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(54) **METHODE ET COMPOSITION POUR L'ADMINISTRATION
D'UN REGIME A BASE DE KERATINE A DES ANIMAUX**

(54) **METHOD AND COMPOSITION FOR MAINTAINING ANIMALS
ON A KERATIN-CONTAINING DIET**

(57) Procédés et compositions permettant de soumettre des animaux à un régime à base de kératine comme source de protéines. Le procédé consiste à alimenter un animal à partir d'une source d'hydrates de carbone et d'une première source de protéines, cette première source comprenant de la kératine et une kératinase pouvant hydrolyser la kératine. On décrit un fourrage pour animaux comprenant une source d'hydrates de carbone et une première source de protéines, cette première source comprenant de la kératine et une kératinase pouvant hydrolyser la kératine. La kératine préférée est constituée par des plumes broyées, et une kératinase préférée pour la mise en oeuvre des procédés décrits est obtenue à partir de la souche PWD-1 *Bacillus licheniformis*.

(57) Methods and compositions for maintaining animals on a diet containing keratin as a source of protein are disclosed. The method comprises feeding an animal a carbohydrate source and a first protein source, the first protein source comprising keratin and a keratinase capable of hydrolyzing the keratin. A composition of the present invention is an animal feed comprising a carbohydrate source and a first protein source, the first protein source comprising keratin and a keratinase capable of hydrolyzing the keratin. A preferred keratin for practicing the present invention is ground feather, and a preferred keratinase for practicing the invention is obtained from *Bacillus licheniformis* strain PWD-1.



Abstract

Methods and compositions for maintaining animals on a diet containing keratin as a source of protein are disclosed. The method comprises feeding an
5 animal a carbohydrate source and a first protein source, the first protein source comprising keratin and a keratinase capable of hydrolyzing the keratin. A composition of the present invention is an animal feed comprising a carbohydrate source and a first protein
10 source, the first protein source comprising keratin and a keratinase capable of hydrolyzing the keratin. A preferred keratin for practicing the present invention is ground feather, and a preferred keratinase for practicing the invention is obtained from *Bacillus*
15 *licheniformis* strain PWD-1.

METHOD AND COMPOSITION FOR MAINTAINING ANIMALS
ON A KERATIN-CONTAINING DIET

Field of the Invention

The present invention relates to methods and compositions which are useful for enhancing the digestibility and utilization of keratinaceous materials fed to animals as a source of dietary protein.

Background of the Invention

Keratinaceous materials are often included in animal feeds as an inexpensive source of dietary protein. Keratins such as feather, horn, hooves, and hair are readily available as agricultural byproducts. A problem with feeding animals such materials, however, is that they are difficult to digest. J. Adler-Nissen, *Enzymic Hydrolysis of Food Proteins*, 100 (1986). Hence, the amount of amino acids taken up into animals from such materials is relatively small compared to the total amount of amino acids present in such materials.

A solution to the foregoing problem is reported in U.S. Patent No. 4,908,220 to J. Shih and C. Williams, issued March 13, 1990. This patent describes a hydrolyzed feather animal feed supplement consisting of feather hydrolyzed by fermenting it with *Bacillus licheniformis* PWD-1 prior to feeding the material to the animal. While the technology disclosed in this application provides a way to substantially increase the digestability of feather, it necessitates a fermentation step which adds to the complexity of manufacturing the feed.

In view of the foregoing, an aspect of an object of the present invention is to provide a way to increase the digestibility of keratin, which keratin is included in an animal feed as a source of dietary protein, without the necessity of fermenting the material prior to feeding.

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Summary of the Invention

The present invention is based on the discovery that a keratinase fed to an animal with a keratin-containing animal feed will enhance the digestibility of that feed. In accordance with one embodiment, the invention provides a
10 method of maintaining an animal on a keratin-containing diet comprising feeding the animal a carbohydrate source and a first protein source, the first protein source comprising keratin, concurrently with a keratinase capable of hydrolyzing the keratin in an amount effective to enhance the digestibility of the keratin in the digestive tract of the animal. The animal may optionally be
15 concurrently fed a supplemental, second protein source.

In accordance with a further embodiment, the invention provides an animal feed comprising a carbohydrate source and a first protein source, the first protein source comprising keratin and a keratinase capable of hydrolyzing the keratin in an amount effective to enhance the digestibility of the keratin.

20 In accordance with a further embodiment, the invention provides a method of maintaining an animal on a keratin-containing diet comprising feeding the animal a carbohydrate source and a protein source, the protein source comprising keratin, concurrently with *Bacillus Licheniformis* PWD-1 keratinase enzyme in an amount effective to enhance the digestibility of the
25 keratin.

In accordance with a further embodiment, the invention provides an animal feed comprising a carbohydrate source and a first protein source, the protein source comprising keratin and *Bacillus Licheniformis* PWD-1

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keratinase enzyme in an amount effective to enhance the digestibility of the keratin.

Detailed Description of the Invention

5 Animal feeds of the present invention comprise a carbohydrate source in combination with a protein source. Carbohydrate sources used to produce an animal feed according to the present invention include, for example, corn, oats, barley, sorghum, or combinations of the same. These grains are preferably ground into a meal for use in the animal feed. Supplementary
10 protein source include, for example, soy meal, fish meal, blood meal, poultry by-product (ground poultry offal), meat meal, and combinations of the same. An animal feed is comprised of from about

13% to about 25% by weight of protein from all protein sources (including both the keratin source and the supplementary protein source). The keratin may be the sole protein source, but is preferably from about 2% to
5 about 15% by weight of the feed. Other nutrients in small amounts, such as vitamins, minerals, antibiotics, and other substances or compounds may be included in the feed as required. The present invention can be used to feed any animal, including cows, sheep, pigs,
10 cats, dogs, ferrets, and birds (e.g., chickens, ducks, and turkeys), but is preferably employed with monogastric animals. The precise composition and physical characteristics of the animal feed will, accordingly, depend upon the species for which the feed
15 is intended, and can be readily determined by those skilled in the art. Thus, the present invention provides a variety of different feeds, including pet feed, poultry feed, and pig feed.

Keratin used as a protein source in
20 practicing the present invention is preferably feather, but may be another keratin material such as hair, horn, or hoove. The material is preferably ground to a consistency suitable for inclusion in an animal feed (e.g., powdered feather or feather meal). If desired,
25 the keratin may be partially degraded by steam or enzymatic means prior to combining with the keratinase, in which case the keratinase serves as a means to further increase the digestibility of the keratin. However, since prior enzymatic digestion adds to the
30 production cost of the feed, the currently preferred embodiment of the present invention employs a keratin which has not been previously subjected to enzymatic degradation.

Keratinases are fed to the animal
35 concurrently with the keratin in an amount effective to enhance the digestibility of the keratin. A suitable ratio of keratinase to keratin is about 1:100 by

weight. The keratinase should be present in an amount at least sufficient to achieve the intended effect, but the upper limit to the amount of keratinase included is not particularly critical. The keratinase may be
5 included in the same composition as the keratin, as in the case of the animal feeds of the present invention, or may be administered separately but concurrently with the keratin (e.g., by inclusion in drinking water). While we do not wish to be bound to any particular
10 theory of operation of this invention, it appears that the keratinase retains activity while it is in the digestive tract of the animal being fed for a time sufficient for it to hydrolyze the keratin and enhance the digestibility thereof.

15 A preferred keratinase for practicing the present invention is obtained from *Bacillus licheniformis* strain PWD-1, which is described in U.S. Patents Nos. 4,908,220 and 4,959,311.

20 This bacteria was deposited with the American Type Culture Collection (ATCC) in Rockville, Maryland, USA in accordance with the Budapest Treaty on March 23, 1988, and assigned ATCC Accession No. 53757. Other keratinases which can
25 be used to practice the present invention are available from a variety of bacterial sources, such as *Streptomyces fradiae*. See generally U.S. Patent No. 2,988,487 to Nickerson; See also Göktan, D., "Decomposition Rates of Keratinous Material Used by
30 Certain Microorganisms," (Abstract No. 207369b), *Microbial Biochem.* 101, 333 (1984); Daniels, G., "The Digestion of Human Hair Keratin by *Microsporum Canis*," *J. Gen. Microbiol.* 8, 289 (1953); Koh, W. et al., "Keratinolytic Enzymes from *Aspergillus flavus* and
35 *Aspergillus niger*," *Bacillus. Aust. J. Biol. Sci.* 274 (1959); Molyneaux, G. S., "The Digestion of Wool by a Keratinolytic *Bacillus*," *Aust. J. Biol. Sci.* 274

(1959); Noval, J. and Nickerson, W., "Decomposition of Native Keratin by *Streptomyces Fradiae*," *J. Bacteriol.* 77, 251 (1959); Kapica, L. and Blank, F., "Growth of *Candida Parapsilosis* with Keratin as Sole Source of Nitrogen," *Dermatologica* 117, 433 (1958); Kapica, L. and Blank, F., "Growth of *Albicans* on Keratin as Sole Source of Nitrogen," *Dermatologica* 115, 81 (1957).

Keratinases used in practicing the present invention may be in crude form or in pure form.

10 Keratinases in crude form may be prepared, for example, by separating bacterial cells which produce the keratinase from their liquid growth media, the liquid growth media comprising crude keratinase.

Alternatively, the cells may be lysed (chemically or
15 physically) in a liquid growth media to produce a crude, cell free extract. Other means of preparing such an extract will be apparent to persons skilled in the art. The crude keratinase may be included in the feed in any form compatible therewith, such as in an
20 aqueous form or in lyophilized form. The lyophilized form is currently preferred.

Pure (or substantially pure) keratinases may be obtained by separating the crude keratinase described above into its individual constituents, in
25 accordance with known techniques. See generally W. Jakoby, Ed., *Enzyme purification and Related Techniques, Methods in Enzymology*, vol. 22 (1971) and vol. 104, pt. C (1984), Academic Press, NY. Numerous suitable separation procedures, such as column
30 chromatography, are known to persons skilled in the art. The individual constituent proteins may, if necessary, be screened for their ability to degrade keratinaceous material, and that constituent which best degrades keratinaceous material comprises the
35 keratinase. Like the crude keratinase, the pure keratinase may be employed in any suitable form, including aqueous form and lyophilized form.

The present invention is exemplified further in the non-limiting examples set forth below.

EXAMPLE 1

5 Preparation of Crude Keratinase Enzyme

A crude keratinase enzyme (KE) capable of degrading feather is obtained from *Bacillus licheniformis* strain PWD-1 in accordance with standard techniques. Being an extracellular enzyme, keratinase
10 is isolated from the culture medium of *Bacillus licheniformis* PWD-1 which is grown on feathers as the sole source of carbon, nitrogen, and energy as previously described. See U.S. Patents Nos. 4,908,220 and 4,959,311. The culture medium is prefiltered to
15 remove residual undegraded feathers and bacterial cells, and then concentrated by membrane ultrafiltration to select proteins having a molecular weight greater than 10,000 daltons. The concentrated preparation is then lyophilized.

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EXAMPLE 2

Preparation of Pure Keratinase Enzyme

A crude lyophilized keratinase prepared as described in Example 1 above is fractionated by CM-
25 cellulose ion exchanger followed by Sephadex*G-100 gel permeation. SDS-polyacrylamide gel electrophoresis analysis indicates that the keratinase has a Molecular weight of about 30,000 daltons.

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EXAMPLE 3

Precision-Fed Assay

A precision-fed assay is carried out in accordance with known procedures. See H. Enbster et al., Poultry Science 65, 487-498 (1985). Fasted adult
35 roosters are gavaged with 20 grams of raw feather powder (RF) or commercial feather meal (CFM), with or without lyophilized keratinase (KE) prepared as

* = Trade-mark

described in Example 1 above. The lyophilized keratinase is mixed with the keratin in a proportion of 1:1000 by weight. The excreta and feed samples are chemically analyzed. The addition of KE increased the total amino acid digestibility of RF from about 30 to about 66% and CFM from about 77 to about 90%.

EXAMPLE 4

Growth Assay of Three-Week Old Chicks

10 This is the most commonly used method in evaluating feedstuff. It is an assay to determine the general bioavailability of the tested protein in supporting the growth of young chicks without knowing the digestibility of individual amino acids. Three
15 week old chicks are divided into three groups. After protein deficiency for three days, they are fed normal 20% (by weight) protein diets with soybean meal (SBM), CFM, and CFM plus KE (1000:1) as the major protein source. Their body weights is monitored for eight days
20 until exhaustion of KE. The weight gains (g/day) is SBM: about 66, CFM: about 50, and CFM plus KE: about 56.

Both Examples 3 and 4 demonstrate that the addition of KE improved the digestibility of a
25 keratinaceous product, indicating the potential of KE as an additive to enhance the nutritional value of CFM.

The foregoing Examples are illustrative of the present invention, and are not to be taken as limiting thereof. The invention is defined by the
30 following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A method of maintaining an animal on a keratin-containing diet comprising feeding the animal a carbohydrate source and a first protein source, said first protein source comprising keratin, concurrently with a keratinase capable of hydrolyzing said keratin in an amount effective to enhance the digestibility of said keratin in the digestive tract of said animal.
2. A method according to claim 1, wherein said animal is a bird.
3. A method according to claim 1, wherein said keratin is selected from the group consisting of animal feather, hair, horn, and hoof.
4. A method according to claim 1, wherein said keratin is ground.
5. A method according to claim 1, wherein said keratin is a feather meal.
6. A method according to claim 1, wherein said carbohydrate source is selected from the group consisting of corn meal, oat meal, barley meal, sorghum meal, and combinations thereof.
7. A method according to claim 1, said method further comprising feeding the animal a supplemental second protein source.
8. A method according to claim 1, said method further comprising feeding the animal a supplemental second protein source selected from the group consisting of soy meal, fish meal, blood meal, ground poultry offal, meat meal, and combinations thereof.
9. An animal feed comprising a carbohydrate source and a first protein source, said first protein source comprising keratin and a keratinase capable of

hydrolyzing said keratin in an amount effective to enhance the digestibility of said keratin.

10. An animal feed according to claim 9, wherein said animal feed is a bird feed.

11. An animal feed according to claim 9, wherein said keratin is selected from the group consisting of animal feather, hair, horn, and hooves.

12. An animal feed according to claim 9, wherein said keratin is ground.

13. An animal feed according to claim 9, wherein said keratin is feather meal.

14. An animal feed according to claim 9, wherein said carbohydrate source is selected from the group consisting of corn meal, oat meal, barley meal, sorghum meal, and combinations thereof.

15. An animal feed according to claim 9, said feed further comprising a supplemental second protein source.

16. An animal feed according to claim 9, said feed further comprising a supplemental second protein source selected from the group consisting of soy meal, fish meal, blood meal, group poultry offal, meat meal, and combinations thereof.

17. A method of maintaining an animal on a keratin-containing diet comprising feeding the animal a carbohydrate source and a protein source, said protein source comprising keratin, concurrently with *Bacillus Licheniformis* PWD-1 keratinase enzyme in an amount effective to enhance the digestibility of said keratin.

18. A method according to claim 17, wherein said animal is a bird.
19. A method according to claim 17, wherein said keratin is selected from the group consisting of animal feather, hair, horn, and hoof.
20. An animal feed comprising a carbohydrate source and a first protein source, the protein source comprising keratin and *Bacillus Licheniformis* PWD-1 keratinase enzyme in an amount effective to enhance the digestibility of said keratin.
21. An animal feed according to claim 20, wherein said animal feed is a bird feed.
22. An animal feed according to claim 20, wherein said keratin is selected from the group consisting of animal feather, hair, horn, and hoof.