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.

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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

divided into two dietary groups and fed the following rations.

Ingredients	Ration A (Full Fed Group), Pounds	Ration B (Limited Fed Group), Pounds
Ground corn	50	16
Ground oats	23	0
Alfalfa leaf meal	10	20
Soybean meal	10	44
Meat and bone scraps	5	17
Limestone	1	1
Steamed bonemeal	1	1
Trace mineralized salt	0.53	1.06
Lederle Aurofac-2A	0.3	0.6
Total	100.83	100.66

All gilts were on pasture from the start of the experiment until sacrificed.

The full fed group was fed ration A *ad libitum*, and the other group was limited to one half this amount of ration B, which gave the latter group about the same quantities of protein, vitamins, and minerals, but decreased the energy intake to approximately one half that of the full fed group. All gilts were bred at the second estrous period, which averaged 106 days after they were put on the rations. Seven gilts on ration A and eight on ration B were sacrificed 3 days after being bred and eight gilts of each dietary group were sacrificed 25 days afterwards.

At the time of sacrifice, samples of the left ventricles of the heart were obtained and placed in iced 0.02*M* phosphate buffer, pH 8.0, until they could be frozen. They were analyzed for succinoxidase activity by the Schneider and Potter (*3*) method within a few days after sacrifice. The lactic dehydrogenase was determined by the method of Green and Brosteaux (*1*), using adrenalin as the hydrogen carrier. The samples were homogenized with the Potter-Elvehjem glass homogenizer. The activity of both enzyme systems was calculated at milliliters of oxygen uptake per gram wet weight per hour. Oxygen uptake was determined at 10-minute intervals over a period of 30 minutes using the Warburg apparatus.

Results and Discussion

In Table I data are presented that were obtained in a preliminary study of the relative activity of succinoxidase in the various divisions of the hearts of a number of swine, weighing approximately 200 pounds each, and produced on practical farm rations. The Schneider and Potter (*3*) method was modified in these preliminary determinations by washing the homogenates at pH 5.5 three times at 3° to 6° C., using a refrigerated centrifuge. The washed residue was then suspended in 0.02*M* phosphate buffer and aliquots were taken for the determination of oxygen uptake. The difference in activity between the ventricular and auricular tissues is highly significant statistically (*4*). As a result of the high activity, amount of sample available, and work load of the left ventricle, this division was believed to be most satisfactory for a study of the influence of dietary factors on the enzymatic activity of the heart in the present investigation.

Table I. Succinoxidase Activity in Swine Hearts

	Division of Heart[a]				
	L.V.	R.V.	M.V.S.	L.A.	R.A.
Q_{O_2}, ml. O_2 uptake/g. wet wt./hr.	15.1[b] ± 4.2	14.1 ± 3.1	14.1 ± 3.0	3.4 ± 0.9	4.2 ± 1.9
No. of swine	10	10	10	5	5

[a] Left and right ventricles, muscular ventricular septum, and left and right auricles.
[b] Mean and standard deviation.

In Table II are presented the data obtained for the weights of the hearts and succinoxidase and lactic dehydrogenase activity of the left ventricle of the gilts at 3 and 25 days of gestation, for the two dietary groups. These data were tested for the significance of the effect of ration and stage of gestation, according to Snedecor (*4*). The gilts fed the full energy ration had hearts that weighed more than those on limited feed, and the difference was significant at the 0.01 level; stage of gestation was without influence. The live weights of the gilts on the high and the limited energy rations at the time of sacrifice were 237 ± 24 and 181 ± 24 pounds, respectively. A highly significant correlation was found between the live weights of the gilts and the weights of the hearts.

As shown in Table II, the stage of gestation had no influence on the succinoxidase activity, but the greater values obtained with the full energy dietary group were significant at the 0.01 level. When the succinoxidase activity was calculated as milliliters of oxygen uptake per milligram of nitrogen in the homogenate per 60 minutes, the full energy dietary group had values of 1.14 ± 0.16 and 1.14 ± 0.14 at the 3- and 25-day intervals, respectively; the corresponding values for the limited energy group were 0.87 ± 0.15 and 0.87 ± 0.12. When calculated on this basis, the effect of the ration was as highly significant as when calculated on the wet weight basis. The succinoxidase system is one of the principal links of the tricarboxylic acid cycle and might be expected to vary in capacity in certain tissues, depending on the amounts of carbohydrate metabolites available. This is believed to have been demonstrated in this study, where the capacity of heart homogenates to take up oxygen through the succinoxidase system has been shown to be significantly greater in animals receiving an adequate energy diet than in those receiving about one half this amount.

In the case of the lactic dehydrogenase (see Table II) all values were in the range of 3.4 to 3.5 ml. of oxygen uptake per gram wet weight per hour, indicating that neither the ration nor the stage of gestation had influence on the activity. When the lactic dehydrogenase activity was calculated as milliliters of oxygen uptake per milligram of nitrogen in the homogenate per hour, the full energy dietary group had values of 0.17 ± 0.01 and 0.15 ± 0.03 at 3 and 25 days of gestation, respectively, while the corresponding values for the limited energy

Table II. Effect of Dietary Energy Level on Weight of Heart, and Succinoxidase and Lactic Dehydrogenase Activity of Left Ventricle of Pregnant Swine at Two Stages of Gestation

	Energy in Ration				
	Full		Limited		Significance[a]
Gestation, days	3	25	3	25	
Weight, grams	259[b] ± 34	256 ± 24	186 ± 29	224 ± 29	Ration—at 0.01 level
Q_{O_2}[c] Succinoxidase	23.7 ± 2.8	21.0 ± 2.9	17.6 ± 3.4	18.5 ± 3.5	Ration—at 0.01 level
Lactic dehydrogenase	3.47 ± 0.24	3.42 ± 0.89	3.35 ± 0.36	3.52 ± 1.60	None
No. of swine	7	8	8	8	

[a] Calculated according to Snedecor (*4*) for effect of ration and stage of gestation. No interaction observed between ration and stage of gestation.
[b] Mean and standard deviation.
[c] Ml. of O_2 uptake/g. wet weight/hr.

dietary groups were 0.16 ± 0.02 and 0.14 ± 0.06. The lactic dehydrogenase activity was markedly lower than corresponding succinoxidase activity values. As the heart obtains a great amount of its energy from lactic acid, it was believed that the high dietary energy ration would give a greater concentration of available lactic acid, resulting in an increased capacity of the lactic dehydrogenase enzyme. As this did not occur in this study to a significant extent, it may indicate that the heart has a considerable capacity to maintain its needs under greatly varying dietary intakes of foods that may supply the lactic acid metabolite.

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ALFALFA CAROTENE

Determination of Carotene in Dehydrated Alfalfa Meal Treated with *N,N'*-Diphenyl-*p*-phenylenediamine

HAROLD A. KALER

National Alfalfa Dehydrating and Milling Co., Lamar, Colo.

The AOAC method for the determination of carotene in alfalfa has been modified to eliminate interference from *N,N'*-diphenyl-*p*-phenylenediamine, a carotene antioxidant. The diamine causes the formation of a yellow color when chromatographed on magnesia as in the official method, interfering with the colorimetric determination of carotene. After chromatography, this color can be removed from the carotene eluate without affecting the carotene by adding dilute alcoholic stannous chloride.

When carotene is determined by the AOAC method (*1*), *N,N'*-diphenyl-*p*-phenylenediamine used to stabilize carotene in alfalfa (*6*) causes high results (*2*). When an acetone-hexane solution containing this diamine is eluted through magnesia as in the AOAC method, the colored product has an absorption spectrum similar to carotene. Beauchene and others (*2*) state that a correction factor for the interference is impractical because it would depend on the amount of the diamine present, size of column, and amount of eluate. When a solution of *N,N'*-diphenyl-*p*-phenylenediamine only is eluted through magnesia, a large amount is adsorbed in a black band at the top of the column. The colored reaction product is slowly eluted as the chromatogram is developed, but apparently not all the diamine is eluted even with high acetone concentrations.

Mitchell and Silker (*7*) avoided this interference by using tricalcium phosphate as an adsorbent. Booth (*3*) used aluminum oxide for the adsorbent and light petroleum (b. p. 80° to 100° C.) to extract the carotene from the leaf meal, taking advantage of the low solubility of the diamine in light petroleum in the absence of acetone. These adsorbents do not form the colored reaction product. Brew (*4, 5*) removed the reaction product from the eluate in the AOAC method by washing the eluate with 85% phosphoric acid. For the purpose of quality control it would be better if the AOAC method could be used with only a slight modification. The method of Mitchell and Silker requires removal of the acetone from the carotene extract by evaporation before it is chromatographed on the tricalcium phosphate.

It appeared to this author that the yellow color was caused by an oxidation product of the diamine, probably *N,N'*-diphenyl-*p*-quinonediimine. It had been found in previous experiments in removing the colored reaction product from the eluate that concentrated phosphoric acid produced a pink color (*5*) and concentrated hydrochloric acid produced a blue color in the acid layer; therefore, *N,N'*-diphenyl-*p*-phenylenediamine was oxidized with a solution of benzoyl peroxide in hexane and found to undergo similar color reactions. A 1% alcoholic solution of stannic chloride also produced a deep blue color upon addition to the diamine-magnesia eluate and to the oxidized diamine in the absence of excess unreacted benzoyl peroxide. The unoxidized diamine, oils, and magnesia eluates of untreated alfalfa extracts gave negative results.

The question arose, whether the diamine in an oil can autoxidize and give the same interference for a sample treated with the oil, even though the carotene extract is not eluted through magnesia. A 2-year-old laboratory sample of a crude vegetable oil treated with the diamine that gave a positive test for the autoxidized diamine by the above color reactions was used to test this possibility. A sample of dehydrated alfalfa meal was treated with the oil and analyzed for carotene by eluting the carotene extract through tricalcium phosphate and Hyflo Supercel (1 + 1 by weight) as in the method of Mitchell and Silker. The oxidized diamine showed up in a narrow band trailing behind the carotene, giving no interference for this adsorbent in the complete absence of acetone. A trace of acetone caused the oxidized diamine to move down with the carotene tailings.

A similar band formed when a few milliliters of a diamine eluate from a magnesia chromatographic column were