

Evaluation of Chicken Feathers Degraded by Keratinase for Poultry Feed Supplement**Okediji E. F.* and Adegunloye D. V.**

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Abstract: Chicken feathers pose a significant environmental challenge due to their high resistance to natural degradation. It accumulates in the environment and constitutes serious problems to human health. The study was conducted to degrade chicken feathers by keratinase from selected bacteria isolated from soil samples containing chicken feather waste and the resulting hydrolysate used as a supplement in broiler feeds. Feather waste was milled and degraded with the isolated bacteria. Thereafter, 4-week-old broilers were grouped into six groups and fed for four weeks. The first group was the positive control, fed with commercial feed; the second group was the negative control, fed without hydrolysate. The third, fourth, fifth, and sixth groups were fed 5% and 10% hydrolysate from two different degraded feathers, respectively, and their weights were taken daily. The blood sample of the experimental broilers were collected for the liver function test. The mean weight of the broilers fed without hydrolysate increased from 0.661 kg to 2.954 kg, while the broilers fed with hydrolysate increased from 0.650 kg to 3.013 kg after four weeks. The ranges of liver function test parameters are aspartate transaminase (73.00-105.00 U/l; Alanine transaminase (10.00-17.00 U/L); Albumin (11.50-13.50 g/l); Total Protein (35.00-40.50 g/l); total bilirubin (14.40-23.65 $\mu\text{mol/l}$); conjugate bilirubin (1.10-2.45 $\mu\text{mol/l}$). The liver function test reveals that the supplement produced from degraded feathers is safe for broiler consumption. Therefore, *Serratia nematodiphila* and *Klebsiella pneumoniae* might be used for large-scale production of keratinase for industrial purposes and to enhance broiler feed nutrients.

Key word: Degradation, Feather, Hydrolysate, Keratinase, Supplement

INTRODUCTION

Keratinases are used to degrade keratin into amino acids or oligopeptides. Many known keratinases can degrade keratin effectively in the presence of multiple genes, cytokines, and growth factors. Keratinase is a protease enzyme that specifically degrades keratin. It is an adaptive enzyme, produced only in response to the presence of an inducer, such as keratin, in the environment. Keratinases, which are serine proteases, are among the most effective and notable proteases. Ultimately, keratin can be fully broken down into free amino acids and sulfides (Fitriyanto *et al.*, 2022).

Extracted from various microorganisms, keratinase has diverse applications across industries including feed, leather, pharmaceuticals, and food, as well as significant ecological uses. Given its promising applications, it is important to explore different bacterial conditions to discover new keratinases (Nnolim *et al.*, 2020). Additional applications of keratin include dehairing, cosmetics, textiles, and biofuel production. The use of keratinase has also shown promise in enhancing drug

delivery to certain tissues and in the hydrolysis of prion proteins (Mursheda *et al.*, 2020). Studies have shown the ability of keratinases to break down prion (PrP) proteins and make meat products safe for consumption by animals (Mane and Hovik, 2022). Despite its many potentials uses across various industries, the production of keratinases from unmodified native hosts is typically considered inadequate to meet the high global demand (Gong *et al.*, 2020). Numerous studies on keratinous waste management have shown that keratin substrates are converted into protein, peptide, and amino acid-rich hydrolysates through enzymatic or full-cell hydrolysis (Gaidau *et al.*, 2021). The accumulation of keratin waste in the environment is significant (Gurunathan *et al.*, 2021). Optimizing enzyme parameters, such as pH, temperature, and media composition, is crucial for enzyme activity (Qingxin, 2021). Different bacterial strains can be isolated and cultured under optimal conditions to enhance keratinase activity.

This present study outlines the isolation of two distinct bacteria, their growth behavior using feathers as a nutrient source, and the

activity of keratinase produced by these strains under optimal pH and temperature conditions. The commercialization of keratinase-based formulations holds the potential to transform various economic sectors while also mitigating pollution that would have been increased by conventional chemical methods (Nnolim and Nwodo, 2021).

MATERIALS AND METHODS

Collection of poultry waste soil samples:

Waste soil samples were collected from poultry waste dumping sites in Akure, Ondo State, Nigeria. The samples of poultry waste were taken from a depth of 3-4 cm with the aid of hand trowel and put in a sterile plastic bag. The collected samples were brought to the laboratory for further processing. The chicken feathers were collected from an abattoir at Oja-Oba, Akure, Ondo State, Nigeria. The chicken feathers were collected for biodegradation and to use the resulting hydrolysate for feed supplement.

Processing of the chicken feathers: The feathers collected were washed properly 3 times with tap water and allowed to dry in the sun for one day. After drying the feathers were pretreated with 75% methanol and air dry for 2 days. The feathers after drying were milled with a manual blender and kept in a dry container for further use.

Preparation of the culture media for isolation, maintenance, and growth of bacteria: The feather meal broth used for isolation, maintenance, and growth of bacteria was made by adding 0.5 g of NH_4Cl , 0.5 g of NaCl , 0.3 g of K_2HPO_4 , 0.4 g of KH_2PO_4 , 0.1 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g of yeast extract, and 10 g of milled feathers in 250 ml of sterile distilled water in a conical flask. The pH was maintained at 7.5. The broth and agar medium were sterilized in an autoclave at 121°C for 20 minutes.

Isolation of microorganisms from poultry soils: Ten-fold serial dilution was carried out on the soil sample with sterile distilled water. One milliliter of the dilution was poured on a plate and about 15 ml of media was poured without bubbles. Then, the

plates were incubated at 37°C for 24 hours. Pure cultures were obtained by repeated streaking on skimmed milk agar plates. The pure isolates were transferred to nutrient agar slants and preserved in a refrigerator at 4°C for further use.

Primary screening of proteolytic bacteria:

The skimmed milk agar medium was then inoculated with the bacteria isolates. The inoculated plates were incubated in the incubator at 37°C for 24 hours, and the observed positive appearance of a clear zone around the colony suggested the proteolytic activity. The bacteria colonies that are proteolytic were further selected for secondary screening. Pure bacterial isolates were kept for further research on nutrient agar medium slants at 4°C .

Secondary screening of keratinolytic bacteria:

The bacterial isolates with a clear zone around the inoculum were further used for secondary screening. The isolates were poured in a culture medium that also contained chicken feathers. The cultures were inoculated and incubated at 37°C on a rotary shaker at a speed of 150 rpm (Zhi *et al.*, 2020).

Identification of bacteria isolates: The potential bacterial isolates were identified using Gram staining, biochemical characterization, and molecular.

Biodegradation of feather and keratinase production process:

The keratinase production was carried out in the basal medium by using a shaking flask method. The composition of the media was (g/l): Milled feather, 10 g, 0.5 g of NH_4Cl , 0.5 g of NaCl , 0.3 g of K_2HPO_4 , 0.4 g of KH_2PO_4 , 0.1 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g and yeast extract. The bacterial inoculum of 2.5 ml was added to 50 ml of medium in 250 ml Erlenmeyer flasks and incubated at 37°C , 150 rpm, for 5 days. After incubation, the fermented medium was centrifuged at 4000 rpm for 20 minutes and the cell-free supernatant was assayed for keratinase activity. After biodegradation the remaining feathers were weighed and the isolates that degraded the feathers were used for further experiments (Ahmadpour *et al.*, 2016).

Optimization of cultural conditions for keratinase activity: The enzyme activity was measured at a varying temperature range of 20–60°C and pH of 2-8 during the biodegradation.

Proximate composition of chicken feathers before and after biodegradation: The proximate composition of the raw chicken feather was determined before biodegradation and the proximate composition of the resulting hydrolysate was also determined after biodegradation. This was done to know the nutrient present in the chicken feather and to also determine the percentage loss of nutrient after biodegradation. The parameters that were determined include fiber, moisture, crude protein, carbohydrate, ash, and fat content

Animal experimental design: Twenty-four male broiler chicks of four-week-old with a weight of 600 g-690 g were obtained. The broilers were wing-banded and weighed individually. The cages were kept in an open-sided house at room temperature. The broilers were grouped into Six (6) groups, positive control, negative control, 5%, and 10% of hydrolysate from two different biodegraded feathers respectively. Soya meal was supplemented using 5% and 10% hydrolysate as shown in Table 1. The boilers were fed with formulated feeds supplemented with the feather hydrolysate. The first group was the positive control test, where the broilers in this group were fed with commercial feeds. The second group was the negative control test, fed with formulated feed without the hydrolysate. The third, fourth, fifth, and sixth groups were fed with the 5% and 10% hydrolysate from the two different biodegraded feathers added to the formulated feed as supplements respectively.

RESULTS

The isolates were identified molecularly and they are *Serratia nematodiphila* and *Klebsiella pneumoniae*. They have the values of 2.38 g and 2.51 g respectively after degradation as shown in Table 2. The proximate composition of the raw chicken

feather and hydrolysate is shown in Table 3. The percentages of fiber, moisture, crude protein, carbohydrate, ash, and fat content of the chicken feather are 3.77%, 5.65%, 82.08%, 4.55%, 2.89%, and 1.06% respectively. The result shows that the percentage content of fat, ash, and fiber is low, while crude protein content is high. The proximate composition of the hydrolysate shows 3.36% fiber, 2.43% moisture, 88.84% crude protein, carbohydrate 1.04%, 3.52% ash, and 0.81% fat content. The result shows hydrolysate is rich in protein which is a good supplement for soymeal in broiler feeds. The maximum keratinase activity by *Serratia nematodiphila* and *Klebsiella pneumoniae* were recorded at pH 8 (0.51) and pH 8 (0.52) respectively, while the minimum keratinase activity by *Serratia nematodiphila* and *Klebsiella pneumoniae* were recorded at pH 10 (0.01) each as shown in Figure 1. *Serratia nematodiphila* and *Klebsiella pneumoniae* have similar keratinase activity. The maximum keratinase activity by *Serratia nematodiphila* and *Klebsiella pneumoniae* were recorded at the temperature of 50°C (0.12) and (0.14) respectively, while the minimum keratinase activity by *Serratia nematodiphila* and *Klebsiella pneumoniae* were recorded at the temperature of 20°C (0.04) and (0.05) respectively as shown in Figure 2. The amount of feed intake and weight gain for four weeks is shown in Figure 3. The percentage weight gained at the end of week four is as follows: positive control; 78%, negative control; 78%, KFH5; 77%, KFH10; 78%, SFH5; 79%, SFH10; 80% as shown in Table 3. Meal supplemented with 5% and 10% feather hydrolysate from *Klebsiella pneumoniae* are represented as KFH5 and KFH10 respectively. Meal supplemented with 5% and 10% feather hydrolysate from *Serratia nematodiphila* are represented as SFH5 and SFH10 respectively. The liver function test was carried out on the broilers after consuming the supplemented feed by feather hydrolysate for four weeks and the result is shown in Table 4. The ranges of liver function parameters are aspartate

transaminase (73.00 U/L -105.00 U/L); alanine transaminase (10.00 U/L -17.00 U/L); albumin (11.50 g/l -13.50 g/l); total protein (35.00 g/l -40.50 g/l); total bilirubin (14.40 umol/l -23.65 umol/l); Conjugate Bilirubin (1.10 umol/l -2.45 umol/l). These parameters' value determines the state of the

liver and the safety of the supplemented feed to the broilers. There was no significant difference when broilers fed with the hydrolysate-supplemented feed were compared to those fed with non-supplemented feed.

Table 1: Composition of the Experimental Diets

Description	Diets (kg)						
	SBP	KFH10	KFH5	SFH10	SFH5	N. CONTROL	
Maize	58.77	58.77	58.77	58.77	58.77	58.77	C
Soyameal	20.57	18.51	19.54	18.51	19.54	20.57	O
Hydrolysate	-	2.06	1.03	2.06	1.03	-	M
GNC	9.99	9.99	9.99	9.99	9.99	9.99	M
Wheatoffal	4.99	4.99	4.99	4.99	4.99	4.99	E
Bonemeal	2.06	2.06	2.06	2.06	2.06	2.06	R
Limestone	2.94	2.94	2.94	2.94	2.94	2.94	C
Salt	0.23	0.23	0.23	0.23	0.23	0.23	I
Broiler premix	0.15	0.15	0.15	0.15	0.15	0.15	A
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	L
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	

Keys: KFH5 and KFH10- Soya meal supplemented with 5% and 10% feather hydrolysate from *Klebsiella pneumoniae*, SFH5 and SFH10- Soya meal supplemented 5% and 10% feather hydrolysate from *Serratia nematodiphila*, N. CONTROL- Negative control, P. CONTROL- Positive control, SBP - Soil bean protein

Table 2: Initial and Final Weight of Chicken Feathers after Biodegradation

Isolates	Initial Weight (g)	Final Weight (g)
S3	10	2.51
C8	10	2.38

Keys: S3 - *Klebsiella pneumoniae*, C8 - *Serratia nematodiphila*

Table 3: Proximate Composition of Chicken Feather

Parameters	(%) Composition	Hydrolysate
Fibre Content	3.77	3.36
Moisture Content	5.65	2.43
Crude Protein	82.08	88.84
Carbohydrate Content	4.55	1.04
Ash Content	2.89	3.52
Fat Content	1.06	0.81

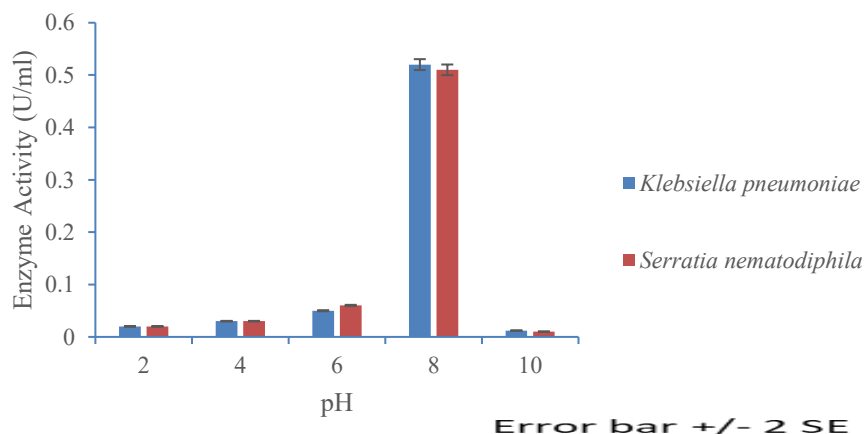


Figure 1: Effects of Different pH on Keratinase Production

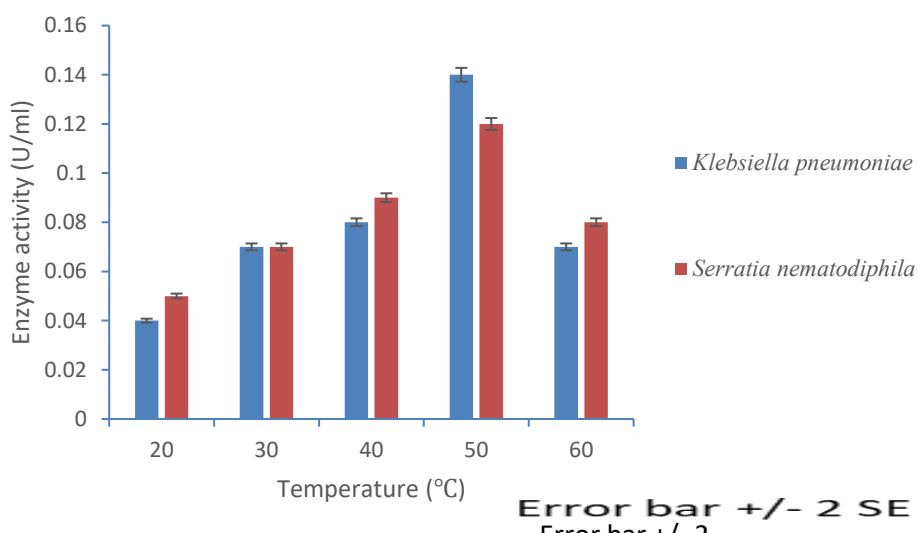


Figure 2: Effects of Different Temperature on Keratinase Production

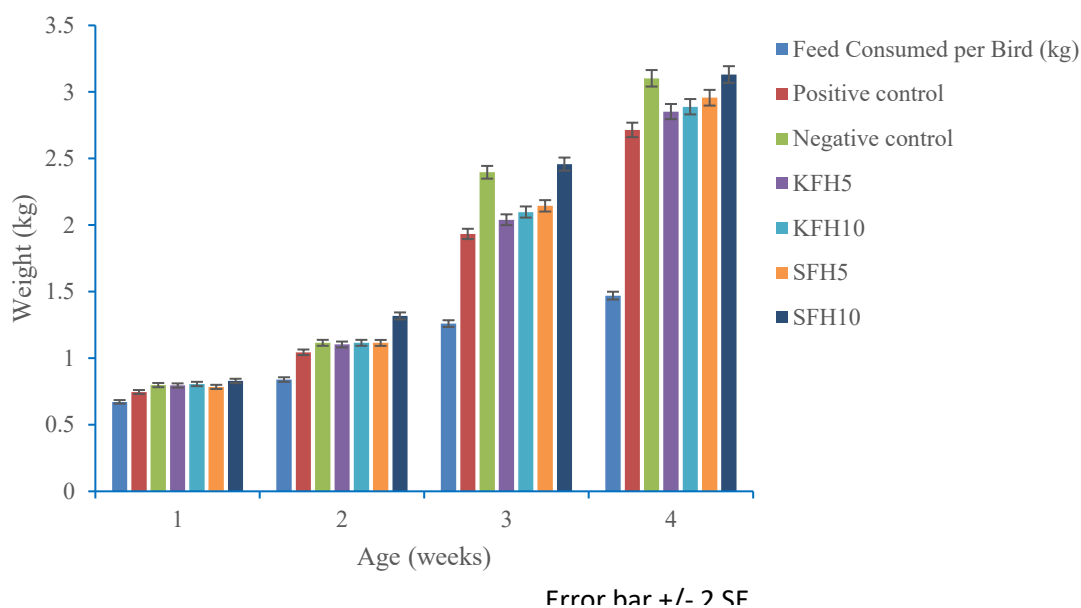


Figure 3: Cumulative Body Weight Per Bird (in kg) for Four Weeks

Table 4: Average Weight Gained by the Broilers after Four Weeks

	Positive control	Negative control	KFH5	KFH10	SFH5	SFH10
Initial weight (kg)	0.635	0.687	0.669	0.659	0.643	0.631
Final weight (kg)	2.900	3.207	3.003	3.014	3.108	3.225
Weight gained (kg)	2.265	2.520	2.334	2.35	2.465	2.594
Weight gained (%)	78	78	77	78	79	80

Keys: PC- Positive control (Broilers fed with commercial feed), NC- Negative control (Broilers fed with formulated feed without hydrolysate supplement), KFH5 and KFH10 - Soya meal supplemented with 5% and 10% feather hydrolysate from *Klebsiella pneumoniae*, SFH5 and SFH10- Soya meal supplemented with 5% and 10% feather hydrolysate from *Serratia nematodiphila*

Table 5: Liver Function after the Consumption of the Supplemented Feed by Broilers

Samples	AST (U/L)	ALT (U/L)	ALP (U/L)	Albumin (g/l)	Total protein (g/l)	Total bilirubin (umol/l)	Conjugate bilirubin (umol/l)
PC	95.00±5.50 ^{ab}	12.50±1.50 ^{ab}	101.60±4.00 ^b	12.50±1.50 ^a	38.00±0.00 ^b	14.45±0.05 ^a	2.45±0.05 ^b
NC	73.00±4.00 ^a	10.00±2.00 ^a	99.40±1.00 ^a	12.50±0.50 ^a	35.00±0.00 ^a	16.15±1.75 ^{ab}	1.10±0.00 ^a
KFH5	103.50±2.50 ^{ab}	13.00±0.00 ^{ab}	108.40±3.00 ^c	13.00±1.00 ^a	35.50±0.50 ^a	19.50±0.50 ^{bc}	2.15±0.05 ^b
KFH10	105.00±3.00 ^b	17.00±1.00 ^b	108.10±5.00 ^c	13.50±0.50 ^a	40.00±0.00 ^c	23.65±0.45 ^c	1.15±0.05 ^a
SFH5	78.00±1.00 ^{ab}	11.50±0.50 ^{ab}	99.45±0.50 ^a	11.50±0.50 ^a	37.50±0.50 ^b	14.40±0.00 ^a	2.30±0.10 ^b
SFH10	95.00±4.00 ^{ab}	16.00±0.00 ^{ab}	107.60±0.00 ^c	12.50±0.50 ^a	40.50±0.50 ^c	16.05±0.05 ^{ab}	2.15±0.05 ^b

Keys: Data are presented as Mean ± S.E (n=2). Values with the same superscript letter(s) along the same columns are not significantly different (P<0.05) according to Tukey's Honestly Significant Test. KFH5 and KFH10- Soya meal supplemented with 5% and 10% feather hydrolysate from *Klebsiella pneumoniae*, SFH5 and SFH10- Soya meal supplemented with 5% and 10% feather hydrolysate from *Serratia nematodiphila*. AST- Aspartate transaminase, ALT- Alanine transaminase, ALP- Alkaline phosphate

DISCUSSION

The bacterial isolates used for the hydrolysate feed supplement were Gram-negative and confirmed molecularly as *Serratia nematodiphila* and *Klebsiella pneumoniae*. *Serratia* species have been reported as better keratinase producers (Veerapura *et al.*, 2019). Thus, in this study, *Klebsiella pneumoniae* appears to be a potential candidate for keratinase production. However, the isolated *Serratia nematodiphila* produced more keratinase enzyme than *Klebsiella pneumoniae* when the keratinous protein substrate (milled feather) was present in the media. The maximum temperature for keratinase production was recorded at 50°C for *Serratia nematodiphila* and it was also recorded to produce enzymes in a wide range of temperatures. This was supported by the findings of Tamreihao *et al.*, (2019) and Veerapura *et al.*, (2019), which show that enzymes are produced in different temperatures from 37°C to 55°C. The optimum activity of the keratinase enzyme was found at pH 8.0 alongside a temperature of 50°C. Similarly, a preferred pH of 8.0 for the activity of keratinase has been reported by Nika *et al.* (2019). *Serratia nematodiphila* and *Klebsiella pneumoniae* might be good candidates for keratinase production and may be employed in feather degrading.

The hydrolysate from the feather degradation was confirmed as a good

supplement for broiler feeds. A review by Qingxin (2019) supports that feathers can be converted to feather meal when degraded. The proximate composition of the chicken feathers are, 3.77% fiber, 5.65% moisture, 82.08% crude protein, 4.55% carbohydrate, 2.89% ash, and 1.06% fat. The proximate composition of the chicken feathers result shows that the percentage content of fat, ash, and fiber was low while crude protein content high compared to other nutrients that composed the chicken feather. The proximate composition of the hydrolysate shows 3.36% fiber, 2.43% moisture, 88.84% crude protein, 1.04% carbohydrate, 3.52% ash, and 0.81% fat content. The hydrolysate is rich in protein which is a good supplement for soymeal in broiler feeds. The carbohydrate is reduced in the hydrolysate compared to raw feather which is believed to have been used up as a source of energy during degradation. The findings were supported by Adelina *et al.*, (2019), which explains that fat and starch were decreased during fermentation because they were used to meet energy needs for the growth of the fermenters.

The mean weight of broilers fed without hydrolysate supplement increased from 0.661 kg to 2.954 kg, while those fed with hydrolysate increased from 0.650 kg to 3.013 kg. This increase in weight gain in broilers fed with hydrolysate supplements shows that the supplement has adequate

nutrients for growth and is suitable for broiler feeds.

The liver enzymes ALT ranges between 10.00 U/L to 16.00 U/L, AST 73.00 U/L to 105.00 U/L, and ALP 99.40 U/L to 108.40 U/L. Albumin (11.50 g/l -13.50 g/l), total protein (35.00 g/l -40.50 g/l), total bilirubin (14.40 umol/l -23.65 umol/l), and conjugate bilirubin (1.10-2.45 umol/l). In the present study, the parameters assessed such as ALT, AST, ALP, albumin, total protein, total bilirubin, and conjugate bilirubin are within the normal range and agree with the findings of Makama *et al.* (2021). There were no significant differences in the parameters when the broilers fed with hydrolysate supplement were compared to broilers not fed with hydrolysate supplement, which implies that the hydrolysate-supplemented feed is a good supplement for broiler feed. The liver enzymes ALT, AST, and ALP showed no significant difference. This indicated that feather hydrolysate supplements from the biodegradation of feathers by *Serratia nematodiphila* and *Klebsiella pneumoniae* had no adverse effect on the normal functioning of the liver.

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CONCLUSION

The study demonstrated that *Serratia nematodiphila* and *Klebsiella pneumoniae* are effective candidates for keratinase production, with *Serratia nematodiphila* showing superior enzyme activity. The optimization of keratinase production conditions, including temperature, pH, and nutrient sources, significantly enhanced enzyme yield. Additionally, the feather hydrolysate produced from these bacterial strains proved to be a viable supplement in broiler feeds, supporting weight gain comparable to traditional soya meal-based diets. The absence of significant differences in liver enzyme levels and other biochemical parameters between broilers fed with hydrolysate-supplemented feed and those fed without it suggests that hydrolysate is a safe and effective alternative protein source in poultry nutrition. This study highlights the potential of using microbial keratinase in sustainable waste management and animal feed production.

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