Indian Journal of Animal Sciences **93** (10): 1015–1020, October 2023/Article
DOI: https://doi.org/10.56093/ijans.v93i10.135704

Effect of feed supplement modifications using keratinolytic bacteria on growth performance and meat chemical composition of domesticated quail

ENDRY NUGROHO PRASETYO[1]✉, MUHAMMAD TAUFIQ HIDAYAT[2] and MAHARANI PERTIWI KOENTJORO[3]

Institut Teknologi Sepuluh Nopember, Gedung H Kampus ITS Keputih–Sukolilo Surabaya 60111, Indonesia

Received: 26 April 2023; Accepted: 5 September 2023

ABSTRACT

This study was carried out to determine a novel strategy for utilization of feather in poultry feed, along with the addition of immobilized keratinolytic *Bacillus* sp. SLII–1 as a supplement. Eighty male quails (day–old) were randomly divided into four diet groups, with each group receiving four different treatments. The results showed that *Bacillus* sp. SLII–I exhibited keratinolytic potential by increasing the protein solubility of feather meal in a liquid medium. The immobilization of *Bacillus* sp. SLII–I using 3% sodium alginate and 0.8% chitosan composition occurred at approximately 4.05×10^7 CFU/g, while the viable cells were maintained in acidic medium (*p*H 3.0) at around 3.95×10^7 CFU/g. Quails aged 19–33 days, fed with feather meal and *Bacillus* sp. SLII–1, demonstrated significantly higher weight gain and lower feed conversion ratio (FCR) as compared to those treated with feather meal without supplements. There were no significant differences in the values of dry matter, ash, and crude protein content of the meat among the quails in all the treatments. Therefore, feed supplemented with immobilized bacteria and feather waste can be utilized as an alternative diet for quails. This way, the use of feed materials can be reduced, and at the same time, chicken feather waste can be effectively utilized.

Keywords: *Bacillus* sp. SLII–I, *Coturnix japonica*, Feather, Feed Supplement, Immobilization, Keratinase

The poultry industry's growth has an increased feed demand but it also generates waste from poultry slaughterhouses. Large amounts of feather debris, around 5–7% of poultry body weight (Tamreihao *et al.* 2019), are produced. Utilizing feather waste in feed diversification offers an alternative to enhance the economic value of feathers and reduce the environmental waste.

Feathers as feed material are ineffective due to their low digestibility. Composed mainly of 90% keratin, a fibrous and water–insoluble protein with strong bonds, feathers limit digestibility and reduce poultry performance when included in meals without processing (Diana *et al.* 2019, Perta-Crisan *et al*. 2021). Adopting a bioconversion technique, such as keratinase, is a suitable strategy to increase the nutritional value of feather. Keratinase breaks down the complex keratin structure into amino acids, peptides, and soluble proteins (Abdelmoteleb *et al.* 2023). Keratinase can be derived from microorganisms. Previous studies have shown that *Bacillus* sp. SLII–I keratinase can convert feathers into meal, replacing soybean meal as a protein source in feed composition (Larasati *et al.* 2017).

Protein meals are usually digested and absorbed in the small intestine. However, the acidic condition of the digestive systems (*p*H 3.0) can hinder effective protein digestion (Mennah-Govela *et al*. 2021). Therefore, it is important to develop a unique strategy for utilizing feathers in poultry feed by adding immobilized *Bacillus* sp. SLII–1 as a supplement. The expectation is that *Bacillus* sp. SLII–1 will hydrolyze indigestible feather keratin, releasing digestible proteins for the poultry's digestive system.

Encapsulation is a promising technique to enhance bacterial resistance to acidity during poultry digestion (Voblikova *et al.* 2023). Chitosan–coated calcium alginate is commonly used for delivering probiotics into the human intestine (Albadran *et al.* 2020, Voblikova *et al.* 2023) due to its secure-handling, non–toxicity, cost-effectiveness, and safety as a feed additive (Albadran *et al*. 2020). Chitosan increases cell stability under acidic conditions (Bedade *et al*. 2019). This research aims to determine the optimal sodium alginate concentration for immobilizing *Bacillus* sp. SLII–1 and assess its impact as feed supplement on the growth performance of Quail (*Coturnix japonica*).

MATERIALS AND METHODS

Growth profile: The growth profile was determined by

Present address: [1]Department of Biology, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember (ITS), Gedung H Kampus ITS Keputih–Sukolilo Surabaya 60111, Indonesia. [2]Study Program of Medical Laboratory Technology, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Jl. Jemursari No. 51–57, Surabaya 60237, Indonesia. [3]The Graduate School of Universitas Brawijaya, Jl. MT. Haryono No. 169, Ketawanggede, Kec. Lowokwaru, Kota Malang, Jawa Timur 65145, Indonesia. ✉Corresponding author email: endry@bio.its.ac.id

incubating a loopful of *Bacillus* sp. SLII–1 culture in 100 mL of Feather Meal Broth (0.5 g/L NaCl, 0.3 g/L K_2HPO_4, 0.4 g/L KH_2PO_4, 10 g/L feather meal) at room temperature (120 rpm) for 48 h. Culture (0.1 mL) samples were taken every 2 h for calculation of the cell density. The cultivation of *Bacillus* sp. SLII–1 was conducted by inoculating it to 100 mL FMB. The culture was incubated at 120 rpm (room temperature) for 48 h. After incubation, FMB was filtered (Whatman filter paper No.1) and the soluble protein content was measured using the Bradford method.

Immobilization of Bacillus sp. SLII–1: Bacillus sp. SLII–1 immobilization was performed using the extrusion method following to Mahmoud *et al.* (2020) with slight modification. After 42 h of incubation, the culture was filtered to separate it from undigested feather waste. Sodium alginate (with varying concentration of 1% (w/v), 2% (w/v), and 3% (w/v) was mixed with 5.5 mL glycerol and 100 mL *Bacillus* sp. SLII–1 culture. Cells suspended in sodium alginate were injected into 50 mL of $CaCl_2$ 0.45 M using a sterile syringe. The formed beads were incubated in $CaCl_2$ solution for 30 min to harden. The beads were washed twice with sterile water and then incubated in 50 mL of chitosan solution with 0.8% (w/v) in 1% (v/v) acetic acid by stirring for 15 min.

Enumeration of immobilized cells and viability: 0.1 g of beads were suspended in 10 mL phosphate buffer at *p*H 7.0. The beads were crushed with a sterile spatula to release all cells and the liquid was processed to enumerate the cells. To test the viability of immobilized *Bacillus* sp. SLII–1 in acidic condition, 0.1 g of beads were suspended in 6 mL phosphate buffer (*p*H 3.0) for 45 min, simulating the poultry gizzard's acidity (Babot *et al.* 2023). After incubation, the beads were separated from *p*H 3.0 and resuspended in phosphate buffer at *p*H 6.5. The beads were then crushed to release all cells.

Evaluation of *Bacillus* sp. SLII–1 viability was performed using the Total Plate Count (TPC) method with serial dilution. In the final step, 0.1 mL of the liquid from the *in vitro* model and nutrient agar were transferred into a petri dish and homogenized by shaking vigorously. The culture was then incubated at room temperature for 48 h and the number of cells was calculated using the formula below:

$$\text{Number of bacteria (CFU/g)} = \frac{\text{number of colonies} \times \text{dilution factor} \times \text{sample volume}}{\text{bead weight (g)}}$$

Growth performance: A total number of 80 male quails (day–old quail/DOQ) were divided into 4 groups with each group receiving different treatments with repetitions. Each group consisted of five quails kept in separate cages (90 × 45 × 25 cm) for 15 days to acclimatize. During this period in the brooder cage, the quails were provided with basal feed and water *ad lib*. The treatment period, from 15–33 day, involved feeding each quail with the experimental feed formulation (Table 1). Weight gain and the FCR were calculated during this interval and consumption data with body weight were collected at the start and the end of the treatment period. The formula used for calculating FCR is given below.

Table 1. Feed formulation

Formulation	A	B	C	D
Feather Meal	2.5%	2.5%	5%	5%
Bacillus sp. *Supplement*	0	4.45×10^8 CFU per kg	0	4.45×10^8 CFU per kg
Standard Feed*	97.5%	97.5%	95%	95%

*Standard feed contained: Yellow corn meal (55%), Rice bran (3%), Soybean (37%), and Fish meal (5%).

$$\text{Weight gain (g/day)} = \frac{\text{(end body weight-initial body weight)}}{\text{day of rear (days))}}$$

Calculation formula for FCR:

$$\text{FCR} = \frac{\text{Feed consumption } (\frac{g}{day})}{\text{Weight gain } (\frac{g}{day})}$$

The data were analyzed using the Analysis of Variance (ANOVA) with a significant level of 90% (Minitab 17 Statistical Software 2010). Significantly different effects from the analysis were further tested with the Tukey test at a 90% confidence level.

Chemical composition of meat: At the end of the experiment (33rd day), two quails from each treatment and replication were randomly selected. The meat samples were cut into smaller pieces and homogenized with a blender at 10°C. All biochemical analyses (dry matter, crude protein, ash) were performed following the AOAC (1990) standards.

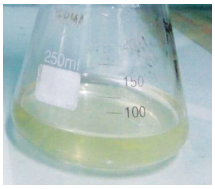
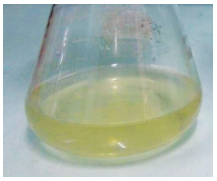
The obtained data were analysed using analysis of variance (ANOVA) with a significance level of 90% and Tukey's test to determine the difference between means, using Minitab 16.0.

RESULTS AND DICUSSION

The initial soluble protein content originated from the autoclaving process. Fagbemi *et al.* (2020) showed that temperature denatured the feather protein. In this study, autoclaving process increased level of raw feather protein from 87% to 99%. Table 2 shows that the keratinolytic of *Bacillus* sp. SLII–1 were digested and broken down into smaller fragments after 48 h. This outcome suggests that *Bacillus* sp. SLII–1 augmented the total soluble protein content in the FMB medium containing insoluble feather meal keratin. The soluble protein concentration and the *p*H in medium reflected the solubilization of feathers and deamination of peptide/amino acids (Bohacz *et al.* 2015).

The genus *Bacillus* exhibits outstanding significance in biotechnology due to its ability to produce high amounts of extracellular enzymes and is also among the most prominent keratinolytic bacteria reported so far (Cavello *et al*. 2021, Martín-González *et al.* 2023). In this keratin degradation pathway, the different proteolytic enzymes contributed to keratin degradation, since the initial attack by keratinases,

Table 2. Degradation of feather and soluble protein content*

Hours	Condition of feather meal	Condition of medium broth without feather meal
0	 Feather meal used was not perfectly smooth	 The growth medium which was separated from the feather meal had a clear colour
48	 Feather meal was smoother	 After incubation, the medium broth separated from the feather meal became more golden

*Soluble protein increased 54% in 48 h.

and disulphide reductases allowed other less specific proteases to act, resulting in extensive keratinolysis, and provided the nutrients for bacterial growth sustainability (Arokiyaraj *et al.* 2019).

In this study, *Bacillus sp. SLII–1* was grown in a minimal medium, where the only carbon source for cell was the feather meal. *Bacillus* sp. SLII–1 synthesized keratinase to hydrolyze keratin as the carbon and nitrogen primary source during its growth in the FMB medium (Larasati *et al.* 2017). Keratinase broke down the disulphide and peptide bonds of keratin, in order to release smaller soluble peptides and amino acids (Ojha *et al.* 2019). This confirms that *Bacillus* sp. SLII–1 is a keratinolytic bacterium with the potential to increase the digestibility of feather keratin as feed.

Keratinase production occurred at the end of the exponential phase of cell growth and keratinase degradation occurred from 24 h to several days. This pattern is associated with the complex mechanisms of the organisms. This result corresponded with the previous findings of Alahyaribeik *et al.* (2020) which discovered that *Bacillus licheniformis* produced the highest amount of soluble proteins (8.28 mg/mL) over four days at 40°C.

The time needed to achieve complete feather degradation by *Bacillus* ranged from 1-10 days. Almahasheer *et al.* (2022) reported that *Bacillus cereus* has completely degraded keratin at 72 h. In another report, *Bacillus* sp. CN2 can efficiently perform keratin degradation after 24 h.

To enhance its role in feather keratin degradation, certain modifications can be applied. De Oliveira Martinez *et al*. (2020) found that when the purified keratinase was combined with refined disulfide reductase, it exhibited higher degradation properties (feather and wool) compared to the activity of each enzyme alone.

Keratinase from microbes is generally an extracellular enzyme when grown on a keratin substrate, although some are bound to cells, forming an intracellular part that includes disulfide reductases, sulphites or thiosulfate groups. These components work synergistically to assist keratinase in degrading keratin by reducing its disulfide bonds. More explicitly, keratinolysis occurs in two steps: sulfitolysis or the reduction of disulphide bonds, and proteolysis. It is speculated that sulfitolysis requires living cells, reductants such as sodium sulfite, DTT, mercaptoethanol, glutathione, cysteine, and thioglycolate or disulfide reductase, in collaboration with keratinolytic proteases for complete degradation of keratin (Moonnee *et al.* 2020, Lai *et al.* 2023).

Biochemical analysis revealed that the full degradation of hair required more than just extracellular keratinase or a combination with intracellular disulfide reductase. Cell attachments were required for the complete degradation of hair, providing the reductant that breaks down the disulfide bridges in a sustainable manner. Therefore, this study employed a different approach in utilizing feather as poultry feed. The use of keratinolytic cells as feed supplements has the potential to improve host performance, since keratin degradation releases essential amino acids (Tamreihao *et al*. 2019).

Immobilization of Bacillus sp. SLII–1: The immobilization of *Bacillus* sp. SLII–1 was carried out using three different concentrations of sodium alginate, resulting in beads with varying shapes and sizes. Table 3 shows that the concentration of sodium alginate affected the bead pore size. This outcome aligns with the principle of chemical bonds in the calcium alginate matrix. The gel formulation within the calcium alginate matrix facilitated cross–linking between guluronic acid residues, connecting with the calcium ions. As the concentration of sodium alginate increased, a great number of crosslinking points between the guluronic acid residues led to the production of rigid and dense beads (Zhang *et al.* 2021). In this study, a higher concentration of sodium alginate resulted in a smaller bead pore size which is related with findings presented in Table 3. Specifically, Table 2 demonstrates that a smaller bead pore size corresponds to a reduced rate of bacterial

Table 3. Immobilization bead test

Concentration of sodium alginate	Concentration of chitosan	Number of encapsulated cells ($\times 10^7$ CFU/g)	Number of viable cells after incubation at *p*H 3.0 for 45 min ($\times 10^7$ CFU/g)
1% (w/v)	0.8% (w/v)	1.89±0.12	1.27±0.01
2% (w/v)	0.8% (w/v)	4.45±0.21	3.65±0.07
3% (w/v)	0.8% (w/v)	4.05±0.21	3.95±0.35

Table 4. Growth performance of quails

Trait	2.5% feather		5% feather		p-value
	No supplement	With supplement	No supplement	With supplement	
Body weight at 15 days of age, g/bird	73.3	70.5	72.35	69.55	0.596
Body weight at 33 days of age, g/bird	150.45	152.8	153.25	154.7	0.648
Weight gain, g/bird	77.15 (c)	82.3 (ab)	80.9 (b)	85.15 (a)	0.023
Feed intake, g/bird	276.15	268.65	270.4	257.35	0.172
FCR	3.58 (a)	3.27 (bc)	3.34 (ab)	3.02 (c)	0.037
Survival rate	100	100	100	100	

Means that do not share the same letter were significantly different at the 10% level with the Tukey test.

release from the bead.

The immobilization formulation also employed the chitosan as a coating for the calcium alginate beads. The addition of 0.8% (w/v) chitosan increased the bead's resistance under acidic conditions. Cationic polymers, such as chitosan, formed gels with sodium alginate through ionic cross–linking. This complex reduced the bead's porosity and demonstrated stability at low *p*H (Dezfooli 2022). The bead is important for delivering the cell into the intestines and helping the cell defend the intestinal environment. Therefore, in this study, encapsulated cells with chitosan–coated alginate material were tested under artificial *in vitro* condition in the testine. The formulation of chitosan–coated alginate was also used by Mahmoud *et al.* (2020) for the immobilization of *Lactobacillus plantarum* TN8 as a supplement for broiler chicken feed. The result showed that this immobilization method led to increased broiler growth. Therefore, the combination of 3% (w/v) sodium alginate and 0.8% (w/v) chitosan was employed as the immobilization formulation for the feed supplement.

Growth performance: Effect of immobilized *Bacillus* sp. SLII–1 to body weight gain and FCR of quail aged 15 to 33 days are shown in Table 4. The use of a 5% feather feed with the addition of *Bacillus* sp. SLII–1 as the supplement resulted in higher weight gain and significantly decreased FCR (by 90%). Additionally, the FCR was more favourable in the 5% treatment, indicating better efficiency in converting feed to weight gain for the quail in this group. This finding aligns with Laboissière *et al.* (2020) who reported that the inclusion of 9% feather meal in quail feed for 21 days have adverse effect on quail performances. Yeh *et al.* (2023) reported that the addition of extruded feather meal up to 10% as a protein source was successful without adverse effects. The results demonstrated that the administration of 5% feather dose into feed, with or without *Bacillus* sp. SLII–1, resulted in higher weight gain as compared to a 2.5% feather content. This result corresponded with the fact that hydrolysates produce very rich in free amino acids such as threonine, cystine, arginine, aspartic acid, lysine and serine (Yeh *et al.* 2023). These result were in correlation with the increased keratinase activity in the quail's intestine. The colonization of *Bacillus* sp. SLII–1 in intestine increased, and this enzyme broke down keratin into essentials amino acids for quails (Ojha *et al.* 2019).

The strategy of using probiotics to enhance the activity of digestive enzymes has been widely supported by research involving various types, such as the use of *C. tropicalis* TKD–3 as probiotics that produce phytase, which, when added to broilers' diet, increased their nutrient digestibility, particularly in terms of apparent metabolic energy (Anggraeni *et al.* 2020). Temiraev *et al.* (2020) discovered that the addition of probiotics increased proteolytic activity. *Bacillus* species, which was a superior enzyme producer also contributed to the growth performance, gut morphology, intestinal microbiota and immune response in broiler chicken (Arif *et al*. 2021).

The mortality rate of 0% in all treatments showed that *Bacillus* sp. SLII–1 was not pathogenic to quail. These results provided suitable strategies containing feather meal and keratinolytic organisms, which was used as an alternative source of economical feed.

Meat chemical composition: Based on ANOVA test, there were no significant differences in all parameters of meat chemical composition (dry matter, ash content, and crude protein) among the feed treatments (Table 5). Although the addition of supplements increased their body weight gain and decreased FCR, no changes were observed in the meat's chemical composition. In contrast, Tang *et al.* (2021) showed that dietary supplementation with *B. subtilis* could improve the meat quality of broilers. Furthermore, additional research is needed to confirm the increased activity of digestive enzymes and the digestibility of the feather in the digestive system. Moreover, the immobilization test employed an *in vitro* approach. It is also essential to assess the composition of microbes to ensure

Table 5. Chemical composition of quail meat

Meat composition	2.5% feather		5% feather		p-value
	No supplement	With supplement	No supplement	With supplement	
Dry Matter (%)	26.36	26.53	26.27	26.05	0.858
Ash Content (%)	1.89	2.07	1.81	2.2	0.702
Crude Protein (%)	22.375	20.4	21.905	20.52	0.309

that the keratinolytic *Bacillus* sp. reaches the poultry's digestive tract, enhancing feather digestibility.

Bacillus sp. SLII–I has the ability to produce keratinase, which is important for feather meal processing. This has economic value as it can serve as a potential substitute for feed source. Additionally, the use of these strain can be considered environmentally friendly, as it helps recycle waste materials, like feathers, into useful product while reducing waste accumulation.

The results showed that *Bacillus* sp. SLII–I has keratinolytic potential, increasing the protein solubility of feather meal in a liquid medium within 48 h. The immobilization of *Bacillus* sp. SLII–I using a composition of 3% sodium alginate and 0.8% chitosan resulted in approximately 4.05×10^7 CFU/g, with viable cells maintained in an acidic medium (*p*H 3.0) at nearly at 3.95×10^7 CFU/g. Quails aged 19–33 days fed with 5% (w/w) feather meal and *Bacillus* sp. SLll–l, exhibited significantly higher weight gain and lower feed conversion ratio (FCR) (α=0.1) compared to those treated with feather meal without supplements. There were no significant difference in the value of the dry matter, ash, and crude protein content of meat among quails in all treatments groups.

ACKNOWLEDGEMENTS

We thank PT. Peksi Gunaraharja (Yogyakarat Special Region, Indonesia) for accommodating and providing quail and cage for experiment.

REFERENCES

Abdelmoteleb A, Gonzalez-Mendoza D, Tzintzun-Camacho O, Grimaldo-Juárez O, Mendez-Trujillo V, Moreno-Cruz C, Ceceña-Duran C and Roumia A F. 2023. Keratinases from *Streptomyces netropsis* and *Bacillus subtilis* and their potential use in the chicken feather degrading. *Fermentation* **9**(2): 96.

Alahyaribeik S, Sharifi S D, Tabandeh F, Honarbakhsh S and Ghazanfari S. 2020. Bioconversion of chicken feather wastes by keratinolytic bacteria. *Process Safety and Environmental Protection* **135**: 171–78.

Albadran H, Monteagudo-Mera A, Khutoryanskiy V V and Charalampopoulous D. 2020. Development of chitosan-coated agar-gelatin particles for probiotic delivery and targeted release in the gastrointestinal tract. *Applied Microbiology and Biotechnology* **104**: 5749–57.

Almahasheer A A, Mahmoud A, El-Komy H, Akqosaibi AI, Aktar S, Abdul A and Borgio J F. 2022. Novel feather degrading keratinases from *Bacillus cereus* group: Biochemical, genetic and bioinformatics analysis. *Microorganisms* **10**(1): 93.

Anggraeni A S, Suryani A E, Sofyan A, Sakti A A, Istiqomah L, Karimy M F and Darma I N G. 2020. Nutrient digestibility of broiler chicken fed diets supplemented with probiotics phytase–producing. *IOP Conference Series: Earth and Environmental Science* **462**: 12003.

Arif M, Akteruzzaman M, Tuhin-Al-Ferdous, Islam S S, Das B C, Siddique M P and Kabir S M L. 2021. Dietary supplementation of *Bacillus*-based probiotics on the growth performance, gut morphology, intestinal microbiota and immune response in low biosecurity broiler chickens. *Veterinary and Animal Science* **4**(14): 100216.

Arokiyaraj S, Varghese R, Ali A B, Duraipandiyan V and Al-Dhabi N A. 2019. Optimizing the fermentation conditions and enhanced production of keratinase from *Bacillus cereus* isolated from halophilic environment. *Saudi Journal of Biological Sciences* **26**(2): 378–81.

Babot J D, Argañaraz-Martínez E, Apella M C and Perez-Chaia A. 2023. Microencapsulation of probiotics with soy protein isolate and alginate for the poultry industry. *Food and Bioprocess Technology* **16**: 1478–87.

Bedade D K, Sutar Y B and Singhal R S. 2019. Chitosan coated calcium alginate beads for covalent immobilization of acrylamidase: Process parameters and removal of acrylamide from coffee. *Food Chemistry* **275**: 95–104.

Bohacz J, Korniłłowicz-Kowalska T, Kitowski I and Ciesielska A. 2020. Degradation of chicken feathers by *Aphanoascus keratinophilus* and *Chrysosporium tropicum* strains from pellets of predatory birds and its practical aspect. *International Biodeterioration and Biodegradation* **151**: 104968.

Cavello I, Bezus B and Cavalitto S. 2021. The keratinolytic bacteria *Bacillus cytotoxicus* as a source of novel proteases and feather protein hydrolysates with antioxidant activities. *Journal of Genetic Engineering and Biotechnology* **19**(1): 107.

De Oliveira Martinez J P, Cai G, Nachtschatt M, Navone L, Zhang Z, Robins K and Speight R. 2020. Challenges and opportunities in identifying and characterising keratinases for value-added peptide production. *Catalysts* **10**: 184.

Dezfooli S M, Bonnot C, Gutierrez-Maddox N, Alfaro A C and Seyfoddin A. 2022. Chitosan coated alginate beads as probiotic delivery system for New Zealand black footed abalone (*Haliotis iris*). *Journal of Applied Polymer Science* **139**(2): e52626.

Diana T F, Pinheiro S R F, Ramos K M, Bonafé C M, Dourado L, Santos A S and Dalolio F S. 2019. Feather meal supplemented with protease in diets for meat–type quails. *Semin Ciências Agrárias* **40**: 3627–28.

Fagbemi O D, Sithole B and Tesfaye T. 2020. Optimization of keratin protein extraction from waste chicken feathers using hybrid pre-treatment techniques. *Sustainable Chemistry and Pharmacy* **17**: 100267.

Lai Y, Wu X, Zheng, X Li W and Wang L. 2023. Insights into the keratin efficient degradation mechanism mediated by *Bacillus* sp. CN2 based on integrating functional degradomics. *Biotechnology for Biofuels and Bioproducts* **16**: 59.

Laboissière M, da Costa M A, de Moraes R, Leandro N S M, Cafe M B and Stringhini J H. 2020. Feather and blood meal at different processing degrees in broiler pre-starter and starter diets. *Brazilian Journal of Science* **49**: e20190036.

Larasati D, Tsurayya N, Koentjoro M P and Prasetyo E N. 2017. Keratinase from newly isolated strain of thermophilic *Bacillus* for chicken feed modification. *AIP Conference Proceedings* **26**: 20022.

Mahmoud M, Abdallah N A, El-Shafei K, Tawfik N F and El-Sayed H S. 2020. Survivability of alginate-microencapsulated *Lactobacillus plantarum* during storage, simulated food processing and gastrointestinal conditions. *Heliyon* **3**: e0351.

Martín-González D, Bordelm S, Solis S, Gutierrez-Merino J and Santos-Beneit F. 2023. Characterization of *Bacillus* strains from natural honeybee products with high keratinolytic activity and antimicrobial potential. *Microorganisms* **11**: 456.

Mennah-Govela Y A, Swackhamer C and Bornhorsy G M. 2021. Gastric secretion rate and protein concentration impact intragastric *p*H and protein hydrolysis during dynamic *in vitro* gastric digestion. *Food Hydrocolloids for Health* **1**: 100027.

Moonnee Y A, Foysal M J, Hashem A and Mia F. 2021. Keratinolytic protease from *Pseudomonas aeruginosa* for leather skin processing. *Journal of Genetic Engineering and Biotechnology* **19**: 53.

Ojha B K, Singh P K and Shrivastava N. 2019. Enzymes in the animal feed industry. *Enzymes in Food Biotechnology* **3**: 93–109.

Perța-Crișan S, Ursachi C Ș, Gavrilaș S, Oancea F and Munteanu F D. 2021. Closing the loop with keratin-rich fibrous materials. *Polymers* **13**: 1896.

Tamreihao K, Mukherjee S, Khunjamayum R, Devi L J, Asem R S and Ningthoujam D S. 2019. Feather degradation by keratinolytic bacteria and biofertilizing potential for sustainable agricultural production. *Journal of Basic Microbiology* **59**: 4–13.

Tang X, Liu X and Liu H. 2021. Effects of dietary probiotic (*Bacillus subtilis*) supplementation on carcass traits, meat quality, amino acid, and fatty acid profile of broiler chickens. *Frontiers in Veterinary Science* **22**(8): 767802.

Temiraev V, Baeva A, Vityuk L, Mamukaev M, Yurina N A, Ktsoeva I I, Bobyleva L A, Zagaraeva E F, Kokov T N and Vologirova F A. 2020. Effect of probiotics on digestive metabolism in growing and laying poultry birds. *Journal of Livestock Science* **11**: 33–39.

Voblikova T and Laricheva K. 2023. *Bifidobacteria* encapsulation and viability of probiotic culture during oral delivery in a milk drink matrix. *International Journal of Food Science* **2023**: 8484835.

Yeh R H, Hsieh C W, and Chen K L. 2023. Two-stage fermented feather meal enhances growth performance and amino acid digestibility in broilers. *Fermentation* **9**(2): 128.

Zhang H, Cheng J and Ao Q. 2021. Preparation of alginate-based biomaterials and their applications in biomedicine. *Marine Drugs* **19**(5): 264.