veloped a striking granulopenic leucopenia, and manifested a markedly lowered clinical resistance to spontaneous infections with high mortality. The susceptibility to experimental infections with *Streptococcus hemolyticus*, Group C, and to influenza virus A, administered intranasally, was likewise increased in contrast with the controls on normal diet.

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Nutritive Value of Keratins. I. Powdered Swine Hoofs.*

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Routh and Lewis[1] recently summarized the literature dealing with the indigestibility of keratins in their natural state. In the same paper they reported that wool which had been powdered in a steel ball mill was readily hydrolyzed *in vitro* by pepsin and trypsin. In subsequent studies Routh[2] found that rats were able to use powdered wool as a source of protein for growth when the material was supplemented with tryptophane, methionine, histidine, and lysine.

The results of these investigations on wool suggested the possibility of converting keratin waste such as horns, hoofs, hair, and feathers into animal feed. This report deals with studies on the nutritive value of powdered swine hoofs fed as a source of protein in the diet of rats and chicks.

Mixed hog hoofs collected at a packing house were ground to a fine, gray powder until all the material passed through a 60-mesh screen.‡ The nitrogen content of the material was 14.5% and it was compared on a weight basis with casein and cartilage without regard to differences in protein content.

Groups of male albino rats 21-22 days of age and weighing 40-50 g were fed the experimental diets given in Table I for 4 weeks. Water and feed were supplied *ad libitum* and body weights were recorded weekly. Typical gains obtained on the different diets are also given in Table I.

Powdered hoofs fed at a level of 18-20% in the diet of rats failed to produce rates of gain comparable to those obtained with equal weights of casein. However, when the level was increased to 30%, growth nearly equal to that of rats fed 18% casein was obtained. Some supplemental effect was obtained when the two proteins were fed together and it is of interest to note that better growth resulted when 15% powdered hoofs were fed with 5% casein than when 9% of powdered hoofs were fed with 9% casein.

The composition of the diets used for the chicks is given in Table II. Day-old White Leghorn chicks were fed the diets for a period of 4 weeks. At the end of 4 weeks the chicks were killed and the gizzards examined for possible lesions.

Powdered hoofs fed at the level of 24% in the diet of chicks produced a higher growth rate and a better development of feathers than an equal percentage of casein. Although the growth rate obtained when 18% casein was supplemented with 10% powdered hoofs was less than that obtained on the diet containing a 10% supplement of cartilage, it was appreciably more than the growth rate obtained with 24% casein. Examination of the gizzards revealed a distinctive condition of the lining in all of the groups fed powdered hoofs. The surface of the linings showed a widespread roughening and cracking. The majority of the fissures were parallel with the normal folds

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

† Wilson and Company Fellow.

1 Routh, J. I., and Lewis, H. B., *J. Biol. Chem.*, 1938, **124**, 725.

2 Routh, J. I., *J. Nutrition*, 1942, **23**, 125.

‡ Prepared by Wilson and Company, Inc.

TABLE I.
Diets and Growth Results in Rat Experiments.

Group	I	II	III	IV	V	VI	VII
	%	%	%	%	%	%	%
Purified casein	18	9	0	20	5	0	0
Powdered hoofs	0	9	18	0	15	20	30
Sucrose	69	69	69	67	67	67	57
Liver powder Fraction B	0	0	0	2	2	2	2
Liver Powder 1-20	2	2	2	0	0	0	0
Avg daily gain in body wt, g	3.6 (3.2-4.2)	2.5 (2.2-3.2)	1.3 (1.0-1.5)	3.6 (3.5-4.0)	3.5 (3.0-3.9)	1.1 (0.4-1.4)	3.2 (3.0-3.5)

All diets contained in addition to the above listed constituents, Salt IV, 4%; corn oil, 5%, cod liver oil, 2%, and vitamin supplement as follows: 2 mg riboflavin, 2 mg thiamine chloride, 2 mg pyridoxine hydrochloride, 1 g choline hydrochloride, and 10 mg calcium pantothenate per kg. Six rats were used in each group.

Liver preparations supplied through courtesy of Wilson Laboratories.

Synthetic vitamins supplied by Merck and Company.

TABLE II.
Diets and Growth Results on Chick Experiments.

Group	I	II	III	IV
	%	%	%	%
Purified casein	24	18	18	0
Powdered hoofs	0	0	10	24
Cartilage	0	10	0	0
Dextrin	58	54	54	58
Avg body wt at 4 wks	94 (50-150)	233 (173-263)	181 (145-225)	121 (93-152)

All the diets contained in addition to above listed ingredients, brewer's yeast, 5%; soy bean oil, 5%; salt IV, 4%; cod liver oil, 2%, and vitamin supplements as follows: 15 mg calcium pantothenate, 3 mg thiamine chloride, 1.5 g choline hydrochloride, 3 mg pyridoxine hydrochloride, 3 mg riboflavin. Twelve chicks were used per group.

Liver preparations supplied through courtesy of Wilson Laboratories.

Synthetic vitamins supplied by Merck and Company.

Yeast supplied by Pabst Brewing Company.

of the linings and did not have the crater appearance of lesions reported by other workers.[3-8] They appear to be similar to the gizzard lesions described by Jungherr.[9]

Discussion: The data on rats and chicks indicate that these animals are able to digest and utilize the protein of powdered hoofs. When 18% and 20% of the diets were supplied by powdered hoofs the protein supply was inadequate for the growth of rats. By increasing the intake of powdered hoofs to 30% of the diet or combining the powdered hoofs with casein an adequate protein source was obtained.

From these facts it appears that one or more amino acids are not available to growing rats in adequate amounts in powdered hoofs. This lack could be due to incomplete digestion of the material or an imbalance of amino acids. With regard to this point it was noted that the group of rats receiving 30% powdered hoofs as the dietary source of protein produced an average daily gain of 3.17 g which greatly exceeds the maximum growth rates reported for powdered wool supplemented with amino acids.[2] From the data of the chick experiments it appears that powdered hoofs alone

[3] Holst, W. F., and Halbrook, E. R., *Science*, 1933, **77**, 354.

[4] Dam, H., *Nature*, 1934, **133**, 909.

[5] Dam, H., and Schönheyder, F., *Biochem. J.*, 1934, **28**, 1355.

[6] Almquist, H. J., and Stokstad, E. L. R., *Nature*, 1935, **136**, 31.

[7] Bird, H. R., Kline, O. L., Elvehjem, C. A., Hart, E. B., and Halpin, J. G., *J. Nutrition*, 1936, **12**, 571.

[8] Almquist, H. J., and Stokstad, E. L. R., *Nature*, 1936, **137**, 581.

[9] Jungherr, E., *Conn. Agr. Exp. Sta. Bul.*, 1935, **202**, 52.

are more adequate for the growth of chicks than purified casein alone. Other workers have shown that the chick has a greater requirement for arginine,[10] glycine,[11] and cystine[12] than the rat. Apparently powdered hoofs supply these amino acids in greater amounts than casein. Therefore, the deficiencies of powdered hoofs in the nutrition of the rat may be due to amino acid imbalance rather than indigestibility. However, the latter possibility cannot as yet be eliminated entirely since the digestive tract of the chick may be more capable of digesting material of this nature than the tract of the rat.

Although gizzard lesions were only observed in those chicks receiving powdered hoofs, it appears from other work performed with practical poultry diets that the lesions are not specific for powdered hoofs.

Conclusions: Rats and chicks are able to utilize powdered pig and hog hoofs as a source of protein for growth. The available protein of powdered hoofs is more adequate for the growing chick than for the growing rat.

10 Arnold, A., Kline, O. L., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1936, **116**, 699.

11 Almquist, H. J., Stokstad, E. L. R., Meechi, E., and Manning, P. D. V., *J. Biol. Chem.*, 1940, **134**, 213.

12 Briggs, G. M., Jr., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1942, **144**, 47.

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Titration and Neutralization of the Western Strain of Equine Encephalomyelitis Virus in Tissue Culture.*

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Titration of virus potency and demonstration of viral neutralizing antibodies are procedures not only involving large numbers of animals but also presenting disadvantages which make quantitative interpretation difficult. In an effort to evolve a simplified approach and particularly to remove the variable of individual animal reactivity, tissue culture methods were utilized for titration and neutralization of the Western strain of equine encephalomyelitis virus (W.E.E.). The method is presented here. The principle on which the study was made possible is based on the finding that tissue failed to grow when it was heavily infected with the virus.

In Vitro Titration. Ten-fold dilutions of W.E.E. virus were made in a buffered salt solution. Instead of injecting the virus into animals, one drop of the material from each dilution was inoculated into tissue cultures consisting of a series of tubes each containing 10 pieces of minced skeletal muscles from a 9-day developing chick embryo and 1 cc of serum ultrafiltrate diluted in buffered salt solution.[1] The different mixtures were incubated at 37.5°C. At the end of 48 hours of incubation, pieces of tissue from each tube were transferred and patched with plasma in micro-culture slides. They were then kept at 37.5°C and the readings were made under the low power microscope 48 hours later. It was found that cells which were not infected (or had overcome the infection) grow out in the plasma patch. Growth was abundant and sheets of fibroblasts were clearly visible. Contrariwise, when virus was present, no growth from the explant was observed.

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1 Simms, H. S., and Sanders, M., *Arch. Path.*, 1942, **33**, 619.