

Cystine Content and Enzyme Digestibility of Powdered Hoof Proteins.

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Routh and Lewis¹ first described the effect of subdivision in enhancing the digestibility of keratin by proteolytic enzymes. Recently Wagner and Elvehjem² obtained growth in rats and chicks with powdered swine hoofs as the main source of protein. Their preparations must therefore have been susceptible to enzymatic digestion *in vivo*. The results obtained in the experiments described in this paper show that (a) the major part of cattle and horse as well as hog hoof powders are digestible by proteolytic enzymes *in vitro*, (b) the undigested residue contains considerably more cystine than does the total hoof protein, and (c) there is an inverse relationship between cystine content and digestibility by pancreatin in different parts of the individual cattle hoofs.

Cattle hoofs were washed with water and then were treated in various ways to reduce them to powder. In one instance, soaked hoofs were shredded with a coarse rasp and the raspings were run through a Premier colloid mill in the presence of large amounts of water, allowed to settle, filtered, washed, dried at 70°, and sieved. For most preparations the cleaned hoofs were dried at 70° for several days until they became brittle, then broken to small pieces by pounding in an iron vessel and finally ground in a porcelain ball mill for 24 to 48 hours. It should be mentioned that the mild grinding procedures differentiate these experiments somewhat from those described by Routh,³ who was able to make wool and chicken feathers nutritionally available by grinding them for several weeks

in a large iron mill equipped with steel balls.

Portions of the powdered cattle hoof were incubated with solutions of pancreatin, pepsin, papain, ficin, and a bacterial protease, after adjustment of the pH to the optimal for the particular enzyme. A considerable proportion of the hoof was digested in each case, showing that, in general, cattle hoof can be digested by proteolytic enzymes. Pancreatin was used in most of the subsequent experiments.

In order to study the influence of particle size on digestibility by pancreatin, various mesh sizes of cattle, horse, and hog hoof powders were treated separately. The results are shown in Table I.

TABLE I.
Effect of Size of Particle on Digestibility of Hoof Preparations* by Pancreatin.†

Mesh size	Cattle hoof	Horse hoof	Hog hoof
Soluble N as percentage of total N.‡			
40-60	—	8	37
60-80	17	11	46
80-100	26	13	55
100-140	30	18	59
140-200	33	24	65
>200	52	39	81

* Dried at 70°, broken and ball-milled.

† 2 g keratin, 5 ml pancreatin solution (4.4 mg N per ml), 45 ml 1% Na₂HPO₄ · 10H₂O, shaken at room temperature for 24 hours. The final pH varied from 6.5 to 7.5.

‡ Not corrected for the amount of N soluble in the buffer alone. Except with hog hoof, this was a negligible factor. 5% of the 80-100-mesh and 10% of the 200-mesh hog hoof powder nitrogen was soluble in the buffer only.

Samples of 200-mesh cattle, horse, and hog hoof preparations were subjected to exhaustive proteolysis by incubation with several changes of pepsin and then pancreatin, each time for 48 hours at 37°. The residues were washed and dried and analyzed for H₂O, N, S, ash, and total cystine content.⁴ The results, corrected for moisture and ash, are shown in Table II.

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

² Wagner, J. R., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 394; *Poultry Sci.*, 1943, **22**, 275.

³ Routh, J. I., *J. Biol. Chem.*, 1940, **135**, 175; *J. Nutrition*, 1941, **23**, 125; 1942, **24**, 399.

⁴ Mecham, D. K., *J. Biol. Chem.*, in press.

TABLE II.
Analyses of Hoof Powders and Residues from Enzyme Digests.*

	Yield,† %	N	S	Total cystine†
Cattle hoof—original	—	16.2	2.2	5.9
residue	19	15.9	3.0	9.0
Horse hoof —original	—	16.3	2.3	5.9
residue	28	15.6	3.4	8.4
Hog hoof —original	—	15.6	1.5	4.4
residue	2.5	12.8	2.3	9.0

* Corrected for moisture and ash.

† The yield results are only approximate since unavoidable losses occurred during handling.

The undigested portion in each case was considerably richer in cystine than the original.

It is well recognized that the physical structure of the cattle hoof is not uniform. Those portions comprising the bottom and rear of the hoof (subunguis) become soft when wet and can readily be pared with a sharp knife, in contrast to the front jutting portion (unguis), which is considerably harder. The hard and soft parts of several hoofs were separated, dried, ground, and analyzed for cystine and for digestibility by pancreatin. The hard portion contained 5.6% cystine, and 51% of the insoluble nitrogen was changed into a soluble form by pancreatin in 24 hours at 37°. The soft portion contained only 2.8% cystine, and 71% of the nitrogen was digested under the same conditions.

Thus, as would be expected from the anatomical structure of the hoof, the powdered hoof material is a mixture of at least 2 and possibly many proteins containing differing numbers of disulfide linkages. The greater number of disulfide cross-links in the unguis might be pictured as contributing to the hoof protein structure the physical stability necessary for resistance to abrasion and shock.

Probably only those fractions resistant to proteolytic enzymes and rich in cystine should be regarded as keratins.

The importance of the disulfide linkage to the enzyme resistance of wool has been described by Geiger, Patterson, Mizell, and Harris.⁵ When the wool fibers were reduced by thioglycolic acid they were readily attacked by proteases. The correlation between cystine content and enzymic resistance in hoof proteins is interpretable in the light of their results.

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Summary. Considerably more than half of dried powdered cattle, horse, and hog hoof material was found to be digestible by proteolytic enzymes. Hog hoof proved the most readily digestible and horse hoof the least. The undigestible residue in each case contained more cystine than the original preparations. The soft part of the cattle hoof contained less cystine and was more readily digested than the horn-like front portion. Thus hoof powder consists of at least two and possibly a number of different kinds of proteins.

⁵ Geiger, W. B., Patterson, W. I., Mizell, L. R., and Harris, M., *J. Res. Nat. Bur. Stand.*, 1941, **27**, 459.