pentoxide and serves to fix ammonia and aid granulation when the superphosphate is used in the production of high-analysis granular fertilizers. The amount of excess acid is no greater than the acid used in the formulation of most grades of fertilizers. Therefore, in most cases, it does not increase the over-all materials cost.

Suppliers of phosphate rock estimate that fine grinding of rock to the size desired for this process would increase the cost of the rock by about 40 cents per ton or about 13 cents per ton of fertilizer containing 10 units of phosphorus pentoxide from the superphosphate. This should be offset by savings in reduced inventories, lower cost of handling materials, and other inherent advantages of a continuous, integrated process. More precise control of acidulation probably would be required; however, this would have the advantage of ensuring uniformity of the physical and chemical properties of the superphosphate.

The continuous den was used in the pilot-plant tests because it was available and because it would permit integration of acidulation and ammoniation into a single continuous process. There is no obvious reason why batch mixing and denning techniques would not be suitable for producing superphosphate for immediate ammoniation. Retention times in batch dens are usually in the range of 5 to 24 hours. Under these conditions, a higher conversion could be obtained with the same acidulation ratio, or the same conversion could be obtained with somewhat less acid.

Studies are in progress in which similar techniques are being applied to processes for the manufacture of concentrated and enriched superphosphates.

Acknowledgment

The authors wish to acknowledge the contributions of G. W. Bolds, who conducted the bench-scale tests and supervised the operation of the acidulation pilot plant, and J. S. Lewis, Jr., and M. M. Norton, who supervised the operation of the ammoniation-granulation pilot plant. They are indebted also to A. B. Phillips, whose advice and comments helped to direct the course of the work. A significant portion of the analytical work was done by Frank Manning, Jr., and Joseph Lee, Jr.

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Received for review December 3, 1956. Accepted March 4, 1957.

SWINE NUTRITION

Digestive Enzymes of the Baby Pig. Pepsin and Trypsin

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The relative proteolytic activities of the gastric mucosa and pancreas of the baby pig were estimated from birth through 6 weeks of age. Although there was a rapid initial rate of development of pepsin during the first week, which lessened as age increased, the total levels of pepsin per unit of tissue were low until the third to fourth week of age. An apparent increase, with age, in the quantity of trypsin per pig was due to increased size of the pancreas, but no definite increase was noted per unit of pancreatic tissue. The relationship of these results to recent research in swine nutrition is discussed.

Quantitative and qualitative changes in digestive enzyme secretions occur in the early life of the pig. Sewall (*13*) was unable to demonstrate peptic activity in the gastric mucosa of fetal pigs nearly at term. Langendorff (*4*) found that, in general, pepsin was present just before birth but in very low concentrations. He pointed out that pepsin appeared prior to birth in herbivora, after birth in carnivora, and that the pig was intermediate in this respect. Mendel (*8*) found no pepsin or sucrase activity in pig embryos nearly at term but maltase was plentiful. Lactase was secreted in the young pig but not in the older pig. Plimmer (*12*) concluded that the secretion of lactase in the young but not in the old was characteristic of all animals. Recently, Heilskov (*2*) demonstrated that lactase secretion in rabbits and cows decreased with age. Bailey, Kitts, and Wood, (*1*) working with the baby pig, found lactase activity in intestinal extracts high at birth, followed by subsequent decrease of activity with age, while the reverse was found for maltase and sucrase. Kitts, Bailey, and Wood (*3*) found that amylase activity of pancreatic extracts increased with the age of the young pigs, while lipase activity was high at birth and remained high as growth proceeded.

Recent investigations on the nutrition of the baby pig, by Lewis and others (*6*), have shown that soybean proteins are very poor substitutes for milk proteins in the formulation of rations for early weaning. Noller, Ward, and Huffman (*11*) have observed similar results with young calves. However, the gain and feed efficiency of young pigs fed soybean protein basal diets were improved by either the addition of proteolytic enzymes to the diet (*6*) or by partial prehydrolysis of soybean protein with enzymes (*5*).

As the ability of the baby pig to utilize various rations might be reflected in the enzyme content of the digestive glands, studies have been made on the enzyme activities of extracts of certain digestive enzyme secretory tissues taken from pigs at various ages. The studies reported

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concern the proteolytic activities of the gastric mucosa and pancreas. The terms pepsin and trypsin, as used herein, include any proteases which contribute to the area of hydrolysis of milk agar plates under the conditions of assay specified.

Materials and Methods

Selection of Pigs. The pigs sacrificed for these experiments were the progeny of hybrid boars mated to three-way crossbred sows (Poland China with Landrace with Duroc). Although the lactation ration was basically a corn–soybean oil meal diet, it was supplemented with animal proteins and balanced with vitamins, minerals, trace minerals, and an antibiotic. The baby pigs were allowed to nurse their dams only and did not have access to any other source of nutrients. Reduced iron tablets containing traces of copper were administered weekly to control anemia.

Originally, six litters of at least seven pigs per litter were selected so that no pig had a birth weight of less than 2.0 pounds. It was intended that one pig from each litter would be removed for sacrifice at weekly intervals, from birth to 6 weeks of age (plus or minus 1 day). However, owing to the loss of some of the pigs, six pigs were sacrificed at birth and at 1 and at 2 weeks; five pigs at 3, 4, and 5 weeks; and two pigs at 6 weeks of age. The sacrificing date of each pig was predetermined at birth by random selection from within the litter.

Removal of Glands. On the day the pigs were sacrificed, they were first allowed to nurse and then were fasted for 6 hours before the sacrificing and body weights were recorded.

The pigs were electrocuted and bled, the body cavity was opened immediately and the desired organs were removed as rapidly as possible. Stomachs and intestines were trimmed of exterior fat and connective tissues, opened, and washed of their contents in cold tap water. The pancreas was also removed, trimmed, and washed. Care was exercised during these processes to minimize the rupturing of cells.

As soon as the glands were washed, they were weighed and placed in a freezer chest containing dry ice. The time from death to the time the glands were placed under refrigeration was 5 to 10 minutes for the stomachs and pancreas and 20 to 25 minutes for the intestines. Shortly after collection, all samples were transferred to a deep freeze for storage at −15° C.

Solvent Drying of Tissues. The frozen tissues were solvent-dried by a modified procedure, based on those described by Morton (*10*) and Meyer, Fischer, and Bernfeld (*9*). All operations were carried out in a cold room at −5° C.

Table I. Pig Body Weights at Time of Sacrifice

Litter No.	Age, Days: Birth	7	14	21	28	35	42
	Kilograms						
1	1.68	2.36	4.90	4.85	8.75	10.16	...[a]
2	1.63	3.08	2.90	...	...	...	...
3	1.13	2.04	3.99	4.44	5.62	7.26	7.98
4	1.31	2.31	3.94	4.35	4.63	8.30	7.35
5	1.59	2.45	2.72	4.44	5.03	7.89	...
6	1.18	2.81	3.40	5.03	7.21	8.44	...
Av.	1.42	2.51	3.64	4.62	6.25	8.41	7.67

[a] Blanks indicate that pigs were not available.

The whole organs were removed from the deep freeze and allowed to warm to −5° C. At this temperature, the tissues became soft enough to cut into small chunks with sharp scissors. The tissue chunks were placed into a semimicro (250-ml.) Monel Waring Blendor container and were homogenized for 3 minutes with four volumes of reagent grade or redistilled C.P. acetone. The homogenate was transferred to a 250-ml. Erlenmeyer flask and any remaining material was washed into the flask by rinsing the blender jar with one volume of acetone. The flask was stoppered with an aluminum foil covered cork, then the flask was shaken slowly for 4 to 5 hours. After shaking, the material was transferred to either a 30-ml. centrifuge tube or a 250-ml. centrifuge bottle (depending upon the volume of the homogenate) and was centrifugated for 30 minutes at 2000 r.p.m. in either a Model U International centrifuge, kept in the cold room, or a Model PR-1 International refrigerated centrifuge, held at approximately −5° C. The supernatant was discarded, the centrifugate was washed back into the 250-ml. flask with the above quantity of fresh acetone, shaken overnight (14 to 18 hours), centrifugated, and decanted. The process was again repeated with the same quantity of acetone and a 4- to 5-hour shaking period.

Centrifuge tubes and bottles containing the final centrifugate were placed in desiccators and the tissue material was dried in vacuo over calcium chloride in the cold room. A new vacuum was pulled on the desiccators every 6 to 8 hours for a period of 72 hours, then the desiccators were removed from the cold room and placed under continuous vacuum for 2 days.

The dry material was weighed, transferred to screw-cap jars, and the contents of each jar were mixed well.

Table II. Pepsin Activity of Stomach Tissue Extracts from Baby Pigs

Litter No.	Age, Days: 1	7	14	21	28	35	42
	Wet Weight of Stomach, G.						
1	10.0	13.4	32.0	35.8	54.7	70.6	...[a]
2	6.1	18.2	14.9	...	...	...	...
3	4.0	15.0	29.8	39.7	41.0	59.6	56.7
4	8.1	17.7	28.9	35.1	35.3	48.9	53.8
5	7.6	16.2	17.5	34.2	38.6	46.6	...
6	6.0	17.2	20.1	33.5	38.2	54.8	...
Av.	7.0	16.3	23.9	35.7	41.6	56.1	55.2
	Dry Weight of Stomach, G.						
1	0.74	1.87	4.70	5.79	8.59	10.98	...
2	0.61	2.46	2.17	...	...	...	...
3	0.29	2.18	4.40	5.90	6.23	9.78	9.43
4	1.09	2.71	4.10	4.89	5.59	7.72	8.20
5	0.98	2.31	2.64	5.14	5.50	7.39	...
6	0.89	2.54	2.84	5.24	6.03	8.65	...
Av.	0.77	2.34	3.48	5.39	6.39	8.91	8.82
	Units of Pepsin Activity/G. of Dry Weight of Tissue						
1	0.08	0.22	0.21	0.47	0.78	1.4	...
2	0.08	0.21	0.20	...	...	...	...
3	0.04	0.22	0.21	0.31	0.88	2.1	1.7
4	0.08	0.22	0.21	0.63	1.00	1.0	1.7
5	0.10	0.18	0.19	0.55	0.38	0.9	...
6	0.07	0.14	0.17	0.73	1.45	1.5	...
Av.	0.07	0.20	0.20	0.55	0.90	1.4	1.7

[a] Blanks indicate pigs were not available.

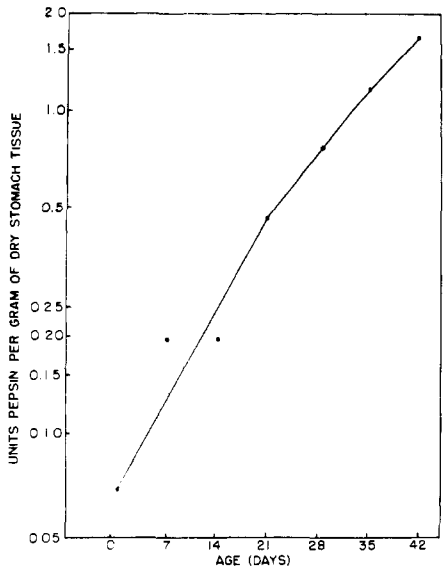

Figure 1. Log (plotted on $\log_{10}$ scale) of pepsin activity of dry stomach tissue from baby pigs of various ages

Estimation of Enzyme Concentration. Relative pepsin or trypsin activities of the dried tissues were estimated by modifications of the disk-plate techniques described by Stark and others (*11*). The substrate for the assay of pepsin and of trypsin consisted of the following:

	%			
	Na_2HPO_4 $7H_2O$	KH_2PO_4	Dried skim milk	Agar (Difco)
Pepsin	...	0.3	0.5	1.0
Trypsin	1.67	0.165	1.0	1.0

Distilled water was added to the dry ingredients in a beaker, the mixture was heated to boiling to dissolve the agar, and the solution was cooled to approximately 55° C. The hydrogen ion concentration of the pepsin substrate was adjusted to pH 2.45 with concentrated phosphoric acid. The hydrogen ion concentration for trypsin (pH 7.4) was left unadjusted. The agar solutions were made up to proper volume and 20 ml. of the substrate-containing agar was measured, with a 20-ml. syringe, into each pressed borosilicate glass Petri plate (94-mm. outside diameter), placed on a level surface. The agar was stirred constantly during the pouring operation in order to maintain even distribution of the substrate. After the agar had solidified, the Petri plates were inverted and stored in a refrigerator for periods never exceeding 1 week.

Filter paper disks (Schleicher and Schuell No. 740-E, 12.7-mm. diameter), six for each sample, were individually grasped with fine-pointed forceps and were touched to the enzyme solution until saturated by adsorption up the disk. Any condensed moisture on the top of the plate was removed. Completed plates were placed upright in stacks of ten or less in a 40° C. incubator for 16 hours. Then they were removed and the diameters of the zones of hydrolysis (clear areas surrounding the disks) were read on a Fisher-Lilly antibiotic zone reader (Fisher Scientific Co., Pittsburgh, Pa.).

The units of enzyme activity in a 4% solution of dried tissue were read directly from standard curves as per cent of the standard. Such units were expressed as units per gram of dry material. The standard curves were made by plotting the diameters of the zones of hydrolysis (in millimeters) obtained with known concentrations of pepsin (1 to 15,000, supplied by courtesy of Cudahy Laboratories, Omaha, Neb.) or trypsin (3 × U.S.P.) against the log of the per cent of enzyme used. Although a linear relationship existed in the pepsin standard over a wide range of enzyme concentrations, a straight line was not obtained for the trypsin standard curve. The greater curvature at the extreme concentrations of pancreatin may have been the result of the increased milk concentration of the substrate-agar, which was necessary for good definition of the zone border for the trypsin assay.

The data were also examined by the standard parallel-line assay analysis. The estimates of enzyme activity derived by this method agreed fairly well with those obtained by graphical readings from the standard response curve, but, at places, there were serious discrepancies from the basic assumptions of a valid assay; therefore, the results from these calculations are not presented. Graphical readings only are given and with certain reservations.

Results and Discussion

Pepsin. The pig weights at time of sacrifice, and the wet weights of the stomachs, dry weights of stomach material, and calculated units of pepsin activity per unit of dried stomach tissue are shown in Tables I and II, respectively. Relative units per kilogram of body weight or gram of wet stomach weight may be calculated from the tables, but are not included here for the sake of brevity.

There is negligible pepsin activity present in the stomach of the young pig at the time of birth (Table II, Figures 1 and 2). If the logarithm of pepsin activity per gram dry weight of stomach tissue is plotted against age (Figure 1), a rapid rate of pepsin development is

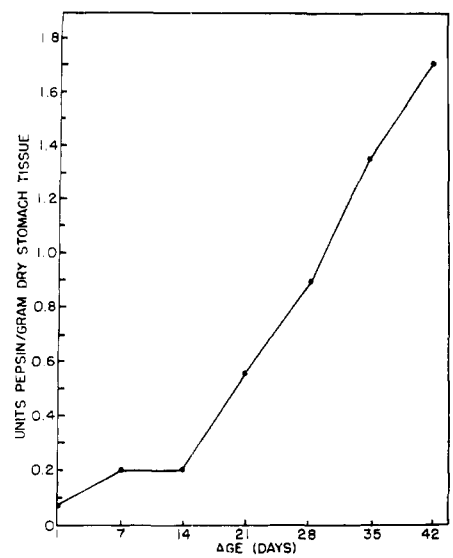

Figure 2. Pepsin activity of dry stomach tissue from baby pigs of various ages

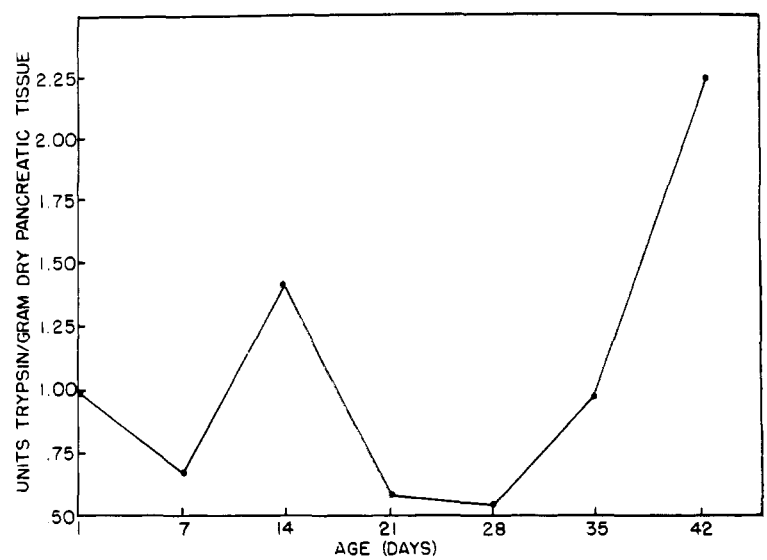

Figure 3. Trypsin activity of dry pancreatic tissue from baby pigs of various ages

shown to occur in the young pig, becoming curvilinear downward with increasing age. However, if results are plotted on Cartesian coordinates (Figure 2), the actual quantity of pepsin activity present can be more clearly demonstrated to be low until the pig is about 3 weeks of age, after which there is a linear increase in the quantity of pepsin activity present (from 3 through 6 weeks of age).

These low levels of pepsin activity may be reflected in poor utilization of vegetable protein (example, soybean oil meal) by early weaned pigs up to 3 or 4 weeks of age, after which relative gains and feed efficiency increase markedly (*6*). According to Liu and coworkers (*7*), the pH of the stomach contents of the baby pig remains relatively acid from birth through 6 weeks of age; hence, one reason for the inability of the very young pig to utilize plant proteins adequately could be the lack of pepsin rather than the unfavorable pH for enzyme action.

Because of the large pig to pig, litter to litter, and laboratory (assay) variations encountered in these studies, the values reported herein should be considered with some reservations. What effects the genetics, prenatal and postnatal nutrition of the pig, environment, or other factors may have on enzyme secretion are at present uncertain.

Trypsin. The pancreas wet weights, dry weights, and estimated units of trypsin activity per gram of dried pancreatic tissue are shown in Table III. Relative units per kilogram of body weight may be calculated by reference to Table I.

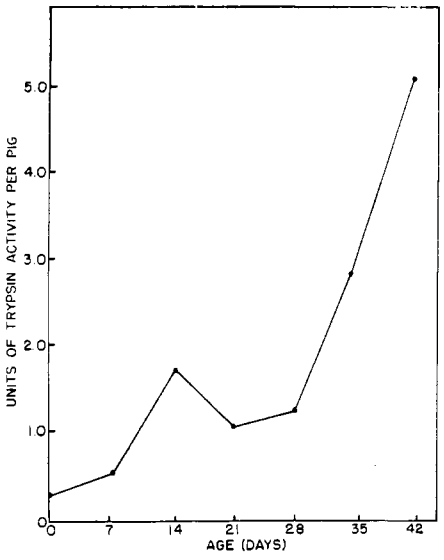

Figure 4. Trypsin activity per pig in baby pigs of various ages

No definite increase in trypsin activity per gram of dry tissue was noted with increasing age (Table III and Figure 3). Levels of enzyme varied considerably between the litters at all ages. For example, of the two 42-day-old pigs, one pig had fifteen times more activity per unit of dry pancreatic tissue than the other pig.

The young pig, at birth, is capable of producing relatively high amounts of trypsin. Any subsequent increase in trypsin activity per animal (Figure 4) is due largely to increased size of the pancreas and not to a greater quantity of enzyme production per unit of tissue. However, the pig to pig variation was very high and, consequently, some pigs may have levels of trypsin inadequate to hydrolyze the less digestible proteins. These observations differ from those for pepsin, where there are increases in both organ size and enzyme activity per unit of tissue.

Table III. Trypsin Activity of Pancreatic Tissue Extracts from Baby Pigs

Litter No.	Age, Days 1	7	14	21	28	35	42
			Wet Weight of Pancreas, G.				
1	3.29	4.5	6.9	9.9	15.0	16.5	...[a]
2	3.60	6.1	4.4	...	...	...	...
3	1.78	3.3	7.8	8.8	11.6	11.7	13.9
4	2.05	4.0	5.1	7.5	10.3	15.7	12.3
5	1.82	4.3	4.8	6.8	9.4	11.5	...
6	1.50	4.8	5.7	8.0	10.2	10.5	...
Av.	2.34	4.5	5.8	8.2	11.3	13.2	13.0
			Dry Weight of Pancreas, G.				
1	0.54	0.80	1.45	2.10	3.27	4.00	...
2	0.49	1.27	0.96	...	...	...	...
3	0.28	0.62	1.47	1.72	2.59	2.74	2.70
4	0.25	0.74	1.06	1.30	2.03	2.61	2.42
5	0.39	0.83	0.90	1.28	2.06	2.56	...
6	0.28	0.98	0.80	1.59	2.08	2.34	...
Av.	0.37	0.87	1.11	1.60	2.41	2.85	2.56
			Units of Trypsin Activity/G. of Dry Weight of Tissue				
1	1.00	0.66	4.20	1.70	0.78	1.40	...
2	0.39	0.78	0.72	...	...	...	...
3	2.60	0.52	0.54	0.22	0.38	1.70	0.28
4	0.36	0.15	2.20	0.38	0.84	0.11	4.20
5	1.10	0.34	0.52	0.25	0.36	0.42	...
6	0.35	0.80	0.30	0.28	0.34	1.10	...
Av.	0.97	0.64	1.41	0.57	0.54	0.95	2.24

[a] Blanks indicate pigs were not available.

Acknowledgment

The authors wish to acknowledge the assistance of Gordon C. Ashton in designing the experiment and in the analysis of the data; of Vaughn Speer, associate in animal husbandry, in preparing pigs for sacrifice; of Don Quinn and his associates at the Swine Nutrition Research Farm; and of the Cudahy Laboratories, Omaha, Neb., in grants, which partially supported this research.

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Received for review October 27, 1956. Accepted March 7, 1957. Journal paper No. J-3070, Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 959.