Detoxication of Tung Meal

JORDAN G. LEE
Department of Agricultural and Biological Chemistry, Louisiana State University, Baton Rouge, La.

The possibility has been explored that treated tung meals which are nontoxic to chicks might be toxic to the albino rat. They are not; both species require the same steps of alcohol extraction, moistening, and steaming if meals are to be completely detoxified. The quality of the tung protein for the rat is low and lysine is the first limiting amino acid. Such detoxified tung meals do not offer much promise as economical sources of protein for cereal supplementation.

In a previous investigation in this laboratory of the detoxication of tung meals the chick was used as test animal in preference to the albino rat (*3*). This choice was made because the author's experience has been that rats refuse food containing tung meal and are perhaps more resistant to the toxicity than chicks, as they survived when fed preparations lethal to chicks. It was found that meals nontoxic to chicks could be produced by moistening and steaming an experimental meal or commercial press cake previously extracted with ethyl alcohol. Mann, Hoffman, and Ambrose (*4*) have since reported they could not obtain tung meals nontoxic to rats. Although their methods of treatment were slightly different, their paper poses the possibility that the meals as prepared in this laboratory would be toxic to the rat. Therefore, it was decided to repeat the essential portion of the chick work using the albino rat as test animal.

Detoxication Tests

Tung nuts obtained locally were shaved, and the flakes were percolated with Skellysolve B at room temperature, air-dried, and ground. Hull particles were removed by hand. This material was then treated as nearly as possible as in the work with chicks. Each test meal was incorporated at a 20% level in a ration also containing casein 20%, fortified corn oil 5%, fortified dextrose 3%, Hubbel, Mendel, and Wakeman salt mix 2%, and dextrose 50%. The fortified corn oil contained 1.0 gram of α-tocopherol, 1 mg. of calciferol, and 1.0 gram of vitamin A ester concentrate per 498 grams of corn oil. The fortified dextrose contained 20 grams of choline chloride, 1.0 gram of niacin, 0.2 gram of calcium pantothenate, 50 mg. of riboflavin, 25 mg. of thiamine hydrochloride, 20 mg. of pyridoxine hydrochloride, 5 mg. of folic acid, 2 mg. of biotin, and 2 mg. of vitamin B_{12} per 279 grams of dextrose. A control ration contained 25% casein and 2% Ruffex (a processed rice hull preparation sold by Fisher Scientific Co. and used as a roughage in experimental diets) with dextrose replacing the tung fraction.

All rations were offered *ad libitum* to individually housed weanling male rats. Five matched pairs of litter mates were used for each comparison. Daily records of body weight and food intake were made during the 21-day test period. All animals were autopsied.

Table I summarizes the data. The actual toxicities of the original meal and of the alcohol (95%)-extracted, unheated meal are difficult to judge because of the determined rejection and scattering of the rations. These animals definitely preferred starvation to the ration. It may be inferred from the lack of mortality and lesser weight loss that the alcohol extraction lessened the actual toxicity, but the results are not clear-cut as with chicks. The food refusal disappeared when the meals were heated and the general pattern then seems to be the same, though less intense, as with chicks. The type of meal that gave greatest growth with chicks—alcohol-extracted, moistened, steamed—also gave greatest weight gain here. Failure to moisten the meal before steaming resulted in less gain and the intestinal tracts of the group fed steamed dry meal showed slight signs of irritation not found when the meal was moistened before heating.

It is unfortunate that the control ration was not as palatable as the rations containing the alcohol-extracted, heated meals, as it vitiates comparison of weight gain. It is clear, however, that in terms of food efficiency the control ration is equal to the detoxified meal and, by inference, superior to the alcohol-extracted meal steamed dry. Though differences in weight gain are small, they were obtained with paired litter mates and are of an all-or-none character.

It may be concluded that the same conditions which produce meals nontoxic to chicks produce meals nontoxic to rats. The rat seems to be less susceptible than the chick to the alcohol-soluble toxic entity and perhaps no more susceptible to the heat-labile toxic material. There is no conclusive evidence to show whether or not the heat-labile toxic material is also the cause of

Table I. Effect of Treatment on Toxicity of Skellysolve B–Extracted Tung Meal

	Treatment and Comparison	Av.[a] Gain, G.	Av. Food Intake, G.	G. Feed / G. Gain
1.	None	−16[b]	63[c]	
	vs.			
2.	Ethyl alcohol–extracted 48 hours, 1 atm.	−5	84[c]	
3.	Moistened, steamed 100° C., 2 hours	101	267	2.64
	vs.			
4.	No. 2 moistened, steamed 100° C., 2 hours	114[d]	278	2.43
5.	No. 2 steamed dry 100° C., 2 hours	103	289	2.80
	vs.			
6.	No. 4	110[d]	282	2.56
7.	Control ration	98	253	2.57
	vs.			
8.	No. 4	111[d]	281	2.53

[a] Over 21-day period.
[b] 60% mortality. No deaths in any other group.
[c] Excessive scattering of feed makes this value unreliable.
[d] Each animal receiving this ration had a greater weight gain than its pair mate.

Table II. Effect on 30-Day Weight Gain of Varying Content of Tung Protein and of Supplementation with Amino Acids

	Ration and Comparison	Av. Gain G.	Av. Food Intake, G.	G. Feed / G. Gain
1.	8.4% crude protein	57	449	7.87
2.	No. 1 + 0.3% methionine	53	450	8.49
3.	No. 1 + 0.1% tryptophan	57	431	7.56
4.	No. 1 + 0.8% lysine.HCl	95	403	4.24
5.	8.4% crude protein	46	312	6.78
6.	10.5% crude protein	61	310	5.08
7.	12.6% crude protein	84	378	4.50
8.	No. 5 + 0.8% lysine.HCl	79	338	4.27

food rejection. The simplest assumption is that they are the same.

These results are actually not in great conflict with those of Mann, Hoffman, and Ambrose. These investigators did not moisten their meals before heating, and that has always been a necessity in this laboratory. Emmel (*1*) and Erickson and Brown (*2*) also found such moistening desirable. The difference in method of alcohol extraction is also of possible significance. The other workers extracted by decantation at room temperature; here, by Soxhlet with the meal temperature ranging from 40° to 45° C. Lee and Watson (*3*) have previously commented on the fact that Emmel was forced to reextract his alcohol-extracted meals with acetone to obtain detoxication. It is entirely possible that extraction of the alcohol-soluble toxin involves breaking of a lipide-protein complex and is not a matter of simple, rapid solubility.

Quality of Tung Protein

To determine the first limiting amino acid of the tung protein, the casein of the ration was replaced by dextrose, leaving the detoxified meal as the only protein source. This gave a ration with 8.4% crude protein. Three other rations were prepared which were identical except for inclusion of the three most commonly lacking amino acids. One contained 0.3% DL-methionine, the second 0.8% L-lysine hydrochloride, and the third 0.1% L-tryptophan. These four rations were fed to three matched litter mate tetrads of female weanling rats for a 30-day period. The animals were observed as before. The results given in Table II show clearly that lysine is the first limiting amino acid. An idea as to the extent of this limitation may be obtained by comparing the feed efficiencies of rations containing 8.4, 10.5, and 12.6% crude protein derived solely from detoxified tung meal with the ration containing 8.4% crude protein plus 0.8% lysine hydrochloride. Five tetrads of matched litter mate weanling females were used as before to obtain these data. None of the rations containing only the tung protein gave as good a feed utilization as did the lysine-supplemented ration.

These results lead to the view voiced in the previous paper. Tung meals, detoxified by ethyl alcohol extraction followed by application of moist heat, do not offer much hope as economical protein supplements for the cereals.

Literature Cited

(1) Emmel, M. W., Univ. Fla., Agr. Expt. Sta., Tech. Bull. **431** (1947).
(2) Erickson, J. L. E., and Brown, J. H., *J. Pharmacol.* **74,** 114 (1942).
(3) Lee, J. G., and Watson, J. A., Jr., *J. Am. Oil Chemists' Soc.* **30,** 32 (1953).
(4) Mann, G. E., Hoffman, W. H., Jr., and Ambrose, A. M., J. AGR. FOOD CHEM. **2,** 258 (1954).

Received for review June 20, 1955. Accepted August 26, 1955.

NUTRITION AND ENZYME ACTION

Influence of Dietary Energy Level on Succinoxidase and Lactic Dehydrogenase of the Heart of Pregnant Swine

R. L. SHIRLEY, J. F. EASLEY, C. E. HAINES, A. C. WARNICK, H. D. WALLACE, and G. K. DAVIS

Department of Animal Husbandry and Nutrition, University of Florida, Gainesville, Fla.

A ration adequate in dietary energy, compared to one containing about one half this amount of energy, and two stages of gestation, have been studied with gilts in regard to the succinoxidase and lactic dehydrogenase activity in the left ventricles of the heart. The full energy dietary group had greater weight of heart and succinoxidase activity in the left ventricles than the limited dietary energy group, at the 0.01 level of significance. Stage of gestation had no influence on the succinoxidase activity or weight of the heart. The lactic dehydrogenase activity was not affected by diet or stage of gestation, and was at a markedly lower level than the corresponding succinoxidase activity.

THE INFLUENCE OF DIETARY ENERGY LEVEL upon growth and vigor of animals has been recognized for many years, but very little work has been done to relate the influence of dietary energy to the enzyme systems of animal tissues. Wainio and others (*7*) studied the effect of protein depletion on the succinoxidase activity of the liver of rats and later (*6*) reported a similar study on the ventricles of the heart. Straub (*5*) purified lactic dehydrogenase in crystalline form from heart muscle, and Nielands (*2*) reported a study of various inhibitors of lactic dehydrogenase activity of heart. The present study was made to investigate succinoxidase and lactic dehydrogenase activities in hearts of pregnant swine at two stages of gestation, when dietary energy was decreased from normal to about one half normal requirement and other dietary factors were kept constant.

Experimental

Thirty-one Duroc gilts, weighing an average of 114 pounds each, were