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A REVIEW: POTENTIALS FOR BIOTECHNOLOGICAL APPLICATIONS OF KERATIN-DEGRADING MICROORGANISMS AND THEIR ENZYMES FOR NUTRITIONAL IMPROVEMENT OF FEATHERS AND OTHER KERATINS AS LIVESTOCK FEED RESOURCES

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Abstract

Advances in microbial enzyme technology, keratinolytic proteases in this case, offer considerable opportunities for a low-energy consuming technology for bioconversion of poultry feathers from a potent pollutant to a nutritionally upgraded protein-rich feedstuff for livestock. A compendium of recent information on microbial keratinolysis in nature and infection (dermatophytoses) has been provided as underscoring feasible harnessing of the biotechnology for nutritional improvement of feathers, and as an alternative to conventional hydrothermal processing. Supporting evidence of a nutritional (amino acid) upgrading sequel to diverse microbial treatments of feathers, and positive results obtained from growth studies in rats and chicks have been presented. The paper concludes with suggestions for avenues of application of biotechnology for nutritional improvement of feather (and other keratins) as feedstuffs for livestock. © *1998 Elsevier Science Ltd. All rights reserved*

Key words: biotechnology, keratin-degrading microorganisms, nutritional improvement, poultry feather.

INTRODUCTION

Poultry feather: a waste, potential and limitations as animal feedstuff

Feathers represent 5–7% of the body weight of the domestic fowl. Although they are of insulatory, locomotory and conformational (structural) importance to the birds, possible biological uses outside the body of the birds appear sub-optimally harnessed, while it seems probable that poultry feather constitutes the most abundant keratinous material in nature. Poultry feather accumulates as a waste after processing the chickens for human consumption; thus the waste carries potent polluting implications, especially with burgeoning global poultry production.

Understandably, poultry feather locks up a great deal of potentially useful protein and amino acids (Table 1) that could be beneficially harnessed as animal feedstuff. This makes recycling of feather a subject of interest among animal nutritionists, because of its potential as a cheap and alternative protein feedstuff. However, limitations to feather utilization arise from its poor digestibility and low biological value and the deficiencies of nutritionally essential amino acids such as methionine, lysine, histidine and tryptophan (Baker *et al.*, 1981; Papadopoulos *et al.*, 1985; Dalev *et al.*, 1997). The amino acids composition of feather is highly variable (Wang and Parsons, 1997). Also, the total essential amino acids, especially, methionine, lysine and histidine concentrations decreased with age in broiler chickens, while the total non-essential amino acids as a percentage of total essential amino acids, increased as the birds aged (Stilborn *et al.*, 1997). The nutritional inferiority of native feather protein derives from the composition and molecular configurations of constituent amino acids that are, basically, to ensure structural rigidity for the role of feathers. The same reason explains why native keratin is insoluble and undegradable by most proteolytic enzymes. Feathers' mechanical stability and resistance to proteolytic digestion are consequences of the tight packing of the protein chain in the α-helix (α-keratin) or β-sheet (β-keratin) into a supercoiled polypeptide chain. There is a high degree of crosslinking of the polypeptide chain caused by extensive formation of disulfide bonds.

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Table 1. Protein and amino acid concentrations (g kg^{-1}) of unprocessed and processed feather meal

Protein and amino acids	Latshaw *et al.*, 1994, unprocessed	Latshaw *et al.*, 1994, processed at 207 kPa for 24 min	Wang and Parsons (1997), processed at 160°C for 15 min
Protein	922.0	866.0	880.0
Alanine	28.8	37.7	39.6
Glycine	51.8	50.7	68.7
Isoleucine	39.4	41.3	42.3
Leucine	56.9	68.8	70.9
Valine	53.0	44.0	59.6
Phenylalanine	34.6	40.1	42.1
Arginine	67.6	62.5	61.0
Histidine	2.3	8.6	5.7
Lysine	15.4	22.6	18.8
Aspartic acid	41.8	55.9	55.2
Glutamic acid	82.2	72.3	97.2
Serine	87.3	72.1	100.0
Threonine	34.5	36.5	40.2
Proline	73.9	74.8	88.4
Cystine	65.8	48.7	42.9
Methionine	7.1	6.3	6.5

The high content of cystein facilitates the formation of cystine bridges. Hydrogen bonding among the polypeptides and the hydrophobic interaction and stabilization of the super coil further confer strength and proteolytic resistance on keratin.

Nonetheless, the conventional method of producing a more readily digestible feather meal employs hydrothermal degradation. According to Papadopoulus (1989), Latshaw *et al.* (1994) and Wang and Parsons (1997), hydrothermal treatment achieves limited and varying nutritional improvement; sustains losses of essential amino acids such as lysine, methionine and tryptophan, and causes the formation of non-nutritive amino acids such as lysinoalanine, lanthionine, etc. (Table 1).

Considering the thermo-energetic cost of conventional processing of feather against the backdrop of its limited nutritional improvement, investigation into alternative technology with prospects for nutritional enhancement, environmental friendliness or compatibility, bioresources optimization and cost-effectiveness seems justifiable.

Biotechnological approaches involving microorganisms and their enzymes appear a conceptually appropriate processing technology. However, there is no compendious literature on the prospects for industrial applications of keratinolytic microorganisms, especially with emphasis on their production of keratinases, properties of keratinases, mechanism(s) and limitations of keratinolysis. Therefore, we reviewed recent information on microbial keratinolysis in order to stimulate the application of the biotechnology in feather processing as animal feedstuff. The upgrading of feather nutritional value should yield a high-protein feedstuff that may greatly spare the use of soyabean and fish meal in livestock diets. Furthermore, bioconversion of feather will predictably benefit the poultry industry, man and the environment.

MICROBIAL KERATINOLYSIS AND KERATINASE PRODUCTION

Evidence for keratinolysis in nature

In spite of the unusual stability of keratins, feathers do not accumulate in nature, thus evidentially confirming the existence of natural decomposers or utilizers. Further evidence, and perhaps a better researched aspect of keratin degradation in nature, is obtained from pathogenic dermatophyte growths (infestation) on keratinous parts of humans and livestock such as hoofs, nails, stratum corneum, horns, hair, etc. Dermatophytes are microorganisms which parasitize keratinous substrates in living animals, and when such microorganisms are cultured in a medium containing keratin they are capable of utilizing it as a source of carbon and nitrogen. The growth of dermatophytes on keratinous substrates indicates their capacity to synthesize proteolytic enzymes that can degrade the complex keratins.

Keratin-degrading microorganisms and justification for their biotechnological application

A myriad of microorganisms, mostly fungi and bacteria, have been identified and reported to utilize keratin, by many authors (Yu *et al.*, 1969; Elmayergi and Smith, 1971; Takiuchi *et al.*, 1984; Asahi *et al.*, 1985; Wawrzkiewicz *et al.*, 1987; Abdel-Hafez and El-Sharoumy, 1990; Malviya *et al.*, 1992; Santos *et al.*, 1996; Simpanya and Baxter, 1996; Singh, 1997). Keratin is degradable by some species of saprophytic and parasitic fungi (Safranek and Goos, 1982; Bahuguna and Kushwaha, 1989; Rajak *et al.*, 1991; Hirchsman *et al.*, 1994), actinomycetes (Noval and Nickerson, 1959; Sohair and Assem, 1974; Naguib *et al.*, 1984; Benedek *et al.*, 1985; Mukhopadhyay and Chandra, 1990; Fasasi, 1997), some *Bacillus* strains (Williams *et al.*, 1990; Takami *et al.*, 1992; El-Shora *et al.*, 1992, 1993; Lin *et al.*, 1992), *Streptomyces*

pactum DMS 40530 (Bockle *et al.*, 1995), and *Steptomyces fradiae* ATCC 14544 (Morihara *et al.*, 1967; Sinha *et al.*, 1991; Kitadokoro *et al.*, 1994). There are still a host of newly isolated microorganisms exhibiting keratinolytic properties. Recently, Lin *et al.* (1995) sequenced and expressed on *Escherichia coli* the gene (*ker* A) which encodes the keratinolytic protease of *Bacillus licheniformis* PWD-1 with 97% sequence identity.

Observing natural degradation and parasitization of keratinous substrates, as well as duplication of the process under laboratory conditions, suggest the feasibility of biotechnological processing for improving the utilization of feather (and other keratins) as animal feed protein. This is consistent with the claims by Lin *et al.* (1992), Dozie *et al.* (1994) and Santos *et al.* (1996).

The application of microbial technology for feather processing holds the following nutritional significance. First, culturing of the microorganisms and keratinase activity may result in a modification of the structure of feather keratin. This may alter its resistance to digestive enzymes of the consuming animals (Elmayergi and Smith, 1971; Barabas, G. *et al.*, unpublished results, 1986; Benedek *et al.*, 1985; Williams and Shih, 1989). Furthermore, there can be nutritional enrichment of the feather meal from microbial protein biomass that may be complementary or additive. Barabas, G. *et al.* (unpublished results, 1986) found higher amounts of lysine, methionine and arginine in fermented than in unfermented feather, leading to their conclusion that it is not only the feather keratin that can be used as the protein source, but the microbial biomass as well. Earlier, Elmayergi and Smith (1971) had reported a marginal increase in methionine and lysine contents of feather fermented by a methionine-secreting mutant of *S. fradiae*. Thirdly, the production of amino acids, especially feed-grade lysine and others, from microbial fermentation of feather is also possible (Mohammed El-Akied, 1987; Williams and Shih, 1989). Supporting evidence for nutritional improvement (amino acids upgrading) of fermented feather is provided in Tables 2 and 3.

Table 2. Amino acids composition (g kg^{-1}) of microbially treated and untreated samples[a]

Amino acids	Treated feather	Untreated feather
Glycine	98.0	162.0
Valine	8.0	20.0
Leucine	45.0	83.0
Isoleucine	21.0	43.0
Arginine	41.0	17.0
Lysine	41.0	18.0
Methionine	4.0	–
Cysteine	46.0	76.0
Threonine	55.0	8.0
Phenylalanine	28.0	43.0
Tyrosine	19.0	16.0
Histidine	4.0	3.0
Tryptophan	–	–
Asparagine	81.0	67.0
Serine	82.0	72.0
Glutamine	105.0	97.0
Proline	222.0	188.0
Alanine	104.0	84.0

[a]Source Barabas, G. *et al.* (1986, unpublished)

Ecology of keratin degrading microorganisms

Keratin-degrading microorganisms are almost ubiquitous in nature, although preferentially (obligatorily or facultatively) thriving on keratinous substrates. Perhaps the best-studied groups are the dermatophytes because of the pathological interest of most investigators (Grappel and Blank, 1972; Higuchi *et al.*, 1981; Wawrzkiewicz *et al.*, 1987, 1991; Apodaca and McKerrow, 1989; Hanel *et al.*, 1991; Porro *et al.*, 1997). Geophillic dermatophytic fungi have attracted considerable interest (Abdul-Fatah *et al.*, 1982; Al-Musallam, 1988, 1990; Al-Musallam *et al.*, 1995; Ashour *et al.*, 1992). Keratin-degrading microrganisms have been isolated from sewage sludge (Ulfig and Korcz, 1983, 1994; Ulfig and Ulfig, 1990; Ulfig, 1991; Ulfig *et al.*, 1996). A zoophilic dermatophyte *Trichophyton gallinae* is highly specific for chicken feather (Wawrzkiewicz *et al.*, 1987).

Non-dermatophytic fungi with keratin-degrading capacity have been isolated for keratinolytic assessment and purification of their enzymes (Malviya *et al.*, 1992, 1993a,b; Dozie *et al.*, 1994). Williams and Shih (1989) isolated *B. licheniformis* from a digester for manure mixed with chicken feather. *Streptomyces sp.* A11, producing proteolytic and keratinolytic activities, was isolated from soil (Mukhopadhyay and Chandra, 1990). Santos *et al.* (1996) reported that

Table 3. Concentration (g per 100 g protein) of selected amino acids in fermented and unfermented feather meal[a]

Amino acid	Feather meal (FM)	FM fermented by parent strain of *S. fradiae*	FM fermented by mutant strain of *S. fradiae*
Methionine	0.37	0.43	0.90
Tyrosine	0.15	0.38	0.74
Lysine	1.77	2.14	3.23
Histidine	0.15	0.21	0.73
Cystine	4.74	3.45	2.18

[a]Source: Elmayergi and Smith (1971).

Aspergillus fumigatus a ubiquitous and opportunistic air-borne pathogen of humans, birds and other animals utilized chicken feather as its sole source of carbon and nitrogen. The keratin-degrading microorganisms thrive under different ecological and environmental conditions, and they demonstrate a wide ranging capacity to solubilize keratinous substrates as well as other compact proteinous substrates. Predominantly, the microorganisms synthesize and export proteases into their substrates to achieve degradation or breakdown of the substrates into assimilable simple nutrients. However, reports on endoproteases or cell-bound enzymes with keratinolytic activity have been documented (Yu *et al.*, 1971; Wawrzkiewicz *et al.*, 1987; Lamkin *et al.*, 1996). Meanwhile understanding of the molecular basis of their subsistence on compact proteinous substrates remains elusive.

PROPERTIES OF KERATIN-DEGRADING PROTEASES (KERATINASES)

Specific proteases elaborated intracellularly or extracellularly by keratin-degrading microorganisms are named keratinases or keratinolytic enzymes. The enzymes have the capacity to act on compact substrates better than other comparable proteolytic enzymes; this distinguishes keratinase from other proteases and peptidases.

Most keratinases are largely inducible, requiring keratin as exogenous inducer. The derepression of keratinase in some microorganisms, such as *Candida albicans* (Kapica and Blank, 1957, 1958) and *Chrysosporium keratinophilum* (Dozie *et al.*, 1994) differentiates their exosecretion from the constitutively expressed cell-bound keratinase of *T. gallinae*.

Many a keratinase is active extracellularly, being exported from the intracellular sites of synthesis; however, evidence for cell-associated activity has been reported for proteinases of *Trichophyton mentagrophytes* (Yu *et al.*, 1971), and *Trichophyton rubrum*, a well-studied keratin degrading microorganism (Lamkin *et al.*, 1996). However, the endoprotease of *T. rubrum* exhibited a broad spectrum for proteinous substrates, unlike that of *T. gallinae*.

Varying microbial keratinolytic activities and enzymatic substrate specificities are described in the literature. This may be due to methodological and species differences. For instance, Lin *et al.* (1992) found that keratin from *B. licheniformis* was capable of hydrolyzing all the protein substrates tested, including bovine serum albumin, collagen, elastin, and feather keratin. Consistent with the above, Bockle *et al.* (1995) observed liberation of peptides from different soluble substrates (casein and gelatin), and insoluble substrates (native and autoclaved) chicken feather. On the contrary, Dozie *et al.* (1994) reported a thermophillic keratinolytic proteinase from *C. keratinophyllum* which hydrolyzed only keratin, but showed no activity on casein, BSA or keratin powder. This indicates that the isolated enzymes were specific only for keratin substrates. In another perspective, Bockle *et al.* (1995) found that the culture filtrate of the keratin-degrading *S. pactum* disintegrated whole chicken feather at the temperature range of 40–70°C far more than the physiological requirement, however the purified enzyme solubilized less than 10% of the native substrate.

A highly specific, intracellular keratinolytic enzyme was elaborated by *T. gallinae* (Wawrzkiewicz *et al.*, 1987). The physicochemical properties of some characterized keratinases from some keratin-degrading microroganisms are summarized in Table 4.

MECHANISM OF MICROBIAL KERATINOLYSIS

Basically microbial keratinolysis is a proteolytic, protein-degrading process since the substrate (keratin) is essentially protein, i.e. 95% protein by weight. The varying physicochemical properties of keratinases notwithstanding, their common action on keratin is indisputable. Most keratinolytic enzymes characterized (Yu *et al.*, 1969; Ebeling *et al.*, 1974; Nakanishi and Yamamoto, 1974; Takiuchi *et al.*, 1984; Asahi *et al.*, 1985; Wawrzkiewicz *et al.*, 1987; Sinha *et al.*, 1991; Malviya *et al.*, 1992; Lin *et al.*, 1992; Morihara and Oda, 1992; Bockle *et al.*, 1995; Santos *et al.*, 1996; Fasasi, 1997) all act as proteinases and are active on keratin. Nevertheless the knowledge of definitive mechanism(s) of keratinolysis and metabolism of keratin-degrading microorganisms is not fully understood. Despite the lack of complete understanding of the mechanistic action of keratinases, evidence abounds for the probable existence of the following processes.

Mechanical keratinolysis

This applies only to fungi and mycelia producing, keratin-degrading microorganisms. Mechanical keratinolysis interprets as the keratin degradation occurring as a result of mycelial pressure and/or penetration of the keratinous substrate. Malviya *et al.* (1992) observed that keratin degradation commenced before the detection of significant extracellular keratinase activity, and this degradation was associated with mycelial growth. This somewhat presupposes mechanical degradation. Fungal mycelial growth and elongation generates pressure or 'weathering' of the compact substrate. Evidence for mechanical degradation (Raubitshek, 1961; Mercer and Verma, 1963; Yu *et al.*, 1968, 1969, 1971; Baxter and Mann, 1969; Kunert and Krajci, 1981; Desmukh and Agrawal, 1982, 1985) as an

Table 4. Properties of keratinases isolated from some keratin-degrading microorganisms

Species	Type	M_r (kDa)	I_p	pH	Temp (°C)	Cofactor	Substrates	References
S. pactum	Serine	30	8.5	7–10	40–75	–	Keratin azure, feather, BSA	Bockle *et al.*, 1995
B. lichineformis		33	7.25	7.5	50	–	BSA, casein, collagen, feather	Lin *et al.*, 1992
C. keratinophilium	Alkaline	69	–	7–10	90	Fe^{2+}	Keratin only. No activity on casein, BSA, gelatin	Dozie *et al.*, 1994
A. fumigatus				6.5–9	45		Autoclaved and native feather, casein	Santos *et al.*, 1996
T. mentagrophytes	Serine	48					Guinea pig hair	Yu *et al.*, 1968
T. rubrum	Serine	27, 35, 93 and 71		8		Ca^{2+}		Apodaca and McKerrow, 1989; Asahi *et al.*, 1985
T. gallinae				8.0			Chicken feather only	Wawrzkiewicz *et al.*, 1987
G. penicolloideus	Cysteine	85					Hair	Malviya *et al.*, 1993a
S. sp.A11	Serine	24 49		7.5	30	Ca^{2+}, Mg^{2+}	Human hair, chicken feather, wool, etc.	Mukhopadhyay and Chandra, 1990
S. brevicaulis	Serine	40–45	7.8	40			BSA, human hair	Malviya *et al.*, 1992
		24–29		35				

integral mechanism of keratinolysis has been previously discussed (Wawrzkiewicz *et al.*, 1987).

Fungal invasion of the host combines mechanical pressure and enzymatic hydrolysis. In a recent study by Figueras *et al.* (1997), the ability of fungal perpendicular penetration of hair without regard to the keratinization of the constituent structures was provided using ultrastructural photomicrography. Mechanical penetration by mycelia may be necessary for exposing more reactive sites for enzymatic cleavage of the peptide bonds.

From available evidence, it may be presumed that mechanical keratinolysis precedes enzymatic hydrolysis, more so since the mycelia actually produce the exoproteases. Also a simultaneous action or synergism between mechanical and enzymatic hydrolysis may be suggested after the initial process. However, the ability of purified keratinase enzymes to initialize, and continue, degradation of feather may cast doubt on the above hypothesis.

Sulphitolysis

Indications for sulphitolysis arise because of the characteristically high cystein content of feather meal. Understandably, the preponderance of cystein in the amino acid profile of feather occurs naturally to confer a rigid biomolecular configuration through the formation of cystine disulphide bridges. Most investigators have contended that one of the possible mechanisms of keratin breakdown is the reduction of disulphide bonds. However, confirmatory studies in this respect are scanty.

Evidence for sulphitolysis

Profound insight into sulphitolysis during keratin degradation was provided by the investigations of Kunert (1972, 1973, 1975, 1976, 1985a,b, 1989, 1992). Contributing evidences were also obtained from the studies by Ruffin *et al.* (1976) and Malviya *et al.* (1993a,b). There is a consensus that a complete hydrolysis of keratin can be achieved only after its denaturation by the cleavage of the disulphide bridges representing the main source of the extraordinary stability and resistance to proteolytic digestion.

According to Kunert (1992), dermatophytes and non-dermatophytes metabolize free or combined cystein as a source of sulphur and nitrogen (Ziegler *et al.*, 1969; Kunert, 1972, 1975, 1988; Malviya *et al.*, 1993a,b). The products of cystine metabolism by fungi were inorganic sulphur and other intermediate products. Kunert (1992) indicated that the excess sulphur is excreted back to the medium in the oxidized form as sulphate and sulphite. The sulphite reacts at neutral to alkaline pH with cystine cleaving it to cystein and S-sulphocystein, according to the equation below (Kunert, 1992):

$$Cys-S-S-Cys+HSO_3^- \rightarrow Cys-SH+Cys-SSO_3^-$$

According to Kunert (1992), this reaction takes place also with cystine combined in proteins, including keratin, hence keratin is denatured prior to the attack by proteases (keratinases) by excretion of sulphite, which causes sulphitolysis of the disulphide bonds. Substantiating evidence of the parallel decomposition of keratin by sulphitolysis of the disulphide bonds has been proffered (Kunert, 1989; Malviya *et al.*, 1992). In fact, Malviya *et al.* (1992) found that keratinase enzyme was not detectable in the culture medium until more than half of the supplied keratin had been degraded. This by implication indicates a non-enzymatic or non-hydrolytic degradation of keratin. The presence of products of sulphitolysis such as peptides of sulphocystein, thiosulphate, sulphate, cystein, in the cultures cultivated on keratin (Kunert, 1972, 1973, 1976; Ruffin *et al.*, 1976; Safranek and Goos, 1982; Malviya *et al.*, 1993a,b) is further indication of disulphide breakdown. However, many investigators (Desmukh and Agrawal, 1982, 1985; and Lin *et al.*, 1992) detected little or none of the intermediate compounds in the cultured media. The explanation for the low or undetectable presence of the intermediate compounds is the preferential utilization of cystein as a source of sulphur by fungi (Kunert, 1987). Studies by Malviya *et al.* (1993a,b), and Mohammed El-Akied (1987) showed that the amount of the residual cystein continuously declined in the cultivation fluid, obviously due to its utilization in fungal metabolism.

Furthermore, physicochemical evidence was obtained from enhanced keratinolysis following the addition of sulphitolysing or reducing chemicals like DDT, DMSO, thioglycolate, dithiothreitol, etc. (Sinha *et al.*, 1991; Takami *et al.*, 1992; Bockle *et al.*, 1995). Kunert (1992) compared the effects of five reducing agents, sodium sulphite, cystein, glutanthione, mercaptoethanol and dithiothreitol on the activity of protease (keratinase) of *Microsporium gypseum* to confirm keratin degradation by sulphite excretion prior to attack by fungal protease. The results showed that sodium sulphite enhance protease activity maximally at a concentration of 30 $mmol^{-1}$ (3·78 $mg\ ml^{-1}$), about 2·9 times. Cysteine, mercaptoethanol and glutathione were in this order less stimulatory, being most effective at a concentration of 0·3–1·0 mmol. The weakest was dithiothreitol. Kunert observed a 3·7, 29, and 43 times enhanced proteolysis after the cleavage of 32%, 66% and 97% of cystine bridges and their conversion into S-sulphocystein, within 24 h. Kunert (1992) discussed the results obtained as revealing that susceptibility to proteolysis increased rapidly and substrate dissolution was microscopically demonstrable and gravimetrically measurable proportionately with the extent of sulphitolysis. Bockle *et al.* (1995) also showed the catalytic effect of DDT addition on the keratinolytic activity of a bacterial proteinase mixture and pure keratinase. It was found by the authors that about 10% degradation was achieved without addition of 1% DDT; whereas the addition resulted in 70% degradation of the keratin. Earlier, Takami *et al.* (1992) found that thioglycolate catalyzed complete solubilization of hair within 1 h at pH 12 and 70°C; and in the absence of the reducing agent the proteinase showed no keratinolytic activity. Enhanced keratinase activity after addition of DDT has also been reported for two serine proteinases of *S. fradiae* (Sinha *et al.*, 1991). The proteinase of *S. pactum* was not active in the presence of thioglycolate, but was active in the presence of DDT. This suggests a differential response of the microbial enzymes to the chemicals reducing the disulphide bonds.

Other indirect (Kunert, 1992) but corroborative data were the identical degradation of sulphitolysed native wool by trypsin and pronase both of which are non-specific proteases. Everett *et al.* (1962) had earlier reported a similar finding. Histochemical reactions have also revealed the presence of a large amount of S-sulpho groups at the sites of hard keratin degradation (Kunert, 1972; Safranek and Goos, 1982).

However, microbial capacity for sulphitolysis (if it exists) differs amongst the microorganisms, and this may be related to their differential parasitic potential.

Proteolysis

Whereas proteolysis or the proteolytic stage of keratinolysis may be over-simplified, and presumed to be similar to peptidolysis; the varying susceptibility of keratinases (proteinases); their different molecular weights, activators and optimal environmental conditions (pH; temperature) collectively shows the paucity of accurate knowledge of the mechanism of protein breakdown by the elaborated enzymes. Indeed, the capacity of non-specific proteinases to degrade sulphitolysed keratin (Kunert, 1992) further complicates the specificity of keratinases. Such inconsistencies in microbial metabolism of complex organic products like cellulose, chitin, polysaccharides, are common.

Nonetheless, the consistently higher proteolysis by microbial keratinase (Kunert, 1992; Lin *et al.*, 1992) when compared with other proteases provides the distinguishing factor. Furthermore the preference of the purified keratinase for long-chain and compact molecules (Lin *et al.*, 1992; Bockle *et al.*, 1995) gives additional evidence in favour of specific keratinases. The substrate specificity of keratinases tends to suggest the likelihood of different modes of action, which, however, remain to be understood.

Sequence of reactions in keratinolysis

It appears difficult to propose with accuracy the sequence of reactions in the degradation of keratins.

However, for fungi and filamentous actinomycetes, it presumably starts off with mycelial growth followed by sulphite secretion for breaking the disulphide bridges and lastly by proteolysis. Beyond the initialization stage, it can be expected that the processes proceed simultaneously, in as much as the organism is still growing. A different mechanism may be proposed when bacterial organisms or purified or crude enzymes, are involved. This basically entails proteolytic breakdown of the keratin substrates. The extensive work by Kunert (1972, 1973, 1975, 1989, 1992) authenticates the hypothetical pathway when the fungal organism is present, while the results by others on isolation and purification of keratinase ostensibly justify the probable mechanism when purified enzymes are used. It also appears very likely that a single mode of action may not be applicable to all classes of keratin-degrading microorganisms.

MICROBIAL TREATMENT OF FEATHER MEAL: FEASIBILITY FOR BIOTECHNOLOGICAL APPLICATIONS

Most investigators agreed that microbial conversion of feather (keratin) represents a biotechnology for improving the utilization of feather as a feed protein. Biodegradation of feather can be achieved by cultivation of keratin-degrading microorganism(s) on feather, and the subsequent elaboration of extracellular keratinase; the use of culture filtrates containg the keratinase or crude enzyme alone without the microorganism, and the use of purified enzyme alone without the microorganism.

Evidence for nutritional upgrading of feather meal

Studies by Elmayergi and Smith (1971) appeared to us as the pioneering attempt to assess the nutritional complementarity between amino acids of feather meal and microbial biomass following the fermentation with *S. fradiae*. The methionine content of the product was higher than the unfermented, though methionine, lysine and tryptophan contents were still low in the fermented product (Table 3). Elmayergi and Smith (1971) showed that all the concentrations of the amino acids were increased considerably after fermentation. However, the results of the feeding trial with chickens (Table 5) indicated no significant difference in the nutritional value between the fermented and unfermented feather meal. This was explained to be the result of product unacceptability by the chickens. The continuation of the experiment with supplementation of methionine up to requirement eventually caused a comparable growth rate of broilers with those fed isolated soyabean.

In a similar study, Barabas, G. *et al.* (1986, unpublished) found that lysine, methionine and arginine contents of feather meal were higher in the microbially fermented feather than in the intact feather. (Table 2). The authors conducted two feeding trials with rats and their findings were that those which received feather hydrolysates did not lose weight but those fed a protein-deficient diet recorded weight loss. Feather hydrolysate digestibility and utilization were confirmed in the studies, though sub-deficiency of methionine would seem to predicate the lower or lack of weight gain of rats fed feather meal. It was concluded from their investigations that not only feather meal (keratin) could be used as protein for animal food, but also the biomass of the enzyme-producing strain as well.

The use of crude keratinase enzyme

Lee *et al.* (1991) reported the pioneering use of a crude keratinase enzyme (KE) as feed additive. The keratinase was prepared from *B. licheniformis*. It was found that the addition of KE increased the total amino acid digestibility of raw feather from 30 to 66%, and commercial feather from 77 to 99%. In the second 8-day experiment, daily weight gains of 3-week-old broilers were 66 g, 50 g, and 56 g day^{-1} on soyabean meal, commercial feather meal and keratinase-supplemented treatments, respectively. It was concluded from both experiments that the addition of KE improved the digestibility of feather products, thus strengthening its applications for nutritional enhancement of feather meal.

Table 5. Effects of feeding unfermneted and fermented feather meal (FM) to growing chicks[a]

Diet	Gain (loss) in weight (g)	Feed consumed (g)
Isolated soyabean	59.0±4.7	166.3±14.2
Unfermented FM	−7.0±0.0	76.3±8.1
Unfermented FM+EAAM[b]	−12.2±2.9	170.0±8.9
Unfermented FM+EAAM−Methionine	−10.0±1.6	71.3±4.9
Unfermented FM+EAAM−lysine	2.7±2.5	90.7±10.2
Fermented FM	20.3±4.0	59.7±7.5
Fermented FM+EAAM	68.4±1.5	165.7±9.8
Fermented FM+EAAM−Methionine	19.0±2.4	55.0±7.8
Fermented FM+EAAM−Lysine	15.3±4.6	70.3±12.8[a]

[a]Source: Elmayergi and Smith (1971).
[b]Essential amino acids mixture.

The use of purified keratinase

The bulk of evidence on the application of purified keratinase enzyme as feed supplement was obtained at North Carolina University by J.C.H Shih and his coworkers. A recent contribution from the investigators (Lin *et al.*, 1996) is the immobilization of purified keratinase enzymes isolated from *B. licheniformis* to minimize enzyme autolysis. The immobilized keratinase demonstrated proteolytic activities against both insoluble feather keratin and soluble casein. It also displayed a higher level of heat stability and increased tolerance towards acidic pHs than the free keratinase, thereby increasing its potential for use in numerous applications

Suggested avenues for nutritional improvement of feather using a biotechnological approach

Considering that biotechnology offers great potential for nutritional improvement of feather, the scopes for applications of microbial technology in feather processing are as follows:

1. Achieving complete structural degradation of feather. This could be feasible through enhanced sulphitolysis by chemical or microbial processes or the combination of the methods. Investigations into cultural and extraneous and intrinsic co-factor(s) that may optimize the enzymic catalysis need to be fully carried out.
2. Enhancing the nutritional value of feather meal, especially its contents of methionine and lysine. Perhaps any or a combination of the following biotechnological tools may be relevant:
   2.1. transpeptidation or amino acid incorporation or covalent bonding of the main limiting amino acids such as lysine (Dalev *et al.*, 1997);
   2.2. genetic manipulation (improvement) via mutagenesis or protein engineering of the keratin degrading microrganism(s);
   2.3. coding of the keratinase gene into innocuous microorganisms, since many active keratinolytic microorganisms are pathogenic.
3. Designing a microbial enzyme complex using a consortium whose collective elaboration of keratinolytic, (sulphitolytic), and peptidolytic enzymes will be significantly high. The possibility of combining microbes capable of secreting limiting amino acids (methionine and lysine) as member(s) of the consortium may also be considered. Examples of such microbial cooperation are found in mixed cultures used as probiotics in animal feeding and the microbial cellulolytic enzyme complex commercially sold as a feed additive. Fungal species known for their ability to produce multi-component enzyme complexes will be a good candidate for consideration.
4. Achieving stability for the keratinase enzyme at high temperature is a prerequisite for biotechnological applications, and activity around neutral pH may be advantageous. Considerably higher rates of keratinolysis may be achieved at elevated incubation temperatures and through the use of additives having reducing properties, i.e. chemicals. Stabilizing effects of some divalent metals on the enzymes leading to their higher activity, have been reported (Dozie *et al.*, 1994; Bockle *et al.*, 1995)
5. Further screening for non-pathogenic microorganisms with extremely high capacity for keratinase production and probable use without the need for isolation and purification of the enzymes. Their biomass could (autolytically) contribute to the protein and amino acids contents of the final products.
6. Harnessing and integration of existing knowledge on pathogenesis of dermatophytes to gain insight into the mechanism of structural degradation of keratins. Such collaborative investigation will elicit effective biotechnological strategies for optimal utilization of the nutrients in poultry feather.

CONCLUSION

Poultry feather and other keratins are potential protein feed resources for livestock, but lacking in essential amino acids and having poor digestibility as a consequence of the structural functions of feathers in the avian species. The natural existence of keratin-degrading microorganisms offers a feasible microbial enzyme technology capable of producing a more nutritionally balanced and digestible product than does the conventional hydrothermal processing. Furthermore, the microbial enzyme technology is low-energy-consuming and environmentally friendly.

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