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# Efficacy of crude and immobilized enzymes from *Bacillus licheniformis* for production of biodegraded feather meal and their assessment on chickens

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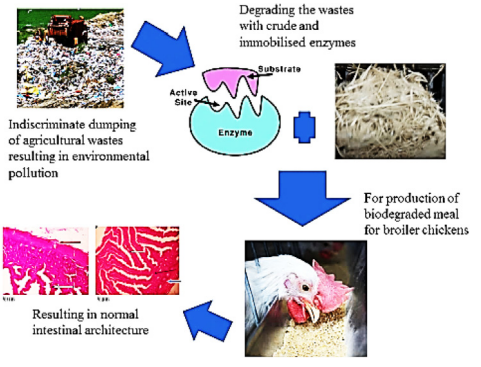
## HIGHLIGHTS

- Keratinase enzymes have a bio-degradative potential for degrading keratin-containing substrates.
- Crude and immobilized enzyme-degraded feather meal were investigated on broiler chickens.
- The thermos-stability showed its maximum keratinolytic activity at 45 °C.
- Feed conversion ratio was statistically similar for feather meal and control diet.
- The microbial biodegradation of feather wastes could be a better approach to overcome high feed cost and environmental pollution arising from solid waste disposal.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Keratinase enzymes are a special type of protease that has a bio-degradative potential for degrading keratin-containing substrates by the enzymes they produced during bioprocessing. The study was carried out to investigate the effect of microbial degraded feather meal on broiler chickens. The strain was previously isolated from a feather dumping site. The effects of crude and immobilized enzyme-degraded feather meal were investigated on growth performance, haematology and intestinal histology of broiler chickens. Maximum activity of the keratinolytic enzyme was at 45 °C, the maximum bio-degradative potential at 48 h of fermentation, while the pH 7 exhibited the maximum keratinolytic activity. The values obtained for feed conversion ratio for birds on feather meal were statistically similar to the value obtained for those on the control diet. The microbial biodegradation of feather

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wastes could be a better approach to overcome high feed cost and environmental pollution arising from solid waste disposal.



## 1. Introduction

Poultry meat forms a major part of human diet most especially protein around the globe with a consumption rate of 14 kg person per year. The poultry feather wastes constitute a major part of the agricultural solid wastes generated after processing and utilization of the viscera. Therefore, management of poultry feather wastes has been a major problem over the years, due to environmental and health hazards they constitute (FAO, 2015; Mazotto et al., 2017). The unimaginable consequences recorded by these environmental pollutions have triggered a public pressure on the policy makers, government, health workers, agro-processing industries to find an alternative to the disposal of poultry feather wastes to useful bio-based products. The clarion-call for the application of biotechnology through enzyme production from keratinolytic microorganisms for the biodegradation of poultry feather wastes could form an eco-friendly, non-chemical approaches, reduction in energy required for animal feed production as well as form an economic feasibility approach by reducing the huge sum of money invested by governments in their management of poultry wastes (Sanchez and Demain, 2010; Demarche et al., 2012; Călin et al., 2017).

Lack of access to financial incentives, high price of animal feed as well as adverse effects of chemical feed supplements are some of the limitations to poultry production in developing countries. Therefore, there is a need to search for an alternative animal feed especially from agro-industrial solid wastes like poultry feather wastes constituting environmental pollution (Khardenavis et al., 2009). The microbial biodegradation of these keratin-containing feather wastes could be an alternative approach to the conventional treatments in the conversion of these feather keratin to a feather meal which is easily digested and could form dietary protein in animal feeding. The conventional treatment including mechanical, chemical, and physical have the potentials to denature the essential amino acids and thereby causing a reduction in the level of protein quality and assimilation (Syed et al., 2009; Mukherjee et al., 2011; Adetunji et al., 2012; Călin et al., 2017).

Keratinase enzymes are a special type of protease that has a bio-degradative potential for degrading keratin-containing substrates by the enzymes produced during bioprocessing. Proteases are enzymes of significant interest that are synthesized in the different biological system most especially from microorganisms. Moreover, the proteases produced from microorganisms are widely utilized in various industrial processes but limited success has been achieved for the biodegradation of protein-rich keratinous wastes which constitute a lot of ecological and health hazards (Dong et al., 2017). Therefore, the application of keratinase enzyme from keratinolytic microorganisms with greater potential to biodegrade keratinase substances especially hard substances could form a permanent replacement to industrially produced proteases. Their uniqueness premise on the fact that production of keratinases in microbial fermentation processes are specific in their activity when compared to the normal protease. This substantiates their utilization in various applications in the industries, and agriculture especially in the production of low-cost and nutrient-rich animal feed (Verma et al., 2017). Therefore, the utilization of keratinous waste from chicken wastes as a fermentation substrate might be a sustainable, clean, eco-friendly and cheaper means for effective production of keratinase enzyme especially for mass production on an industrial scale (Mazotto et al., 2011). Furthermore, keratinase enzymes produced from *Bacillus spp*. have been recommended as a generally recognized as safe bacterium with a greater utilization in protein requisite in food and medical application (Pohl and Harwood, 2010). They are mainly produced extracellularly during solid-state fermentation and submerged fermentation from different microorganisms. These microorganisms can utilize keratinous wastes materials as a sole carbon and nitrogen source for their growth. Among the keratin biodegrading microorganisms which include fungal species (Călin et al., 2017), actinomycetes (Verma et al., 2016) and bacteria (Mazotto et al., 2017) especially from *Bacillus licheniformis* (Liu et al., 2012).

The genus *Bacillus* are in fact the most frequently enquired group of keratinolytic bacteria, the other *Bacillus* species other than *B. licheniformis* that has been reported for keratinolytic activity by previous researchers include *Bacillus cereus* and *Bacillus subtilis* (Łaba and Szczekała, 2013), *Bacillus megaterium* (Forgacs et al., 2001), *Bacillus polymyxa* (Laba and Rodziewicz, 2010), *Bacillus pumilus* (Huang et al., 2003), *Bacillus halodurans* (Prakash et al., 2010; Shrinivas and Naik, 2011) and *Bacillus polyfermenticus* (Dong et al., 2017).

The application of microbial biotechnology offers a lot of benefits in the utilization of immobilized enzymes when compared to the crude enzymes during bioprocessing with many industrial applications. The immobilized biocatalysts have a lot of benefits when entrapped on solid or gel materials which include, continuously and repeated utilization of the enzyme, effortlessness of product separation, enhancement of enzyme stability in different conditions, quick termination of the reaction. The utilization of immobilization biocatalysts has been reported in various fields, which include chemical, drugs, agricultural and food industries (Saieb et al., 2015; Franssen et al., 2017). Therefore, the purposes of the present study were to compare the efficacy of the keratinase enzyme and the immobilized keratinase enzyme in the biodegradation of chicken feather wastes and to determine the effect of the biocatalyst from *Bacillus licheniformis* on the nutritional enhancement of the chicken feather wastes as well as to determine the efficacy of the microbial degraded feather meal performance haematology, and intestinal histology of broiler chickens.

## 2. Material and methods

### 2.1. Microorganisms and growth conditions

The *Bacillus licheniformis* (LMUB05) used in this study was previously isolated from the feather dumping site of Landmark University Farm. The plates were incubated at 37 °C for 48 h on nutrient agar and stored at 4 °C in the refrigerator.

### 2.2. Preparation of substrate

The biodegradation of the chicken feather was performed by using three different treatments containing the 200 mL each of crude enzyme, immobilized enzyme as well as the sterilized water, which were added to the bioreactor. The basal medium was prepared as follows: 6 g/L of $NaNO_3$; 6 g/L of NaCl; 0.3 g/L of $CaCO_3$; 2 g/L of $KH_2PO_4$; 0.15 g/L of $MgSO_4$, 0.03 g/L of $FeSO_4.7H_2O$, 0.03; 20 g/L of chicken feather. Aeration was delivered to the bioreactor by the means of the aerator so as to enable agitation and hydrolysis of the feather for 72 h. After hydrolysis, fermented broth in the bioreactor was discarded and the feathers were later dried in the oven for 2 d at 45 °C and ground to a particle size 1 mm (Adetunji et al., 2012). These products were designated as microbial biodegraded feathers meal.

### 2.3. Inoculum development

The obtained strain (LMU05) was revived by streaking a loopful of pure culture plates on nutrient agar and they were incubated at 37 °C for 48 h. A loopful of pure culture was then introduced into the inoculum medium containing 1% sterilized feather meal and 0.2% yeast extract (pH 7.5). The liquid culture was then incubated at 37 °C using a rotatory incubator at 100 rpm for 24 h.

### 2.4. Production of keratinase enzyme

The keratinase enzyme was produced by inoculating one millilitre of the inoculum into nineteen millilitre of fermentation medium composed of 6 g/L of $NaNO_3$; 6 g/L of NaCl; 0.3 g/L of $CaCO_3$; 2 g/L of $KH_2PO_4$; 0.15 g/L of $MgSO_4$, 0.03 g/L of $FeSO_4.7H_2O$, 0.03; 20 g/L of chicken feather in 100 mL flask. The medium was sterilized at 121 °C for 15 min. The submerged fermentation was carried out for 120 h by incubating the medium at 37 °C on a rotatory shaker at 100 rpm. The whole content was centrifuged at 5000 rpm at 10 °C for 20 min. The supernatant was cold sterilized using a membrane filter and this was later used as the crude enzyme which was stored at 4 °C.

### 2.5. Immobilization of keratinase

The immobilization of the keratinase was carried by preparing using a modified protocol developed by Sinem et al. (2017). The calcium alginate gel beads were prepared by dropping sodium alginate solution (2%) in 90 mL of sterilized water. 10 mL of crude keratinase enzyme was added to the sterilized alginate solution in order obtain 2% final concentration. The resultant mixture was later dropped into a cross linking solution (100 ml of 2% calcium chloride solution in 250 Erlenmeyer flask in order to obtain a spherical beads (4–5 mm diameter) of calcium alginate gel entrapping crude keratinase enzyme . The flask was later placed under continuous stirring for 2 h in a rotary shaker at a shaking speed of 100 rpm. Afterwards, the mixture was allowed to stand for two hour to ensure proper solidification of the beads. The mixture was later decanted and the rest of the liquid was filtered. The beads were then washed several times using distilled water in order to get rid of unbound enzyme. The conical flask was corked and later stored at 4 °C.

### 2.6. Determination of biodegradative potential of keratinolytic activity from Bacillus licheniformis

This was evaluated using a modified protocol developed by Chaturvedi et al. (2014). The component of the reaction mixture consisted of 1 mL of the keratinase crude and immobilized enzyme, 2 mg of microbial degraded feather powder and 2 mL of phosphate buffer adjusted to a final pH of 7.5. The mixture of microbial degraded feather powder and the buffer made up the control. The reaction was performed at 40 °C for 3 h and the mixture was shaken on rotatory shaker at 100 rpm. Afterwards, the reaction was terminated by adding 2 mL of 10% trichloroacetic acid, while the centrifugation was used to separate the precipitated protein at 5000 r/min for 15 min. The rise in absorbance at 280 nm of the filtrate of the test sample relative to that of the control was taken as a measure of release of protein. This was converted into keratinase units (1 U D 0.01 absorbance increase for 1 h reaction time).

### 2.7. Effects of pH, temperature and incubation period on keratinase production

The effect of pH, temperature and incubation period on the crude and immobilized enzymes were evaluated with chicken feather as the main substrate. The keratinase activity was carried out using a protocol developed by Mohanapriya et al. (2014). The influence of pH was carried out by evaluating the enzyme activity, by using citrate buffer with pH 4.0, and 5.0, phosphate buffer with pH 6.0, 7.0, 8.0, and bicarbonate buffer with pH 9.0, 10.0 and 11.0, 12.0. The best temperature suitable for the enzyme production was evaluated by incubating the cultural media at different temperatures varying from 25 °C to 95 °C (25, 35, 45, 55, 65, 75, 85, 95 °C). The effect of incubation period on the activity of keratinase production was performed by at various periods up to 24 h, 48 h, 72 h, 96 h, 120 h separately. The residual activity of the enzyme was carried out at the end of each reaction.

### 2.8. Experimental animals

Two-week old Arbor acre broiler chicks ($n = 144$) procured from a reputable hatchery in south western Nigeria were used for the study in a completely randomized design. The experimental protocol was approved by the Animal Ethics Committee, Department of Animal Science, Federal University Gashua, Nigeria. Four experimental diets were formulated, which served as the treatments. The control diet was soy–corn diet, while soybean mean was substituted at 2.5% for commercial feather meal (Agro Bar-Magen Feed Additives, Israel), crude enzyme degraded feather meal and immobilized enzyme degraded feather meal in the other treatment diets respectively. The effects of the diets were assessed on growth performance, organ/part weights to body weight ratio, haematological parameters and intestinal histology of broiler chickens. The feeding trial lasted for 21 days. Each treatment group contained 36 birds with 6 replicates each. Fresh feed and cool, drinking water were provided for the animals daily. Feed intake was measured weekly as well as the body weight, while body weight gain and feed conversion ratio were calculated from the data obtained. The gross nutrient composition of the experimental diet is presented in Table 1.

### 2.9. Histology studies

Representative sample birds were selected in each group and were euthanized and the intestines were excised into universal specimen bottles separately according to groups. The intestines were preserved in 10% buffered formalin solution prior to the analysis. The histological studies were carried out according to the procedure of Galighor and Koziff (1976) and Avwioro (2010). The tissues were cut into about 4 mm thick into pre-labelled cassettes and were further immersed in 10% formal saline for 24 h to fix. The tissues were processed automatically with automatic tissue processor (Leica TP 1020). The tissues were allowed to passed through various reagents including; stations 1 & 2 containing 10% formal saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 & absolute 11) for the purpose of dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 h and the tissues stayed in each station for 1 h.

Each processed tissue had a solid support medium using a semi-automatic tissue embedding centre. The molten paraffin wax was dispensed into a metal mould and the tissue buried and oriented in it. A pre-labelled cassette was placed on this and it was transferred to a cold plate to solidify. The tissue block formed was separated from the mould. The blocks were trimmed to expose the tissue surface using a rotary microtome at 6 micrometre. The surfaces were allowed to cool on ice before sectioning. The tissues were sectioned at 4 micrometre and the sections were floated on water bath (Raymond lamb) set at 55 °C and these were picked using clean slides. The labelled slides were thereafter dried on a hotplate (Raymond lamb) at 60 °C for 60 min and stained with haematoxylin and eosin.

### 2.10. Carcass cut

Two birds from each replicate were randomly chosen at the end of the feeding trial, slaughtered and organs, visceral and parts percentage to live weight were calculated.

### 2.11. Haematological studies

Blood samples were collected from each treatment into pre-labelled heparinized test tubes according to treatments. The haematological parameters: packed cell volume (PCV), white blood cell counts (WBC), neutrophils, lymphocytes and haemoglobin were determined according to standard procedures (Schalm et al., 1975; Campbell, 1988; Aiello, 1998).

### 2.12. Statistical analysis

The data were subjected to one-way analysis of variance and analysed using SPSS (version 21). Significant means were separated using Duncan's multiple range test (Duncan, 1955).

**Table 1**
Gross nutrient composition of experimental diets.

| Feed ingredients (g/kg) | Control diet | Commercial feather meal-based diet | Crude enzyme degraded feather meal-based diet | Immobilized enzyme degraded feather meal-based diet |
|---|---|---|---|---|
| Yellow maize | 60.00 | 60.00 | 60.00 | 60.00 |
| Soybean meal | 28.50 | 26.00 | 26.00 | 26.00 |
| Palm oil | 1.50 | 1.50 | 1.50 | 1.50 |
| Wheat bran | 6.00 | 6.00 | 6.00 | 6.00 |
| Feather meal | 0.00 | 6.00 | 6.00 | 6.00 |
| Premix[a] | 0.25 | 0.25 | 0.25 | 0.25 |
| Dl-methionine | 0.20 | 0.20 | 0.20 | 0.20 |
| Limestone | 1.50 | 1.50 | 1.50 | 1.50 |
| Di-calcium phosphate | 1.85 | 1.85 | 1.85 | 1.85 |
| Sodium chloride | 0.20 | 0.20 | 0.20 | 0.20 |
| Nutrient analysis | | | | |
| Protein | 18.67 | 19.47 | 19.49 | 19.50 |
| ME (Kcal/kg) | 2950.35 | 2912.85 | 2912.85 | 2912.85 |
| Fibre | 4.13 | 4.03 | 4.03 | 4.03 |
| Fat | 3.89 | 3.86 | 3.89 | 3.91 |
| Lysine | 0.75 | 0.70 | 0.70 | 0.70 |
| Methionine | 0.51 | 0.46 | 0.45 | 0.45 |
| Calcium | 1.02 | 1.02 | 1.02 | 1.02 |
| Phosphorus | 0.61 | 0.61 | 0.61 | 0.61 |

[a] 2.5 kg contains 8,000,000 i.u. vitamin A, 1,600,000 i.u. vitamin D3, 15,000 i.u. vitamin E, 2000 mg vitamin K, 3000 mg vitamin B2, 20 g vitamin C, 20,000 mg niacin, 6000 mg pantothenic acid, 1500 mg vitamin B6, 10,000 mg vitamin B12, 500 mg folic acid, 400 mg biotin, 150,000 mg choline chloride, 100 mg cobalt, 600 mg copper, 10,000 mg iodine, 20,000 mg iron, 90,000 mg manganese, 100 mg selenium, 20,000 mg zinc, 1300 mg antioxidant.

**Table 2**
Growth performance parameters of broiler chickens fed with feather meal-based and control diets.

| Parameters | Control diet | Commercial feather meal-based diet | Immobilized enzyme-degraded feather meal-based diet | Crude enzyme-degraded feather meal-based diet | *P*-value |
|---|---|---|---|---|---|
| Feed intake (kg) | $0.99\pm0.08^{ns}$ | $0.99\pm0.02^{ns}$ | $0.96\pm0.09^{ns}$ | $0.96\pm0.04^{ns}$ | 0.795 |
| Initial weight (kg) | $0.48\pm0.05^{ns}$ | $0.46\pm0.06^{ns}$ | $0.48\pm0.05^{ns}$ | $0.47\pm0.07^{ns}$ | 0.965 |
| Final weight (kg) | $1.03\pm0.03^{a}$ | $0.93\pm0.04^{b}$ | $0.89\pm0.04^{bc}$ | $0.83\pm0.05^{c}$ | 0.001 |
| Weight gain (kg) | $0.55\pm0.02^{a}$ | $0.47\pm0.03^{b}$ | $0.41\pm0.01^{c}$ | $0.36\pm0.04^{c}$ | 0.001 |
| FCR | $1.81\pm0.23^{c}$ | $2.11\pm0.18^{bc}$ | $2.34\pm0.05^{b}$ | $2.68\pm0.19^{a}$ | 0.002 |

Values are means ± standard deviation, means with different superscripts within the same row are significantly ($P < 0.05$) different, ns = non-significant, FCR = feed conversion ratio.

## 3. Results

The results from the thermostability assay carried out during this study showed that the keratinase enzymes that was immobilized exhibited a better stability during keratin biodegradation when compared to crude enzyme produce from *Bacillus licheniformis* (LMUB05). The enzymes exhibited maximum activity (or thermostability) at 45 °C, from the immobilized enzyme had a relative activity of 125.0 ± 4.6% while the crude enzyme had 83.0 ± 2.8% relative activity. Moreover, it was observed that the maximum bio-degradative potential was observed at 48 h after the fermentation began. The crude enzyme had 110 ± 4.6% relative activity while the immobilized enzyme had 127 ± 3.8% relative activity. The pH of 7 exhibited the maximum keratinolytic from the immobilized enzyme with 120 ± 3.0% relative activity while the crude enzyme had 76 ± 2.1% relative activity (Fig. 1a,b,c).

Feed consumption and initial weight were not different across the treatments (Table 2). Feather meal depressed final body weight and body weight gain of the broiler chickens. Birds on crude enzyme-degraded feather meal had the least final body weight and body weight gain. The values obtained for feed conversion ratio for the experimental animals on treatment diets and commercial feather meal were statistically similar to the value obtained for those on control diet, while the least value was obtained by those on crude enzyme-degraded feather meal, although the value was statistically similar to those on immobilized enzyme-degraded feather meal.

Table 3 presents the organ/part weights to body weight ratios of broiler chickens fed the control diet and feather meal-based diets. The organ weight to body weight ratio of visceral, liver and wings did not differ across the treatments. Dressed weights of the chickens on crude and immobilized enzyme-degraded feather meal compared well with those of control diet. The value obtained for head and legs weight to body weight ratio for birds on immobilized enzyme-degraded feather meal was statistically similar to the values obtained for the commercial feather meal and the control group. However, birds on crude enzyme obtained the highest value when compared with the control. The values obtained for intestine weight to body weight ratio obtained for the experimental groups were statistically similar to the control. Birds on crude enzyme-degraded feather meal had the highest ratio for gizzard weight to body weight, which was similar to the value obtained for the control, while those on immobilized enzyme had the least value, which compared with the commercial feather meal-based diet.

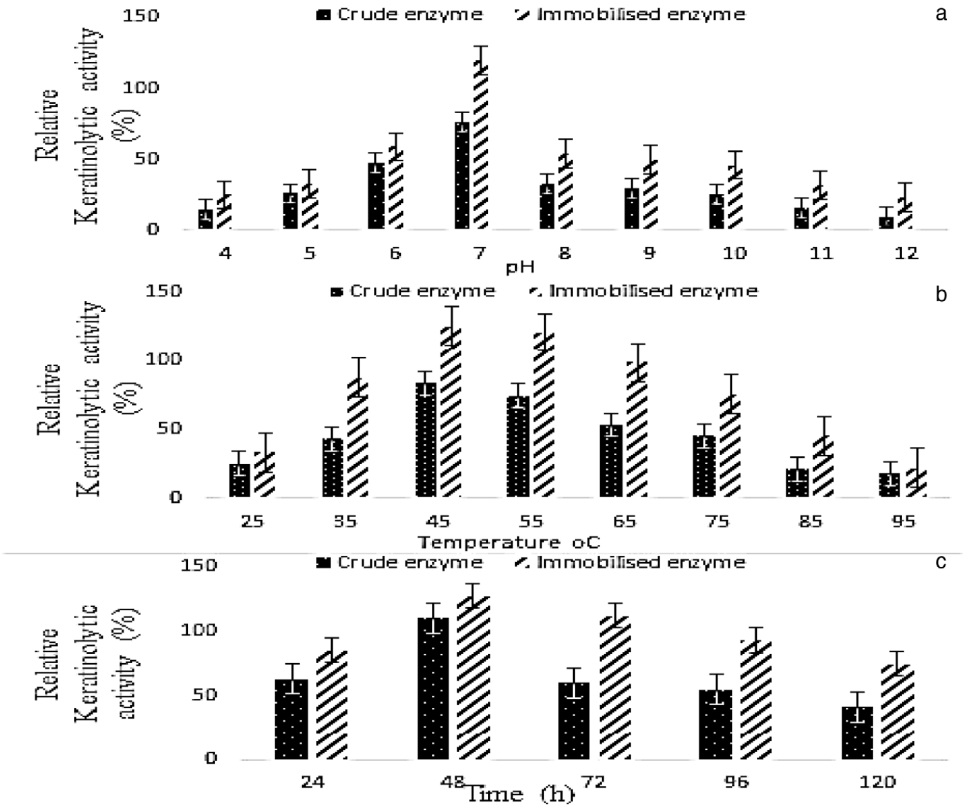


**Fig. 1.** Effect of pH (a), temperature (b) and time (c) on keratin biodegradation on *Bacillus licheniformis* (LMUB05).

**Table 3**
Organ weight to body weight ratios of broiler chickens fed with feather meal-based and control diets.

| Parameters | Control diet | Commercial feather meal-based diet | Immobilized enzyme degraded feather meal-based diet | Crude enzyme degraded feather meal-based diet | *P*-value |
|---|---|---|---|---|---|
| Dressed weight | $0.7\pm0.10^{a}$ | $0.54\pm0.04^{b}$ | $0.61\pm0.01^{ab}$ | $0.68\pm0.04^{a}$ | 0.024 |
| Head + legs | $0.07\pm0.02^{b}$ | $0.08\pm0.01^{ab}$ | $0.06\pm0.03^{b}$ | $0.11\pm0.01^{a}$ | 0.056 |
| Visceral | $0.15\pm0.05^{ns}$ | $0.12\pm0.02^{ns}$ | $0.13\pm0.03^{ns}$ | $0.15\pm0.03^{ns}$ | 0.586 |
| Liver | $0.02\pm0.01^{ns}$ | $0.02\pm0.01^{ns}$ | $0.02\pm0.01^{ns}$ | $0.03\pm0.01^{ns}$ | 0.81 |
| Intestine | $0.05\pm0.01^{ab}$ | $0.04\pm0.01^{b}$ | $0.06\pm0.01^{a}$ | $0.06\pm0.01^{a}$ | 0.065 |
| Wings | $0.07\pm0.01^{ns}$ | $0.06\pm0.01^{ns}$ | $0.06\pm0.02^{ns}$ | $0.08\pm0.01^{ns}$ | 0.358 |
| Gizzard | $0.05\pm0.01^{ab}$ | $0.04\pm0.01^{bc}$ | $0.03\pm0.01^{c}$ | $0.06\pm0.02^{a}$ | 0.005 |

Values are means ± standard deviation, means with different superscripts within the same row are significantly ($P < 0.05$) different, ns = non-significant.

Table 4 shows the haematological parameters of broiler chickens fed feather meal-based diets and control diet. The values of packed cell volume for commercial feather meal and immobilized enzyme-degraded feather meal were statistically similar to the control diet and crude enzyme-degraded feather meal. Haemoglobin concentrations followed a similar pattern. The WBC counts were significantly higher for birds on immobilized enzyme-degraded feather meal, followed by the control. Birds on crude enzyme-degraded feather meal had the least value. Neutrophils were higher for feather meal-based diets than the control, while they obtained lower values of lymphocytes than the control.

The photomicrographs of the small intestinal section of broiler chickens fed the control diet, commercial feather meal, and crude and immobilized enzyme-degraded feather meal-based diets are shown in Fig. 2A–D. The intestinal photomicrographs of chickens fed the control diet showed mucosal layer with moderate infiltration of lamina propria and glands with inflammatory cells (a), the crypts and villi were also inflamed (b), submucosal layer (c) showed mild infiltration while the circular muscle layer (d) appeared normal. The intestinal photomicrographs of chickens fed commercial feather meal showed normal mucosal layer with normal glands without inflammation (a), the villi (b), submucosal layer (c) and the circular muscle layer (d) appeared normal. The intestinal photomicrographs of chickens fed immobilized enzyme-degraded feather meal indicated normal mucosal layer with mild infiltration of glands by inflammatory cells (a), the villi (b), submucosal layer (c) and circular muscle layer (d) appeared normal, while the intestinal photomicrographs of chicken fed crude enzyme-degraded feather meal revealed mucosal layer with mild infiltration of lamina propria and glands with inflammatory cells

**Table 4**
Haematological parameters of broiler chicks fed with feather meal-based and control diets.

| Parameters | Control diet | Commercial feather meal-based diet | Immobilized enzyme-degraded feather meal-based diet | Crude enzyme-degraded feather meal-based diet | *P*-value |
|---|---|---|---|---|---|
| PCV (%) | $32.0\pm1.3^{a}$ | $30.0\pm2.8^{ab}$ | $28.0\pm2.6^{ab}$ | $24.0\pm1.2^{b}$ | 0.062 |
| WBC ($x10^{3}mm^{3}$) | $7.4\pm1.2^{b}$ | $3.2\pm0.16^{c}$ | $8.3\pm0.3^{a}$ | $2.9\pm0.1^{d}$ | 0.001 |
| Neutrophils (%) | $28.0\pm1.16^{c}$ | $60.0\pm3.5^{a}$ | $69.0\pm4.0^{a}$ | $66.0\pm2.6^{ab}$ | 0.001 |
| Lymphocytes (%) | $72.0\pm2.17^{a}$ | $38.0\pm1.46^{b}$ | $31.0\pm1.0^{b}$ | $34.0\pm1.94^{b}$ | 0.001 |
| Haemoglobin (mg/dl) | $11.4\pm1.1^{a}$ | $10.7\pm1.0^{ab}$ | $9.96\pm0.8^{ab}$ | $8.54\pm0.71^{b}$ | 0.062 |

Values are means ± standard deviation, means with different superscripts within the same row are significantly ($P < 0.05$) different, ns = non-significant, PCV = Packed Cell Volume, WBC = White Blood Cell counts, RBC = Red Blood Cell counts.

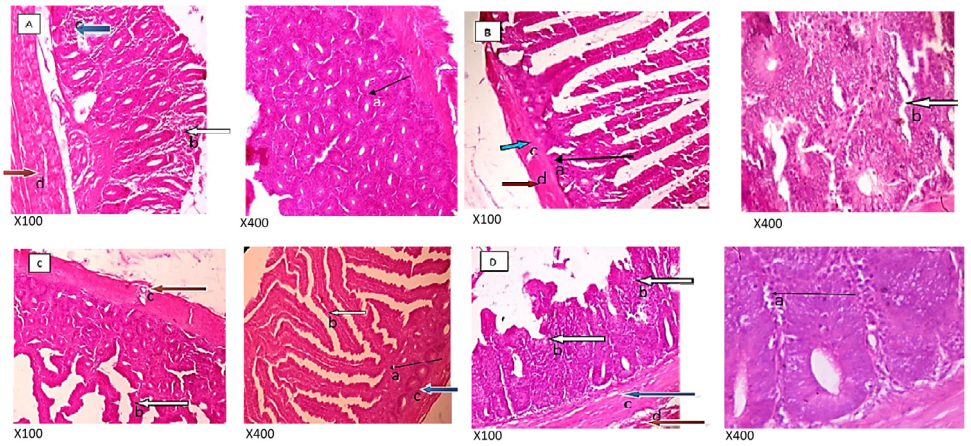


**Fig. 2.** Intestinal photomicrographs of chickens fed the control diet (A), commercial feather meal (B), immobilized enzyme-degraded feather meal (C) and crude enzyme-degraded feather meal (D).

(a), the crypts and villi appeared mildly sloughed and inflamed (b), submucosal layer (c) and the circular muscle layer (d) appeared normal.

## 4. Discussion

Poultry feathers and other agricultural wastes are allowed to accumulate in developing countries because little is known about their usefulness, which has necessitated this study. Keratinase enzymes being special types of protease have a biodegradative potential for degrading keratin-containing substrates by the enzymes produced during bioprocessing. The proteases produced from microorganisms are widely utilized in various industrial processes but limited success has been achieved for the biodegradation of protein-rich keratinous wastes which constitute a lot of ecological and health hazards (Dong et al., 2017). However, immobilization had been observed to have a greater effect on the stability of the keratinase enzyme (Taskin, 2013). Ochetim (1993) reported that FCR of broiler chicks ranges between 2.31 and 2.74. The authors further noted that 4.5% dietary inclusion of boiled feather meal depressed FCR while the inclusion levels up to 3% had no significant effect on broiler chickens. At 4.5% inclusion level, the final weight was also depressed. Dressing percentage was also not different for all the inclusion levels, but birds fed diets containing 0, 1.5 and 3% boiled feather meal had higher carcass yields. The variations observed in the performance characteristics of the experimental animals in the present study could be attributed to the imbalance amino acid profile in feather meal (Wessels, 1972; Ochetim, 1993). The slight variations observed in this study with that of the previous studies may be due to differences in the duration of the study. Hence, studies with longer durations may be recommended.

Also, Grazziotin et al. (2008) observed lower dietary intake and body weight gain of keratin-based diets by growing rats when compared with a standard casein-based diet containing similar protein concentration. However, a higher body weight gain than those fed soybean protein was observed when methionine was added to the feather hydrolysate diet of the rats. Rutkowski et al. (2003) reported lower values for body weight gain and FCR for broiler chicks fed autoclaved and enzymatically treated feather meal for the first 2 weeks of the study when compared with the control but the variations became negligible as the study progressed. The FCR ranged between 2.32 and 2.42 at 42nd day of the study. The depressed body weight gain observed in experimental animals fed feather meal-based diets has been attributed to the low digestibility of lysine in feather meals or its high inclusion in the diet (Rutkowski et al., 2003).

The small intestine is made up of the duodenum, jejunum, and ileum. It is also the main site for digestion and absorption of nutrients. The effects of dietary inclusion may be pronounced on the small intestines of the animals than other organs. Also,

serious impairment in the structure and function of the small intestine may affect digestion and absorption of nutrients, which consequently may likely affect the performance of the animals. Wang et al. (2007) earlier reported that dietary supplement of bee pollen in the diet of broilers promoted the development of all three parts of the small intestine during early developmental stages. The mucosa was more developed in the pollen group and the intestinal villi in the pollen group were longer and thicker than those of the control group. A reduction in feed consumption or feed restriction has been linked with a decrease in villi height of the intestinal epithelium (Yamauchi and Tarachai, 2000; Hu and Guo, 2008). Although, growth performance was slightly better for immobilized enzyme-degraded feather meal-based diet, the histological observation for crude enzyme-degraded feather meal may not be associated with the processing technique or strain of microbes used, because a similar observation was made for the control diet.

Adejumo and Onifade (2005) noted that feather meal at 14 and 18% inclusion levels depressed feed consumption, weight gain and feed efficiency in growing rabbits. Haemoglobin concentration, mean cell volume, and mean cell haemoglobin concentrations were also higher at 14 and 18% inclusion rate, but reduced liver function enzymes. The authors recommended lower inclusion rate. The result of histology presented in the present study shows that enzyme-degraded feather meal poses no threat to the intestinal structure and function of the broiler chickens. Feather meal has also been reported not to influence feed intake, body weight gain and FCR in broiler chicks exposed to feather meal-based diets for 14, and 21 days respectively (Caires et al., 2010). The authors concluded that feather meal inclusion can be used with no negative influence on broiler performance or carcass yield. It also reduces feed cost. A reduction of cost of production is really a positive approach for handling of agricultural wastes in developing countries as well as a means to reduce huge investment on disposal of agricultural wastes in developed countries.

Previous researchers have shown that the best keratinase activities were observed at the pH ranges of 6.5 to 7.5 from different microorganisms including actinomycetes, bacteria, and filamentous fungi (Jeong et al., 2010; Sahoo et al., 2012). The value obtained in this study corresponds to the previous report from various scientists that the highest keratinolytic activities vary from 30 °C to 80 °C. This shows that the biotechnological potential of the keratinolytic strain used during this study can be utilized in various extreme environments (Xu et al., 2009; Chaturvedi et al., 2014). Moreover, it was observed that the immobilization had a greater effect on the stability of the keratinase enzyme which follows a similar trend with the previous work by Taskin (2013).

## 5. Conclusion

This study has shown the efficacy of immobilization of keratinase enzyme from *Bacillus licheniformis* (LMUB05) when compared to the crude enzyme. The crude and the immobilized enzyme may enhance the biodegradation of agro-industrial wastes like chicken feather wastes used during this study and their conversion to animal feed ingredients. The result observed from the pH tolerance and thermal stability of the immobilized keratinase from *Bacillus licheniformis* (LMUB05), shows that they could be utilized for the conversion of microbial degraded feathers into useful peptides and amino acids. The results from the feeding trial revealed that immobilized enzyme from *Bacillus licheniformis* microbial degraded feather meal performed slightly better than the crude enzyme from *Bacillus licheniformis*-degraded feather meal. The effect may be clearer with studies with longer duration and feeding manipulations. It also suggested that feeding modification may enhance the growth performance of keratinase degraded feather meal-based diets.

## Conflict of interest

We declare that we have no conflicting interest concerning this manuscript.

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