its long-recognized toxic nature, the first scientific report on toxicity of tung seeds appearing to be that of Mutschler and Krauch in 1879 (*18*).

At present the meal is being used as a fertilizer only; this represents a relatively low-priced outlet. Tung meal has been used as an insecticide (*14, 21*), but it does not appear to be so employed to any great extent. The possible use of meal protein as a plastic has been investigated (*16*), but did not result in a quantity outlet for the meal. Undoubtedly a reliable procedure for the detoxification of tung meal on a large scale would be attractive, thus converting it from a low-priced fertilizer into a more valuable animal feed. The present publication deals mainly with the results of a series of experiments, devised on the

basis of previously recorded data, designed to detoxify tung meal.

Publications on Tung Toxicity

The literature on tung toxicity through 1947 has been thoroughly reviewed by Watson (*22*); more recently Balthrop (*4*) and Lee and Watson (*15*) have published similar reviews. Because of these previous reports only a summary of the most significant publications on the toxicity of oil-free tung meal is presented here.

Table I. Effect of pH of Solvent on Peptization of Nitrogenous Constituents of Hexane-Extracted Tung Meal HE

HCl Added, Millimoles/ Gram Meal	NaOH Added Millimoles/ Gram Meal	pH of Supernatant	% of Total Meal N Peptized
4.83		1.50	63.5
2.41		2.06	45.4
0.97		3.81	12.3
0.48		4.79	11.7
0.24		5.45	13.1
0.10		5.96	15.7
0.05		6.16	16.5
0.00 (water)	0.00	6.42	17.7
	0.05	6.73	18.8
	0.09	7.05	20.2
	0.24	7.94	30.3
	0.47	9.34	47.2
	0.95	10.37	88.9
	2.36	11.74	91.6
	4.73	12.31	92.1

Godden (*13*) fed rats, dairy cattle, poultry, and pigs with feeds composed of a basal ration mixed with varying amounts of a tung meal that had been obtained by solvent extraction at temperatures below 45° C. (benzoline of boiling point 90° to 105° C.), steaming for 40 minutes, and air drying at 20° to 30° C. The animals did not eat much of the tung meal diets; diarrhea or dysentery (scouring) was observed in cattle and pigs; post-mortem examination of sacrificed pigs indicated irritation of the intestinal mucosa. None of the animals died, although the rats were kept on a ration containing 25% of the tung meal for 21 days. Using rats, Erickson and Brown (*10*) found that oil-free meal obtained by petroleum ether extraction of tung seed kernels was lethal to five animals within 86 hours when fed at a level of 19.5% of the ration. The oil-free meal could be detoxified by extraction with warm (64° to 71° C.) 95% ethyl alcohol, by heating with steam in a pressure cooker for 2 hours at 110° C., or by heating in a hot air oven for 15 hours at 100° to 119° C. Diets containing 27% of a commercial press cake were palatable, and no harmful effects were observed.

Rusoff, Mehrhof, and McKinney (*20*) found that a solvent-extracted tung meal when fed to growing chicks at levels from 5 to 20% appeared to be unpalatable but gave no evidence of toxicity. This meal was prepared from press cake by extracting with a "special extraction naphtha" (boiling range 94° to 112° C.) for 2.5 hours; the solvent was removed by a 2.5-hour steam process, during which time the pressure was increased from 10 to 90 pounds. The moist meal was then stored in burlap bags for a considerable period before use, during which time it undoubtedly underwent some fermentation. McKinney (*16*) stated that solvent-extracted tung meal was definitely toxic to chickens, while solvent-extracted press cake was unpalatable but of doubtful toxicity. Autoclaving the extracted press cake for 2 hours at 110° C. yielded a meal nontoxic to chickens. Employing 2-day and 3-week-old chicks, Davis, Mehrhof, and McKinney (*8*) concluded that ground press cake, raw or autoclaved (for 1.5 hours at 116° or 128° C.), was unsafe to use in feeds, causing heavy mortality at 10 and 15% levels of ration.

In 1947 Emmel (*9*) summarized in detail investigations from which he concluded that the toxic components of both commercial tung meal and fresh defatted tung kernels were: a saponin, and a second toxic principle which could be extracted with ethyl alcohol. Using chicks 4 to 18 days old, he found that commercial tung meal (fed at 20% level of ration) could be partially detoxified by: (1) extraction with water; (2) autoclaving for 4 hours at 15 pounds' pressure; and (3) autoclaving for 1 hour at 14 pounds' pressure in the presence of 5% aqueous hydrochloric acid. Complete detoxification could be achieved when the commercial meal, defatted by extraction with ether, acetone, naphtha, or hexane, was extracted in a Soxhlet for 6 to 8 hours with 95% ethyl alcohol (to remove the second toxic principle mentioned above) and subsequently subjected to the hydrochloric acid–autoclaving treatment described. The toxicity of commercial tung meal was found to decrease with age in 1 to 2 years of storage. Neither the saponin nor the second toxic component was obtained in the pure state; the presence of a saponin in an aqueous extract of the meal was inferred from several qualitative tests—liberation of reducing material on acid hydrolysis, hemolytic properties, etc. The toxic nature of the alcoholic extract was proved by the mortality it caused when fed to chicks.

Using a meal prepared by petroleum ether (boiling point 60° to 71° C.) extraction of tung seeds, Watson (*22*) came to the conclusion that at least two toxic components were present: a thermostable principle which could be extracted with ethyl alcohol, benzene, acetone, ethyl ether, chloroform, carbon tetrachloride, or trichloroethylene; and a thermolabile principle which could be inactivated by moistening the meal and steaming for 2 hours. A combination of ethyl alcohol extraction and steaming rendered the meal nontoxic to chicks. Toxic aqueous extracts of the meal could not be obtained, and an enzymatic test failed to indicate the presence of a saponin in such extracts. Attempts to concentrate the thermolabile principle failed, but the crude thermostable principle, obtained from an ethyl alcohol extract of the meal, was purified to yield an oily, toxic material which gave only one band on a chromatographic column. This material was not identified, but some of its properties were recorded.

Bryan (*6*) claimed that the toxic principle of the meal was an allergenic albumin, partially extractable with water. The globulins were found to be nontoxic. The development of an economic method of detoxification was claimed, but the nature of the procedure was not divulged.

Table II. Effect of Salts of Peptization of Nitrogenous Constituents of Hexane-Extracted Tung Meal HE

Salt	Concn. of Salt, Normality	pH of Supernatant	% of Total Meal N Peptized
NaCl	0.10	6.35	26.8
	0.30	6.45	41.2
	0.50	6.65	43.1
	0.75	6.65	43.1
	1.00	6.65	43.1
Na_2SO_3	0.25	8.25	50.3
	0.50	8.60	58.6
	0.75	8.80	59.4
	1.00	8.92	57.2

Balthrop and Gallagher (*5*), using rats and dogs, found that tung meal could be partially detoxified by boiling for 10 minutes with 0.083*N* hydrochloric acid, treating with a 10% acetic acid solution, and soaking in a saturated solution of magnesium sulfate. The effectiveness of these reagents was attributed to their ability to denature a toxic protein. Recently Lee and Watson (*15, 22*) have concluded that commercial press cake and hexane-extracted kernels can be detoxified for chicks by successively extracting with warm 95% ethyl alcohol for 48 hours, moistening, and steaming for 2 hours at 100° C. The biological value of the protein in such detoxified meals was found to be low as compared with casein or soybean meal protein.

Experimental Work

The experiments described in this report represent primarily an effort to re-

Table III. Analytical Data on Tung Products

Sample and Description	Analyses ("as Is" Basis), % H_2O	N	Ash	Crude fiber	Lipides
HE, hexane-extracted kernels	11.32	7.44	7.31	9.38	0.70
HE-10, HE autoclaved 30 min. at 10 lb./sq. inch	10.38	7.08	7.00	8.93	0.30
HE-21, HE autoclaved 30 min. at 21 lb./sq. inch	9.43	7.14	7.49	9.49	0.34
HEA, HE extracted with ethyl alcohol	13.80	7.34	7.49	10.03	0.24
HEA-10, HEA autoclaved 30 min. at 10 lb./sq. in.	10.86	7.64	7.70	9.67	0.14
HEA-21, HEA autoclaved 30 min. at 21 lb./sq. inch	10.69	7.65	7.69	9.93	0.08
C, ground commercial press cake	7.26	3.03	3.83	37.09	0.38
C-10, C autoclaved 30 min. at 10 lb./sq. inch	8.13	3.23	3.89	36.25	0.71
C-21, C autoclaved 30 min. at 21 lb./sq. inch	8.16	3.15	3.91	38.61	0.72
CA, C extracted with ethyl alcohol	10.45	3.39	4.21	38.16	0.19
CA-10, CA autoclaved 30 min. at 10 lb./sq. inch	10.51	3.17	3.98	38.63	0.27
CA-21, CA autoclaved 30 min. at 21 lb./sq. inch	10.13	3.39	4.25	37.33	0.27
Tung protein from HE	9.51	14.47	0.39	...	..
Meal residue from protein preparation	8.93	2.92	10.29	18.78	..

solve some of the apparent contradictions in the literature concerning the detoxification of tung meal. Two preparations were employed: a meal made by extracting flaked tung seed kernels (shell-free) with commercial hexane (boiling point 60° to 71° C.), termed HE; and a sample of commercial expeller press cake, termed C. A portion of each meal was autoclaved for 30 minutes at 10 pounds per square inch (114° C.), and another portion was autoclaved for 30 minutes at 21 pounds per square inch (127° C.). The two original preparations were extracted with 95% ethyl alcohol at room temperature (24–26° C.), and each alcohol-extracted meal was subjected to the two autoclaving procedures described for the original meals. All materials were investigated for toxicity, employing rats as test animals. The alcohol extractions were conducted at room temperature in an effort to differentiate the results of this treatment from the alterations produced by the heat treatments.

The oil obtained from the hexane extraction of the kernels (preparation of meal HE) also was tested for toxicity; this appeared advisable because of the disagreement in the literature (*22*) on this phase of the general toxic properties of tung products. A protein preparation, obtained from meal HE by extraction with dilute sodium hydroxide, was examined for toxicity; this was done in view of previous reports (*5*, *6*) which indicated that the poisonous component resides in a protein fraction.

Preparation and Treatment of Hexane-Extracted Meal Tung seeds, commercially decorticated, were hand-picked to remove the hard seed coats. Any intact seeds were cracked by hand and the coats discarded. The kernels (containing approximately 60.9% oil and 6.9% moisture) were flaked and then extracted with commercial hexane (boiling point 60° to 71° C.) in a batch extractor, the meal temperature being maintained at about 22° C. A detailed account of this particular extraction has been published (*17*). The meal was freed of solvent by spreading on trays and air-drying for 24 hours. This material was pulverized in a laboratory-scale hammer mill (Raymond), heating during this operation being minimized by not employing a screen in the mill.

Meals HE-10 and HE-21. These were prepared from meal HE by autoclaving for 30 minutes at 10 (114° C.) and 21 pounds per square inch (127° C.), respectively, followed by air-drying at room temperature. The preparations then were ground in the hammer mill.

Meal HEA, Alcohol-Extracted. A 3.08-kg. portion of meal HE was placed in a glass vessel, 10 liters of 95% ethyl alcohol was added, and the mixture was subjected to mechanical stirring for about 5 hours. After standing overnight the liquid was drawn off, an immersion filter being employed to retain meal and fines. This procedure was repeated 15 times, 7 to 10 liters of ethyl alcohol being employed each time, and the duration of contact between meal and alcohol being at least 24 hours for each extraction. The last extract was water-white, and the extracted meal weighed 2.81 kg. after air-drying for 3 days.

Meals HEA-10 and HEA-21. These were prepared by autoclaving two portions of meal HEA at 114° and 127° C. exactly as described.

Treatment of Commercial Press Cake A sample of this material, formed in a commercial screw-type expeller press from tung seeds of the current crop (1950–51) was pulverized in the hammer mill (without a screen). The material was reground to pass through $^1/_{16}$-inch mesh screen.

Meals C-10 and C-21. These were prepared by autoclaving portions of meal C at 114° and 127° C. as described.

Meal CA, Alcohol-Extracted. A 6.00-kg. portion of meal C was batch-extracted with 95% ethyl alcohol in a manner similar to that described for meal HE. Thirteen portions of alcohol were used; the last extract was still highly colored, and the extracted meal weighed 5.73 kg. after air-drying for 5 days.

Meals CA-10 and CA-21. These meals were prepared by autoclaving portions of meal CA at 114° and 127° C. as described.

Tung Oil A portion of the hexane solution of tung oil, obtained in the preparation of meal HE, was freed of solvent (refractive index test) by gentle warming under vacuum. Some characteristics of the oil were: refractive index (sodium *D* line; 25.0° C.) 1.51733; refractive index (mercury *G* line; 25.0° C.) 1.54469; refractive dispersion 273.6 × 10^{-4}; Brown heat test 10.5 minutes; acid number 1.35.

Protein from Meal HE Before obtaining a protein preparation from the tung meal, it was deemed important to obtain information on the degree of peptization of the meal nitrogen as influenced by pH and the presence of salt. As sodium chloride and sodium sulfite have been used as peptizing agents to characterize seed proteins (*11*), these two salts were chosen for this study. The procedure employed was essentially that used by Fontaine and Burnett (*11*).

Portions of the meal 2.50 grams were weighed into 250-ml. centrifuge bottles, 100-ml. portions of each solvent to be tested were added to each bottle, and the mixtures were shaken intermittently for 3 hours at room temperature. Clarification was effected by centrifuging for 15 minutes at 1975 times gravity (at bottom of bottle), and the nitrogen contents and pH values of the supernatants were determined. Table I shows the influence of pH of solvent on the peptization of the meal nitrogen; Table II shows peptization obtained in the presence of varying concentrations of

sodium chloride and of sodium sulfite. In Figure 1 the pH-peptization data given in Table I are compared with similar data previously obtained for peanut meal (*11*) and cottonseed meal (*12*).

On the basis of the peptization data it was decided to employ sodium hydroxide to extract the protein from the tung meal. A 908-gram portion of meal HE was slurried with 9080 ml. of water at room temperature and the pH of the mixture elevated to 10.75 by the slow addition of 200 ml. of approximately 20% sodium hydroxide solution. After stirring for 0.5 hour the liquor was clarified by centrifuging successively in a basket head and in a Sharples-type continuous supercentrifuge. The protein in the liquor was precipitated by lowering the pH to 4.75 with 3*N* hydrochloric acid. The protein curd was freed of mother liquor by a combination of filtration through cheesecloth and centrifugation. The wet curd was dialyzed (cellulose sausage casing) against running distilled water for 3 days at about 6° C., a rocker arrangement being employed to give constant agitation. The dialyzed material was freed of water by vacuum-drying from the frozen state (lyophilization), and the powdery product allowed to equilibrate with atmospheric moisture by exposing to the air for about 24 hours. A yield of 105 grams of the protein preparation was obtained.

The above procedure was repeated using the same quantities of meal and reagents, and the second portion of protein obtained was mixed with the one previously prepared. The two, ground together in the hammer mill, constituted the "tung protein" used for the toxicity tests.

The meal solids remaining after the alkaline extractions were mixed, washed twice with tap water, dried in a circulating oven for 48 hours at 50° C., and then ground in the hammer mill. This "meal residue" was also investigated for toxicity.

Analyses of Meal Preparations These analyses were performed according to the methods of the American Oil Chemists' Society (*3*), and the results are presented in Table III.

Determination Of Short-Term Chronic Toxicity The tests were carried out on young female albino rats, which were fed a basic diet having the following percentage composition: corn meal 73, casein 10, linseed oil meal 10, alfalfa 10, bone ash 1.5, sodium chloride 0.5, and cod-liver oil (U.S.P.) 3; the percentage analysis was nitrogen 2.71% (× 6.25 = 16.94% protein), moisture 10.4%, fat 4.6%, fiber 1.4%, ash 3.5%, and N.F.E. 62.6%.

Rats in groups of five were placed on one of the tung diets as listed in Tables IV and V, by replacement of part of the (mixed) basic diet with an equal amount of the tung product under study. The procedures of feeding, housing, and handling the rats were essentially those previously described (*1, 2*). The general design and duration of the experiments are given in Tables IV and V. Free access to the diets and to water were permitted at all times, and the rats and feed consumed were weighed every 3 or 4 days. Toxicological evaluations were made on the basis of growth, food intake, consistency of fecal droppings, and gross post-mortem appearance of organs (all surviving animals being killed at the end of the experimental periods). Data on growth and food consumption of the animals on the various diets are presented in Tables IV and V.

As it is likely that the residual oil in tung meal is in an autoxidized state, it was considered desirable to simulate this condition when the oil was tested for toxicity. Hence, the 10% tung oil diet was exposed to air for 10 days prior to feeding in order to facilitate autoxidation of the oil. During this time the diet was stirred frequently to break up the hard mass that formed. All other diets containing 2.5 and 5% tung oil were prepared from this diet.

Results and Discussion

The hexane-extracted kernel preparation, meal HE, was tested at two different times for toxicity as reported in Tables IV and V. The results obtained were essentially the same in each case—the rats refused to eat, and lost weight, and, when younger animals (average weight 38.9 grams) were used, a diet containing 20% of meal HE killed all five members of the group in 11 days. There was no definite evidence of diarrhea, and the only postmortem finding was hyperemia or inflammation of the gastrointestinal tract. Autoclaving meal HE for 30-minute periods at 10 (114° C.) and 21 pounds per square inch (127° C.), yielding preparations HE-10 and HE-21 respectively, resulted in reduction of the toxicity, as judged by growth and food consumption (Table V), but did not completely destroy or inactivate the toxic principle(s), as gastrointestinal inflammation of varying intensity was a consistent finding. Table V also shows that alcohol extraction of meal HE at room temperature, yielding preparation HEA, resulted in decreased toxicity, but that the extraction procedure alone was not as effective as the heat treatments. Autoclaving the alcohol-extracted meal, yielding HEA-10 and HEA-21, caused a slightly greater decrease in toxicity than did autoclaving the original meal HE. All of the meals derived from HE consistently produced inflammation of the gastrointestinal tract; thus none of the treatments chosen resulted in satisfactory detoxification of the hexane-extracted tung kernels. Purgation was not observed; there was a transient, slight softening of the stool, and a persistent foul odor.

The ground commercial press cake C was tested at two different times for toxicity; the results, given in Table V, show that this material is definitely less toxic than the hexane-extracted kernel preparation HE. Autoclaving Meal C caused a further reduction in toxicity, especially when the more drastic condition of 21 pounds per square inch (127° C.) was employed to yield meal C-21, food consumption and growth in this instance being equal to those recorded for the controls. However, all three preparations (C, C-10, and C-21) induced irritation of the gastrointestinal tract of varying intensity as indicated by hyperemia. Materials CA, CA-10, and CA-21, prepared by alcohol extraction of the commercial press cake C and subsequent autoclaving, were also of reduced toxicity, judging from growth and food intake; furthermore, very

Table IV. Toxicity of Hexane-Extracted Tung Meal HE and Tung Oil

Material Tested	Concn. in Diet, %	No. of Female Rats		Days on Diet	Average Growth				Average Food Consumption G./Rat/Day
		Start	End		Weight at start, g.	Weight at end, g.	Weight Change G.	Weight Change %[a]	
Tung meal HE	0	5	5	13	39.2	82.4	43.2	110	7.8
	5	5	4	13	38.8	30.0	− 8.8	−23	Ca. 2.0[b]
	10	5	3	13	38.6	27.7	−10.9	−39	Ca. 1.7
	20	5	0[c]	..	38.8	...	...	..	Ca. 1.4
Tung oil[d]	0	5	5	46	60.2	173.8	113.6	189	9.8
	2.5	5	5	46	61.2	165.2	104.0	170	9.3
	5.0	5	5	46	61.4	156.2	94.8	154	8.7
	10.0	5	5	46	62.6	147.8	85.2	136	8.2

[a] % weight change = $\frac{\text{weight change (g.)}}{\text{weight at start}} \times 100$.

[b] Tung meal in concentrations as low as 5% of diet must be extremely unpalatable, as rats refused to eat.

[c] For rats on 20% tung meal diet during first 6 days, average body weight dropped from 38.8 to 32.6 grams. Two survived 10 days when average body weight dropped to 29.5 grams. On 11th day all rats had succumbed.

[d] Tung oil in these diets had been allowed to autoxidize as described in text.

slight to no enteritis was observed when these alcohol-treated materials were fed. There was no definite evidence of diarrhea in any of the rats fed press cake C or its derived preparations.

The protein preparation from meal HE, as well as the meal residue obtained incident to the preparation of the protein, displayed toxic properties as indicated in Table V, although neither material appeared as toxic as the original meal. Gastroenteritis in rats on the tung meal protein diet was not consistent with the level of this protein in the diet, being seen at the low and high dietary intakes, but not at the intermediate level. In rats on the meal residue diet, the degree of gastroenteritis appeared to vary with the concentration of the meal residue in the diet. It is to be noted in Table III that the meal residue retained a considerable proportion of the original nitrogen content of meal HE, indicating that the residue itself probably had a rather high protein content.

Using weight gain, food consumption, and lack of gastroenteritis as criteria, it appears that the only meal preparations obtained approaching nontoxicity were those resulting when commercial press cake was extracted with ethyl alcohol (CA), or when the alcohol-extracted cake was subsequently autoclaved (CA-10 and CA-21). The fact that these procedures did not alter substantially either the ash or crude fiber contents of the press cake C (see Table III) tends to show that these two constituents did not contribute to the toxic nature of the ground commercial press cake.

Data on growth and food consumption obtained when female rats were fed diets containing tung oil are presented in Table IV. Inhibition in growth of the rats on the diets appears to be proportional to the concentration of tung oil in the diet, and the food consumption was somewhat lower for animals on 5 and 10% tung oil diets as compared with those on the basic diet alone. Without additional studies, it cannot be stated definitely whether the inhibition of growth is partly due to the tung oil or to voluntary restriction in food consumption. Fecal droppings of the rats on the 10% tung oil diet were harder than those of the rats on the other levels of tung oil and on the basal diet. On post-mortem examination, grossly all of the organs of the animals which had received the tung oil diets were indistinguishable from the organs of the controls; other than inhibition in growth and restriction of food consumption, no other recognizable signs of toxic reactions were observed, even though these rats were kept on the oil diets for 46 days, a period of much greater duration than those employed for testing the meal preparations.

The over-all results obtained in these short-term toxicity experiments are in fair agreement with the conclusions drawn by Emmel (*9*), Watson (*22*), and Lee and Watson (*15*), who employed chicks as test animals. Most certainly autoclaving the hexane-extracted kernels HE resulted in a partial detoxification of the material for rats; this might be attributed to destruction of Emmel's heat-sensitive "saponin" or of Watson's "thermolabile" component. Heat-denaturation or partial hydrolysis of the toxic protein of Bryan (*6*) and others also might account for this result.

The failure to obtain a completely detoxified preparation by the ethyl alcohol extraction of the defatted kernels is in contrast with the results of Erickson and Brown (*10*), who also used rats as test animals. However, these authors subjected their preparation to the alcohol treatment at an elevated temperature (64° to 71° C.), while in the present experiments the extraction was performed at room temperature. The failure to obtain a nontoxic meal from the defatted kernels by heat treatment alone, or by a combination of ethyl alcohol extraction plus heat, is in apparent disagreement with Erickson and Brown and Lee and Watson. Both groups employed moist heat treatments which might be considered more drastic (2 hours at 110° C. and steaming for 2 hours, respectively) than the half-hour period at 127° C. used in the experiments reported here. Davis *et al.* (*8*) also failed to detoxify tung meal for chicks by the rather rigorous procedure of autoclaving the meal for 90 minutes at 22 pounds per square inch.

The reduced toxicity of the commercial press cake C as compared with the hexane-extracted kernels HE is to be expected in view of the heat treatment the partially decorticated seeds undergo when processed, and in view of the demonstrated destruction by heat of part of the toxicity of the unheated, defatted tung kernels. The occurrence in the press cake of a toxic component, stable toward heat but capable of being removed by ethyl alcohol extraction, as claimed by both Emmel (*9*) and Watson (*22*), appears to have been verified by the experiments reported here. The failure to detoxify the press cake by autoclaving alone seems to conflict with McKinney's report (*16*), which claims complete detoxification of solvent-extracted press cake for chickens by autoclaving for 2 hours at 110° C. McKinney does not specify the exact nature of the solvent, however; and the heat treatment would appear more drastic than the autoclaving used here. It is likely that any variation in the temperatures employed during processing will cause variation in the toxicity of the resulting press cake; this would make it difficult to compare the findings of various investigators without carefully considering the variables involved in the production of the particular sample of press cake being studied.

The carry-over of part of the toxicity of the hexane-extracted meal HE into a

Figure 1. pH-peptization curves for solvent-extracted meals

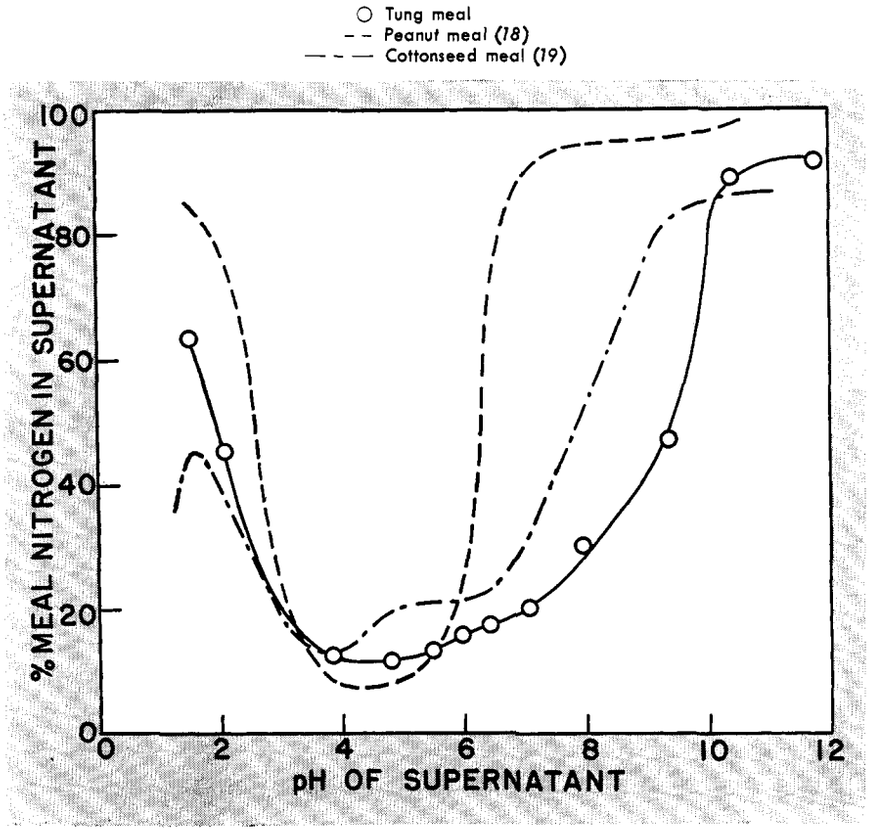

Table V. Toxicity of Various Tung Preparations

Material Tested	Concn. in Diet, %	Av. Food Consumption G./Rat/Day	Average Growth[a] Weight at start, G.	Weight at end, G.	Weight Change G.	Weight Change %[b]	%/Day g. test Material Consumed[c]
Control A	0	10.7	74.2	132.0	57.8	78	...
HE, extracted kernels	5	3.8	72.0	51.6	−20.4	−28	−6.8
	10[d]	3.2	72.0	46.6	−25.4	−35	−6.6
	20[e]	2.2	71.6	48.4	−23.2	−32	−6.6
HE-10, He autoclaved at 10 lb.[f]	5	10.2	72.4	124.6	52.2	72	6.7
	10	7.4	72.0	99.4	27.4	38	2.4
	20	6.4	71.8	85.8	14.0	20	0.8
HE-21, HE autoclaved at 2 lb.[f]	5	8.9	70.8	115.6	44.8	63	6.7
	10	9.2	71.6	113.0	41.4	58	3.0
	20	7.4	72.0	90.4	18.4	26	0.8
HEA, HE alcohol-extracted	5	4.5	72.4	61.8	−10.6	−15	−3.0
	10	4.3	72.8	59.0	−13.8	−19	−2.1
	20	3.5	73.8	43.4	−30.4	−41	−2.9
HEA-10, HEA autoclaved at 10 lb.[f]	5	9.9	71.2	123.6	52.4	74	7.0
	10	9.7	70.8	122.2	51.4	73	3.6
	20	9.8	72.8	120.8	48.0	66	1.6
HEA-21, HEA autoclaved at 21 lb.[f]	5	9.7	71.6	123.8	52.2	73	7.1
	10	10.7	72.8	127.2	54.4	75	3.4
	20	9.7	72.4	115.8	43.4	60	1.5
C, comm. press cake	5	10.7	74.2	127.4	53.2	72	6.3
	10	10.3	75.2	124.2	49.0	65	3.0
	20	9.0	73.8	103.0	29.2	40	1.1
C-10, C autoclaved at 10 lb.[f]	5	10.3	75.2	133.6	58.4	78	7.1
	10	10.4	75.6	124.2	48.6	64	2.9
	20	11.8	75.8	126.4	50.6	67	1.4
C-21, C autoclaved at 21 lb.[f]	5	11.1	75.6	140.4	64.8	86	7.3
	10	11.2	77.0	140.0	63.0	82	3.5
	20	11.4	75.4	131.2	55.8	74	1.5
Control B[g]	0	10.8	74.4	143.6	69.2	93	...
C, comm. press cake	5	10.4	70.8	125.2	54.4	77	7.1
	10	10.1	70.6	124.6	54.0	77	3.7
	20	8.5	70.8	98.2	27.4	38	1.1
CA, C alcohol-extracted	5	10.6	72.6	131.0	58.4	80	7.2
	10	11.7	72.6	137.8	65.2	90	3.7
	20	11.5	72.8	129.00	56.2	77	1.6
CA-10, CA autoclaved at 10 lb.[f]	5	11.8	73.2	140.8	67.6	92	7.5
	10[h]	11.1	72.4	129.7	57.3	79	3.4
	20	13.0	72.6	136.8	64.2	88	1.6
CA-21, CA autoclaved at 21 lb.[f]	5	11.1	71.8	137.6	65.8	90	7.7
	10	11.6	72.2	132.4	60.2	83	3.4
	20	12.5	73.0	139.6	66.6	91	1.7
Protein from meal HE	1	8.9	72.6	121.8	49.2	68	3.6
	2	7.5	72.2	93.0	20.8	29	0.9
	4	4.7	72.4	65.2	− 7.2	−10	−2.6
Meal residue from protein preparation	5	9.1	73.6	114.4	40.8	55	5.7
	10	6.1	73.4	77.4	4.0	6	0.5
	20	4.3	74.8	59.8	−15.0	−20	−1.2

[a] Five female rats per group; 21 days on diets unless otherwise noted.

[b] $\%\ \text{weight change} = \frac{\text{weight change (g.)}}{\text{weight at start}} \times 100.$

[c] Calculated by use of expression:

$$\frac{\%\ \text{weight change}}{(\text{Days on diet})\left(\frac{\%\ \text{tung in diet}}{100}\right)(\text{av. food consumption})}$$

[d] 17 days on diet.

[e] 11 days on diet.

[f] All autoclaving periods of 30-minute duration.

[g] All feeding experiments listed below control B carried out simultaneously with this control. All other feeding experiments carried out simultaneously with control A.

[h] Data on four rats.

protein preparation made using this meal is not incompatible with the assumption of Bryan (*6*) and others that the toxic component is a protein, but the evidence as presented here is not conclusive in view of the recognized ability of protein to adsorb tenaciously smaller organic molecules.

The relatively nontoxic character of the hexane-extracted tung oil is in partial agreement with the contradictory literature as reported by Watson (*22*).

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