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# Effect of chicken feet collagen ointment (*Gallus gallus domesticus* L.) on incision wound healing in mice (*Mus musculus* L.)

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ASC, hydroxyproline, collagen, incision wound healing, protein.



**Abstract**

Chicken feet (*Gallus gallus domesticus* L.) are a by-product of a chicken slaughterhouse that has the potential to be a source of collagen for wound healing therapy. Collagen plays an important role in skin tissue regeneration, protein synthesis, and connective tissue formation during the healing process. This study aimed to characterize chicken feet collagen using Fourier Transform Infrared (FTIR), evaluate the physicochemical properties of collagen ointment preparations, and analyze their effect on the percentage of incision wound closure, protein levels, and hydroxyproline in mice (*Mus musculus* L.). The study was conducted as a laboratory experiment with a complete randomized design, including negative controls, two positive controls, and three groups of collagen ointment at concentrations of 5%, 10%, and 15%. Collagen is extracted using the Acid Soluble Collagen (ASC) method and is formulated in the form of an ointment. FTIR characterization shows the presence of amide groups A, B, I, II, and III as characteristic of collagen. The ointment preparation meets physical and chemical requirements, including homogeneity, semi-solid consistency, and pH according to the skin. Collagen ointment increased the percentage of incision wound closure across all groups without a significant difference among treatments. Tissue protein levels declined progressively from the untreated control through the treated groups, reaching the lowest value at the 5% concentration (P1). Hydroxyproline levels, by contrast, did not follow a uniform pattern: the 5% ointment (P1) showed hydroxyproline levels comparable to the positive controls, whereas the 10% and 15% ointments (P2, P3) showed hydroxyproline levels comparable to the untreated negative control, suggesting that healing in the P2 and P3 groups remained in an active, collagen-synthesizing phase at the time of sampling. These results suggest that chicken feet collagen has the potential to be an active ingredient in topical preparations to support the healing of incision wounds.

## Introduction

Wounds are among the disorders caused by changes in the normal structure of the skin, resulting in damage at the cellular and tissue levels. This can affect the anatomy and physiology of the skin. There are several causes of injury, such as scratches from sharp objects, interactions with harmful chemicals, blows from blunt objects, or animal bites (Amfotis *et al.*, 2022). Wounds are a serious problem, because they become one of the entry portals for microorganisms or foreign entities to enter and attack the tissues, causing infection. In treating the injured area to avoid infection, proper treatment of the wound is needed. Several methods can be used to speed up recovery and restore normal skin condition, such as wound dressing, medicines, bioengineering technology, or ointments (Polverino *et al.*, 2024).

Some methods have their own advantages and disadvantages, so it's important to know the type of wound and the right method to treat it. The first method introduced was wound dressing, which was classified into 3 types: traditional, passive, and modern wound dressings (Mirhaj *et al*., 2022). Other methods, such as the use of bioengineering technology through tissue engineering. The principle is to replace damaged tissue with elements that contain components of cells resembling living tissue. This method has several drawbacks, in addition to the techniques used are quite complicated, the price required also tends to be higher (Tottoli *et al*., 2020). In addition to some of these methods, the healing method with ointment has also been widely used because of its quick and easy application. Ointments and creams are semisolid preparations that contain active ingredients to accelerate closure in the wound area. However, improper formulations can cause irritation, which can worsen skin conditions. Therefore, it is important to know the types of active ingredients used in wound healing methods (Stan *et al*., 2021).

The active ingredients in the ointment formulation can be natural or synthetic. In this case, active ingredients sourced from nature have several advantages, including being environmentally friendly and biocompatible and biodegradable (Nammas, 2024). One of the natural ingredients that comes from animals and has the potential to be an agent in accelerating the wound healing process comes from chicken feet. This part is often wasted in the chicken slaughterhouse, so it ends up as a preparation or addition to cooking. In 100 g of chicken feet contains 14.96 g of fat; 77.59 g protein; and 56.31 g of collagen, of which 72.25% of the protein contained in 100 g of chicken feet is collagen (Araújo *et al*., 2018). Collagen is one of the important components that make up the space between cells, and it plays an important role in providing elasticity and strength to the body's constituent tissues. In addition, collagen plays a role in regenerating tissues due to injury events (Owczarzy *et al*., 2020).

The importance of collagen as an active ingredient in accelerating wound healing has been demonstrated in several studies. Felician *et al.* (2019), which states that jellyfish collagen (*Rhopilema esculentum*) applied topically can cut the wound closure time. Collagen peptides derived from jellyfish extract function to improve the *remodeling* phase and accelerate wound healing. Other research related to the use of collagen as a wound healing agent was also reported by Nugroho *et al.* (2022), which explains the role of collagen extracted from the skin of tilapia (*Oreochromis niloticus*) as an active ingredient in accelerating the wound closure process through the administration of a 15% collagen ointment formulation. The results of the study proved that the wound closure rate of the treatment group was better, when compared to the positive control using bioplacenton. In another study, such as the one conducted by Atianingsih (2021), it was stated that collagen sourced from chicken feet in the form of hydrogel preparations with a 5% formulation can have a positive effect on the burn healing process in rabbits.

There have been many studies that explain the use of collagen extracted from various animal sources as an active ingredient for wound healing. However, few studies have utilized collagen sourced from poultry, such as chickens, especially from chicken feet, where the abundance is very high, likely because chicken feet are a low-value slaughterhouse by-product that has traditionally been directed toward food use rather than being processed for higher-value biomedical raw material. Previous research using chicken feet is still limited to the purpose of extracting collagen as a compactor and thickening agent for food [citation needed – author to add a supporting reference]. Therefore, this study aimed to determine the yield obtained from the extraction process on the skin of chicken feet, to characterize the extracts obtained according to the FTIR test, as well as to determine the biological effectiveness of chicken feet collagen ointment on the percentage of incision wound closure, protein levels, and hydroxyproline levels in mice, the latter two serving as biochemical indicators of tissue remodeling and ongoing collagen synthesis during the healing process.

## Materials and Methods

This study used a laboratory experiment method with a Complete Random Design (RAL) consisting of negative control groups, positive controls 1 and 2, where positive control 1 (K+1) received a commercial bioplacenton ointment and positive control 2 (K+2) received the ointment base without collagen extract, allowing the effect of the collagen extract itself to be distinguished from the effect of the ointment base and from a standard commercial reference, and 3 treatment groups given collagen ointment at concentrations of 5%, 10%, and 15%, with each group consisting of 8 replicates. This research has received code of ethics approval No.240/KEPK-FFUNMUL/EC/EXE/12/2025 from the Faculty of Pharmacy, Mulawarman University. All reagents used in this study were of analytical grade (Sigma and Merck).

## 1. Research Subject

White male mice (*Mus musculus* L.) that are 2 – 3 months old and reach an average body weight of 25 – 30 g. Mice were acclimatized to the laboratory environment for 7 days prior to treatment, with free access to standard feed and water.

## 2. Research Methods and Design

### a. Sample Preparation and Soaking with 0.1 M NaOH Solution

The chicken feet are cleaned of blood and impurities, and then the bones and skin are separated. After that, the skin of the chicken feet is washed with water and dried at room temperature. In the next stage, the sample is weighed as much as 1 kg, then cut into pieces with a size of 1 – 2 cm. Next, the chicken feet skin was soaked in a 0.1 M NaOH solution with a ratio of sample and solvent (1:8). Soaking was carried out for 12 hours with a change of NaOH solution every 2 hours (Nugroho *et al*., 2022).

### b. Chicken Feet Skin Collagen Extraction

The extraction stage was carried out after the sample was dried at room temperature for 2 hours, then immersed in a solution of acetic acid with a concentration of 0.5 M with a ratio of sample and solvent (1:30). Soaking with an acetic acid solution is carried out for 48 hours with stirring every 4 hours, a duration chosen to allow sufficient time for the acid to disrupt the cross-links between collagen fibrils and maximize solubilization of collagen from the tissue matrix (Nugroho *et al*., 2022).

### c. Precipitation with NaCl

First of all, the sample obtained from the extraction results is filtered using a filter cloth (100 mesh). The filtrate is then collected, and NaCl is added until the concentration reaches 1 M. The sample is left to precipitate with NaCl for 12 hours, after which it is centrifuged at 3000 rpm and 4°C for 1 hour (Zaelani *et al*., 2019).

### d. Dialysis and Drying

The dialysis stage begins by collecting the precipitate; the sample is then placed into a Visking tube and dialyzed for 24 hours using 0.1 M acetic acid, followed by dialysis against distilled water for 24 hours, with the water changed every 4 hours. The resulting wet collagen is then collected and dried using *a food dehydrator* for 14 hours (Zaelani *et al*., 2019).

### e. Detection of Collagen Peptide Groups in Samples

At this stage, a sample of chicken feet extract and KBr powder of 1 mg was weighed. The next stage, is to mix the two materials with a mortar, then put them into pellets and compacted. After that, the pellets were put into the sample container, then analyzed using the FTIR tool at a wave number between 4000–400 $cm^{-1}$ (Nugroho *et al*., 2022).

### f. Ointment Preparation

All ingredients were weighed according to the formulation. The ointment base [author to specify composition and proportions, e.g., lanolin, petroleum jelly/Vaseline, and methylparaben] was combined in a mortar with the chicken feet collagen extract at each of the three concentrations (5%, 10%, 15%), then ground until homogeneous and transferred into an ointment jar. Positive control 2 (K+2) consisted of the ointment base alone, without collagen extract.

### g. Evaluation of Ointment Physicochemical Properties

Evaluation of the ointment preparations encompassed organoleptic (colour, aroma, texture), homogeneity, pH, adhesion, spreadability, and emulsion-type testing, following standard physicochemical criteria for topical semi-solid preparations.

**h. Incision Wound Closure Measurement**

Measurement of the closure of the incision wound in mice was carried out through observation, and measurements were carried out using a digital caliper every 3 days over a 12-day period, namely on the 3rd, 6th, 9th, and 12th days. The percentage of wound closure was calculated using the formula P (%) = [(L0 - Lt) / L0] x 100%, where L0 is the initial wound length (day 0) and Lt is the wound length at the measurement day; a fully closed wound therefore corresponds to 100% closure.

**i. Protein Levels Measurement**

Protein levels were measured in mouse skin tissue collected on day 12, the final day of the observation period, when the animals were euthanized for tissue sampling. The total protein measurement was carried out using the Lowry method. The initial stage was to prepare 0.05 g of skin tissue. Then, a tube containing a PBS solution of 200 µL is prepared, and the tissue is inserted into the *tube* and homogenized for 30 seconds at a speed of 3000 rpm (Domaszeswska-Szostek *et al*., 2021). The sample is then centrifuged for 10 minutes at room temperature at 6000 rpm. From the supernatant, 100 µL was pipetted, then 100 µL NaOH 2 N was added. The sample was then incubated for 10 minutes at 100°C, then cooled at room temperature to add Lowry B and Lowry A reagents. After that, the sample was homogenized and left at room temperature for 30 minutes, then measured with a spectrophotometer at a wavelength of 600 nm (Waterborg, 2009).
In the next stage, BSA standard curves are made at several concentrations, namely concentrations of 0, 10, 20, 50, 100, 200, 500, and 1000 µg/mL. The first stage is taken with a standard BSA solution of 2000 µg/mL, then pinched as 0, 50, 125, 250, 500, 1,250, and 2000 µL. After that, each solution is put into a test tube, then diluted using aquadest. The next stage, 100 µL of standard solution is injected into a *microcentrifuge tube*, then 100 µL of NaOH 2N is added. After that, the Lowry B reagent and the Lowry A reagent were added. The solution was then incubated for 30 minutes in dark conditions until a blue color was formed, then the absorbance value was measured using a spectrophotometer at a wavelength of 600 nm (Waterborg, 2009).

**j. Hydroxyproline Levels Measurement**

Measurements of hydroxyproline levels were taken on day 12, the same terminal sampling timepoint used for the protein assay, by taking 0.05 g of skin tissue. After that, the isolated tissues were incubated at 60°C for 24 hours. In the next stage, the tissue sample is put into a test tube to be hydrolyzed at 60°C for 24 hours using 3 mL of HCl 6 N. Then, the sample solution that has reached room temperature is transferred as much as 2 mL into the Eppendorf tube, and then the sample solution is centrifuged at a speed of 10,000 rpm for 5 minutes. The next stage, the supernatant is put into a test tube to be evaporated at 60°C for 30 minutes. After that, a total of 500 µL of sample solution was added to 30 µL of Chloramine T and 470 µL of pH 6 citrate buffer, then homogenized. For 20 minutes, the sample was left at room temperature, then $HClO_4$ was added to a concentration of 0.4 M, as much as 250 µL. Then, 250 µL of Ehrlich solution was added and homogenized, and then it was left to sit for 90 minutes at 60°C. The result of the reaction will cause the formation of a deposit, so that the sample solution is then centrifuged for 5 minutes at a speed of 3000 rpm. In the final stage, the sample was measured with a spectrophotometer at a wavelength of 557 nm (Gupta *et al*., 2016).

After measurements using spectrophotometers, the next stage is the manufacture of a standard solution of hydroxyproline. The standard solution is made in a concentration of 0; 6,3; 12,5; 25; 50; 100; 200; and 400 µg/mL. First, a solution of 500 µL of hydroxyproline was prepared, then put into the test tube, then 30 µL of Chloramine T was added. After that, a pH 6 citrate buffer of 470 µL was added, then homogenized using a vortex. The solution was then left to sit for 20 minutes. The next stage was the addition of 250 µL of 0.4 M $HClO_4$ solution and 250 µL of Ehrlich solution. In the final stage, centrifugation was carried out at a speed of 3,000 rpm for 5 minutes, then the supernatant was measured for absorbance using a spectrophotometer at a wavelength of 557 nm (Evani, 2020).

**k. Data Analysis**

Data obtained from the measurement of wound closure percentage, protein content, and hydroxyproline were analyzed using GraphPad *Prism* 10 and SPSS *Statistics* 22 software. Analysis was carried out on the values of the percentage of wound closure, protein content, and hydroxyproline content.

## Results and Discussion

This study aims to determine the yield value of the chicken feet skin extraction process using *the Acid Soluble Collagen* (ASC) method, as well as evaluate its biological effectiveness on the healing process of incision wounds in mice. The parameters observed included collagen characteristics based on FTIR analysis, wound closure percentage, and skin tissue protein and hydroxyproline levels.

### 1. Chicken Feet Skin Extract Yield

The results of chicken cartilage extraction using *the Acid Soluble Collagen* (ASC) method, the yield of chicken feet extract is obtained as follows.

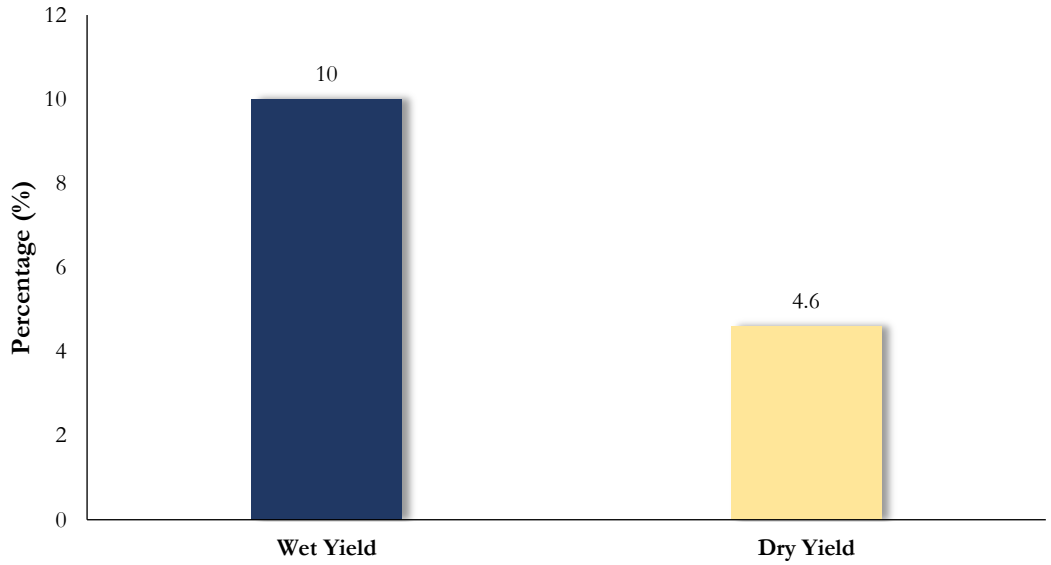


**Figure 1. Percentage of Yield of Chicken Feet Extract**

Figure 1 shows the percentage value of chicken feet extract yield obtained from the extraction process. Based on these results, a wet yield value was obtained, which was 10% or 100 g. Meanwhile, the dry yield obtained was 4.6% or 19 g, reflecting the collagen mass after the solvent and water removal process. The percentage of dry yield was lower compared to the results reported by Zhou *et al.* (2016), where the percentage of dry yield obtained is 14.49%. In this case, the different yield values indicate that with the same method, the yield can vary significantly. Thus, the results of this study provide an idea that technical factors and raw material conditions have the potential to affect the yield value, so that the results obtained are not always identical to the previous report.

### 2. Character Collagen

Detection of collagen extracted from chicken feet skin using the FTIR instrument was carried out to identify whether collagen had been successfully isolated based on the characteristic functional groups of collagens. The results of the FTIR scanning are presented in the following figure.

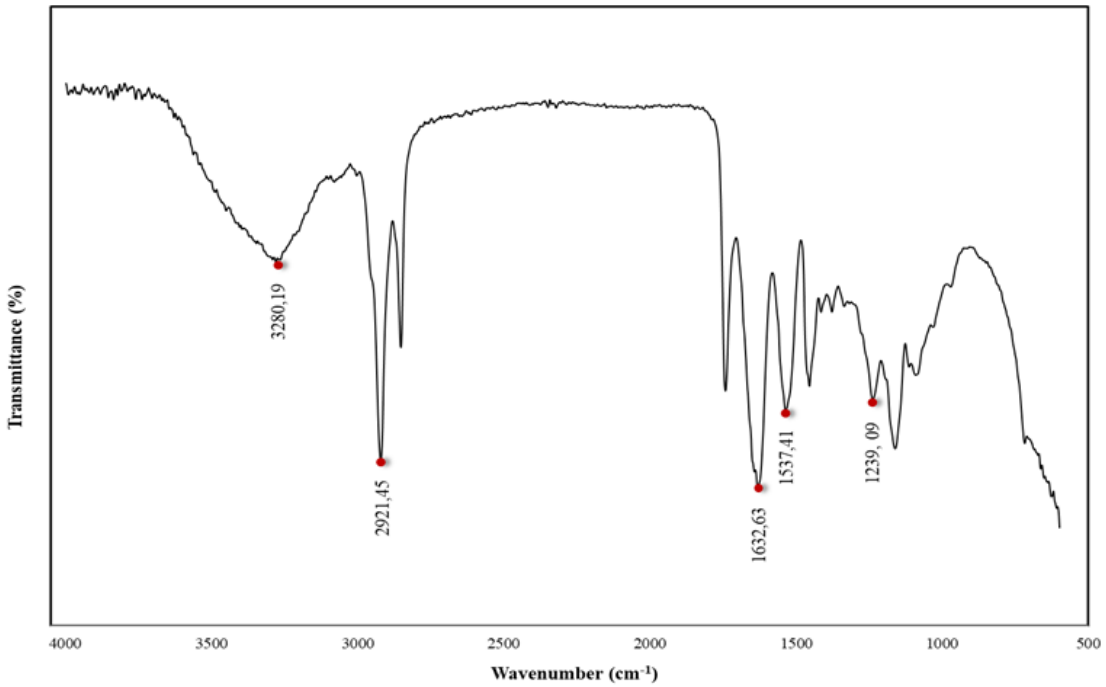


**Figure 2. FTIR Spectrum Chicken Feet Extract**

Based on the FTIR spectrum of chicken feet collagen on the IR spectrum transmission percentage curve with a range of 700 – 4000 $cm^{-1}$ (Figure 2), showing several peak points at wave numbers 3280.19; 2921.45; 1632.63; 1537.41; and 1239.09 $cm^{-1}$. The five peak points represent the amide group which is characteristic of collagen compounds, namely amide A, amide B, amide I, amide II, and amide III. Amida A is generally detected in the range of 3400 – 3440 $cm^{-1}$, while amide B is in the wavelength range of 2924 – 2932 $cm^{-1}$ (Zhou *et al.*, 2016). Meanwhile, amides I, II, and III were detected in the range of 1600 – 1700 $cm^{-1}$, 1480 – 1575 $cm^{-1}$, and 1200 – 1400 $cm^{-1}$ (Zaelani *et al.*, 2019). The typical peak points of collagen detected against chicken feet skin extract samples are related to the functional groups in the sample shown in (Table 1).

**Table 1. Results of FTIR Analysis of Chicken Feet Skin Extract**

| Yes | Number of Waves | Literature | Ribbon Display | Cluster | Functional Group |
|---|---|---|---|---|---|
| 1 | 3280.19 $cm^{-1}$ | 3280 $cm^{-1}$ (Prokopova *et al.*, 2024) | Width (overlap) | N-H | Peptide group |
| 2 | 2921.45 $cm^{-1}$ | 2925 $cm^{-1}$ (Prokopova *et al.*, 2024) | Sharp to medium | CH2 | Alkyl chain |
| 3 | 1632.62 $cm^{-1}$ | 1630 $cm^{-1}$ (Stani *et al.*, 2020) | Strong or dominant | C=O | *Peptide* backbone |
| 4 | 1537.40 $cm^{-1}$ | 1535 $cm^{-1}$ (Hua *et al.*, 2020) | Medium | N-H/ C-N | Peptide group |
| 5 | 1239.09 $cm^{-1}$ | 1240 $cm^{-1}$ (Stani *et al.*, 2020) | Medium to narrow | C-N | Amide III |

The detection results showed the presence of the N-H group at wave numbers 3280.19 $cm^{-1}$ and 1537.40 $cm^{-1}$, the $CH_2$ group at 2921.45 $cm^{-1}$, 2852.69 $cm^{-1}$, and 1455.44 $cm^{-1}$. In addition, other clusters contained in the sample, namely the C=O cluster detected at wave numbers 1742.57 $cm^{-1}$ and 1632.62 $cm^{-1}$, the C-N group at wave numbers 1537.40 $cm^{-1}$ and 1239.09 $cm^{-1}$, and the existence of the C-O group at wave numbers 1162.14 $cm^{-1}$ and 1091.86 $cm^{-1}$. The existence of these various groups indicates the appearance of amide bands that are related to the structure of collagen. Some of the amide bands detected in the samples include A, B, I, II, and III.

Amida A is one of the bands that arises due to the free stretching activity of the N-H group by infrared light. According to Oslan *et al.* (2022), the number of waves shifting towards a lower value indicates that the free N-H group has bonded to another atom on another collagen chain. This is evident in the detection results for chicken foot skin extract, which show that the wave number at 3280.19 $^{cm-2}$ is lower than the general collagen wave number reported by Zhou *et al.* (2016), which falls within the range 3400 – 3440 $cm^{-1}$. In this case, it is known that the N-H group in the sample is thought to have bonded to the O atom in another collagen chain, so that its value has shifted. The second band that was successfully detected was the amide B band, which appeared due to the vibration of the asymmetrical stretch of the $CH_2$ group on the collagen side chain. Based on the results of the FTIR test, a wave number of 2921.45 $cm^{-1}$ was obtained, which shows that the sample is in the range of wave numbers 2922 – 293 $cm^{-1}$, which is characteristic of the collagen structure (Ahmed *et al.*, 2019).

The third band that indicates the characteristic of collagen is the amide I band, which is formed when there is a vibration of the carbonyl group stretch (C=O). According to Ahmed *et al.* (2019), the range of FTIR test wave numbers at 1600 – 1700 $cm^{-1}$ is related to the secondary structure of proteins, especially amide band I. In line with the report, the results of the FTIR test on chicken feet skin samples that were successfully detected at wave number 1632.63 $cm^{-1}$ show that the carbonyl group detected as one of the amide bands characteristics of collagen structure, so that the sample is positive and has an amide band I. The fourth band from the FTIR test results is the amide band II, which comes from the combination of the bending vibration of the N-H group and the stretching of the C-N group. Based on the detection results, a wave number of 1537.41 $cm^{-1}$ was obtained indicating that the sample was in the typical range of the amide II group of the collagen structure. These results are in line with the results of Zaelani *et al*'s research. (2019), where the range of wave numbers 1500 – 1600 $cm^{-1}$ is a special range that characterizes the presence of amide II groups in collagen. The fourth band that is an important component in the structure of collagen is amide III, which is detected due to the bending of the N-H group, as well as the strain of the C-N group. The combination plays a role in maintaining the triple helical structure stabilized by the hydroxyl group. According to Ahmed *et al.* (2019), the range of wave numbers that indicate the presence of amide band III, namely at 1320 – 1220 $cm^{-1}$. This is in line with the detection results carried out on the sample, where the wave number 1239.09 $cm^{-1}$ belongs to the typical amide III band range of the collagen structure.

Based on the results of the FTIR analysis, it is known that chicken feet skin extract is positive for collagen. These results are in line with the report of Zhou *et al.* (2016), who reported that collagen extracted using the *Acid Soluble Collagen* (ASC) method showed five typical peak points of collagen structure markers. The five peak points include amide peaks A, B, I, II, and III, respectively at wave numbers 3297 $cm^{-1}$, 2930 $cm^{-1}$, 1630 $cm^{-1}$, 1552 $cm^{-1}$, and 1238 $cm^{-1}$. Meanwhile, according to Suparno and Prasetyo (2018), collagen extracted using the *hydro-extraction* method has five consecutive peak points at wave numbers 3426.65 $cm^{-1}$, 2924.37 $cm^{-1}$, 1637.13 $cm^{-1}$, 1409.51 $cm^{-1}$, and 1241.01 $cm^{-1}$. The similarity of the peak position of absorption in the results of the study shows that collagen from chicken feet skin extract is in accordance with collagen in general. The existence of these five amide groups indicates that the typical structure of collagen, especially related to peptide bonds and polypeptide chains, where the constituent chains are still well maintained. These FTIR results confirm that the extract meets the qualitative identity criterion for collagen, in that all five characteristic amide bands were detected at wavenumbers consistent with literature values. This qualitative identification is a necessary first step toward use as a pharmaceutical raw material, but is not by itself sufficient: standardization for pharmaceutical use would additionally require quantitative measures such as the degree of deacetylation/purity, molecular weight distribution, and batch-to-batch reproducibility, none of which were assessed in the present study and which we recommend as directions for future work.

## 3. Wound Closure Measurement

Figure 3 presents visual documentation of the healing process of incision wounds in mice during the 12-day observation period. Changes in wound morphology were systematically observed to assess the progress of incision wound closure in mice.

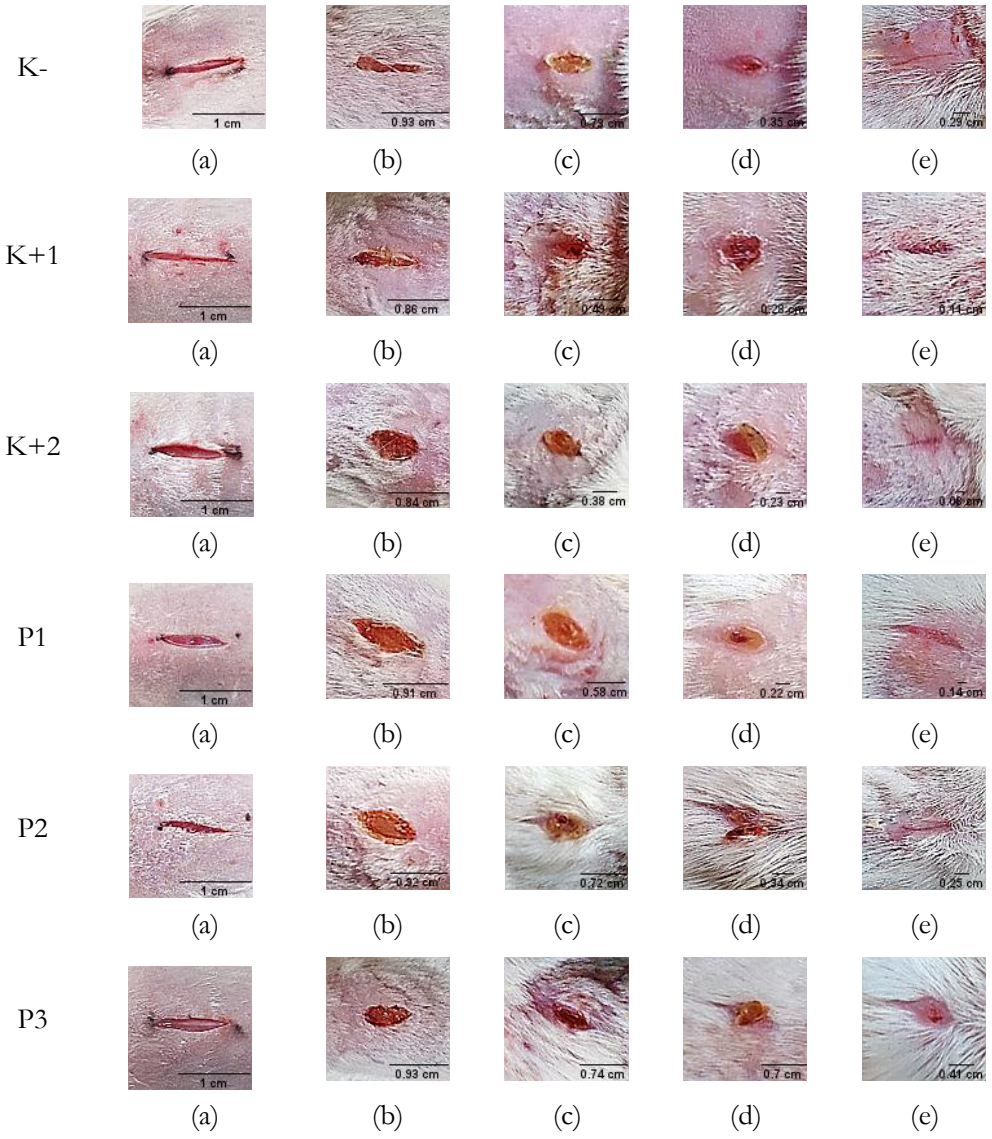


**Figure 3. Observation of Incision Wounds in Mice for 12 Days**

**Information**: (a) Day 0, (b) Day 3, (c) Day 6, (d) Day 9, (e) Day 12. Rows correspond to treatment groups: K- (untreated), K+1 (bioplacenton), K+2 (ointment base), P1 (5% chicken feet collagen ointment), P2 (10% chicken feet collagen ointment), and P3 (15% chicken feet collagen ointment).

Measurements of the closure of the incision wound in mice were carried out every 3 days over a 12-day period, namely on the 3rd, 6th, 9th, and 12th days. The results of the percentage of wound closure were then analyzed using the GraphPad *Prism* 10 and SPSS *Statistics* 22 software presented in Table 2.

**Table 2. Average Incision Wound Closure Percentage in Mice on Days 3, 6, 9, and 12**

| | Average Wound Closure Percentage (%) Day to | | | |
|---|---|---|---|---|
| | **3** | **6** | **9** | **12** |
| K- | $_{1}9{,}00\pm2{,}07^{a}$ | $_{2}46{,}20\pm8{,}61^{a}$ | $_{3}66{,}20\pm7{,}67^{a}$ | $_{4}86{,}80\pm6{,}07^{a}$ |
| K+1 | $_{1}10{,}40\pm2{,}11^{a}$ | $_{2}47{,}60\pm7{,}30^{a}$ | $_{3}81{,}60\pm4{,}81^{a}$ | $_{3}94{,}00\pm3{,}67^{a}$ |
| K+2 | $_{1}15{,}40\pm1{,}91^{a}$ | $_{2}50{,}40\pm6{,}94^{a}$ | $_{3}72{,}00\pm3{,}70^{a}$ | $_{4}93{,}40\pm2{,}85^{a}$ |
| P1 | $_{1}16{,}60\pm2{,}99^{a}$ | $_{2}51{,}20\pm4{,}72^{a}$ | $_{3}83{,}60\pm5{,}76^{a}$ | $_{3}97{,}20\pm2{,}80^{a}$ |
| P2 | $_{1}15{,}00\pm2{,}07^{a}$ | $_{2}49{,}00\pm6{,}41^{a}$ | $_{3}72{,}20\pm4{,}76^{a}$ | $_{4}88{,}20\pm7{,}36^{a}$ |
| P3 | $_{1}14{,}60\pm2{,}40^{a}$ | $_{2}43{,}40\pm6{,}67^{a}$ | $_{3}59{,}60\pm8{,}51^{a}$ | $_{4}85{,}20\pm7{,}51^{a}$ |

**Description**: K- (no treatment), K+1 (bioplacenton), K+2 (ointment base), P1 (chicken feet collagen ointment 5%), P2 (chicken feet collagen ointment 10%), and P3 (chicken feet collagen ointment 15%). The mean±SE value followed by the same superscript letter (a) in the same column indicates no significant difference ($p<0.05$), while different subscript numbers (1, 2, 3, 4) on the same line indicate a significant difference ($p<0.05$).

Based on the results of the observation of the average percentage of incision closure in mice (Table 2), it shows that the entire group of mice gradually increased the percentage of wound closure from day 0 to day 12. This indicates that the wound healing process runs gradually in the control group mice (K-, K+1, K+2) and treatment group mice (P1, P2, P3). In mice in the negative control group (K-), who were not given any treatment, there was a gradual increase in the percentage of wound closure. The increase occurred significantly from day 3 to day 12 (Figure 3 Group K-). A progressive increase in the percentage of incision wound closure (Table 3) in the test animals showed that although healing occurred naturally, the average wound closure in mice of the negative control group (K-) on day 3 did not differ significantly compared to the rest of the treatment group. This is in line with Gouletsou *et al.* (2024) which states that mice in the negative control group have an average healing speed that is not significantly different from mice in the treatment group, but when compared to the average value of wound length in the negative control group is still higher than that of the treatment group.

Mice of the K+1 group (given bioplacenton) showed a good healing process, in which there was an increase in the percentage of wound closure (Table 2). Based on the data obtained on days 3 to 12, it showed no significant differences between the K+1 (given bioplacenton) and K- (no treatment) groups. Nonetheless, biologically, it appears that bioplacenton provides progress in healing incision wounds during the 12-day observation period (Figure 3 Group K+1). This event is likely caused by the active ingredient bioplacenton (*neomycin sulfate*), which at some stage of wound healing can prolong the inflammatory phase before entering the proliferation stage, thus slightly delaying the time of wound closure. This is in line with research conducted by Sukmawan *et al.* (2021), which stated that the percentage of incision wound closure in mice in the negative control group and mice in the positive control group (bioplacenton administration) did not show a significant difference. The incident was further explained by Jensen *et al.* (2025) which states that *neomycin* contained in bioplacenton has the potential to cause allergies, so it can affect the speed of wound healing.

In the test animal group treated with ointment-based (K+2), there was a significant increase in the percentage of wound closure (Table 2) from day 3 to day 12 (Figure 3 Group K+2). The results of the analysis between treatments on day 3 showed that the average value of the incision wound closure percentage was not significantly different from that of the negative group (K-) mice. However, the mean value of the incision-wound closure percentage showed a tendency that the cut-off group (K+2) mice had a higher wound closure rate and showed significantly different results, when compared to the average 3rd day wound closure percentage of the K+1 group mice. This indicates that the ointment base, which consists mostly of lanolin, may have a positive effect on the wound healing process, although the results are not statistically significant compared to the untreated group. This is in line with the results of research by Al-Zubaidy and Naqi (2022), who stated that lanolin, as one of the components of ointment preparations, has the ability to protect the skin, reduce inflammation, and improve the skin's protective layer. Lanolin can lock in moisture and reduce excess water loss, as well as have a reconstructive effect on damaged skin tissue.

The wound healing response in the mice treated with 5% and 10% chicken feet collagen ointment (P1 and P2), showed fairly stable dynamics throughout the 12-day observation period. Based on the results obtained, it can be seen that there was an increase in the percentage value of wound closure (Table 2) from day 3 to day 12 (Figure 3, Groups P1 and P2). However, comparisons between treatment groups on days 3 and 9 showed that the scores obtained did not differ significantly, either between P1 and P2 mice versus P3 group mice or compared to control groups (K-, K+1, K+2). Meanwhile, observations on day 12 (near the end of the wound closure phase) showed an average value of the percentage of incision closure that did not differ significantly between all groups of test animals. This is in line with the results of the research of Wardani *et al.* (2017), which stated that the value of the percentage of wound closure throughout the 12-day observation period did not show a significant difference when compared to the entire mouse treatment group. This is likely caused by the condition of the wound that has dried, then formed a scab and covered the tissue underneath. The scab that forms can affect the measurement results macroscopically. In addition,

the use of chicken feet collagen ointment at concentrations of 5% and 10% may still not be optimal enough to cause a noticeable healing effect, although not significant, the average percentage of incision wound closure on day 12 of the P1 group is still higher than that of the K+1 group who use commercial ointment (bioplacenton). Similar results were reported by Pratiwi *et al.* (2020), who found that the administration of fish collagen hydrolysate ointment at concentrations of 5% and 7.5% still did not provide optimal effects when compared to the positive control group. This can be seen based on the expression of the resulting wound-healing agent (FGF-2), where between the negative control and treatment groups showed no significant differences.

The group of mice treated with 15% (P3) concentration of chicken feet collagen ointment, at the observation of day 3 showed a relatively comparable wound healing pattern to the K- group mice and the entire treatment group (P1 and P2), as seen through (Table 2). Meanwhile, observations on day 9 showed that the average value of the percentage of wound closure of the P3 group showed no significant difference from the K+2 group (ointment base), but showed the average value of the smallest wound closure percentage. This makes the P3 group mice have the slowest wound healing rate, when compared to the entire test animal group (Figure 3 P3 Group). However, based on the analysis of each treatment during the 12-day observation period, there was an increase in the percentage of wound closure from day 3 to day 12. This phenomenon indicates that increasing collagen concentration to 15% is still not able to provide a noticeable difference in the effect on wound healing speed. The higher concentration is not proportional to the effectiveness of the ointment in the wound healing process. This is in line with the results of Nugroho *et al.* (2022), which states that tilapia peel collagen ointment at a concentration of 15% still does not provide optimal effects in wound healing. The results showed that the observed wound length was not significantly different when compared to the control group, so the higher the concentration did not always have a good effect. This is further explained by Mathew-Steiner *et al.* (2021), which states that collagen can accelerate the inflammatory process through the regulation of MMPs, but in some conditions such as excess collagen from the outside, it can potentially prolong the inflammatory phase and inhibit the wound healing process.

## 4. Measurement of Protein and Hydroxyproline Levels

Measurement of protein levels and hydroxyproline levels of mouse skin tissue was carried out on day 12. The average data was analyzed using the GraphPad *Prism* 10 and SPSS *Statistics* 22 applications, so that the results seen in Table 3 were obtained.

**Table 3. Average Protein and Hydroxyproline Levels**

| Groups | Protein (µg/mL) | Up to Hydroxyproline (µg/mL) |
|---|---|---|
| K- | 759,33±30,97$^{c}$ | 310,09±72,22$^{b}$ |
| K+1 | 422,67±69,13$^{b}$ | 137,71±14,31$^{a}$ |
| K+2 | 238,78±49,08$^{ab}$ | 136,76±10,73$^{a}$ |
| P1 | 149,89±10,55$^{a}$ | 103,91±15,53$^{a}$ |
| P2 | 301,55±30,08$^{ab}$ | 303,90±26,16$^{b}$ |
| P3 | 265,45±45,72$^{ab}$ | 364,38±26,51$^{b}$ |

**Description**: K- (no treatment), K+1 (bioplacenton), K+2 (ointment base), P1 (chicken feet collagen ointment 5%), P2 (chicken feet collagen ointment 10%), and P3 (chicken feet collagen ointment 15%). The mean±SE value followed by different superscript letters (a, b, c) in the same column indicates a significant difference ($p<0.05$).

Based on the results of the analysis of the measurement of protein content (Table 3), it was found that mice in the control group included the K- group with a protein content of 759.33±30.97 µg/mL, the K+1 group of 422.67±69.13 µg/mL, and the K+2 group of 238.78±49.08 µg/mL. Meanwhile, the protein levels of mice in the treatment group using chicken feet collagen ointment were at a concentration of 5% to 15%, including the P1 group of 149.89±10.55 µg/mL, the P2 group of 301.55±30.08 µg/mL, and the P3 group of 265.45±45.72 µg/mL. This shows that the K- group mice are the group with the highest protein levels, while the P1 group mice have the lowest protein levels. Significant differences in protein levels between the two groups showed a variation in metabolic activity and tissue regeneration processes between the groups of mice. High levels of K-group mouse protein are likely to occur because the final phase of proliferation or the beginning *of remodeling* is still ongoing, fibroblast activity and collagen structural protein synthesis are

still relatively high. In contrast, P1 group mice that exhibit lower protein levels are likely to occur because the wound healing process has entered a remodeling (maturation) phase accompanied by decreased protein synthesis activity and increased extracellular matrix reorganization. This is in line with the findings of Wang *et al.* (2022), which states that in the transition phase of proliferation to *remodeling*, protein levels in tissues will tend to increase. This happens because proteins in the extracellular matrix tissue are used to create injured or damaged tissue structures. However, after the 8th day, the level will tend to stop increasing after entering the maturation stage. In addition, based on these data, mice in groups K+2, P2, and P3 had protein levels that did not differ significantly, indicating that the treatment in the three groups of mice had a relatively similar effect on the protein levels of skin tissue. This may indicate that after treatment, tissue protein levels tend to reach a stable state because they have entered the tissue recovery phase (*remodeling*). This condition is further described by Gonzalez *et al.* (2016), which states that during the process of maturation and *remodeling*, fibroblasts disappear from the wound area due to the process of emigration and cell death, thus causing scarring with reduced cells. This can affect protein levels in skin tissue.

Based on the results of the measurement analysis of hydroxyproline levels (Table 3), it was known that mice in the control group included the K- group with hydroxyproline levels of 310.09±72.22 µg/mL, the K+1 group of 137.71±14.3 µg/mL, and the K+2 group of 136.76±10.73 µg/mL. Meanwhile, the hydroxyproline levels in mice in the treatment group were given chicken feet collagen ointment at a concentration of 5% to 15%, including the P1 group of 103.91±15.53 µg/mL, the P2 group of 303.90±26.16 µg/mL, and the P3 group of 364.38±26.51 µg/mL. This showed that the P3 group mice (15% chicken feet collagen ointment) had the highest average levels of hydroxyproline, followed by P2 group mice (10% chicken feet collagen ointment), and K- group mice (no treatment). High levels of hydroxyproline in P3, P2, and K- group mice reflect an ongoing increase in collagen synthesis. This suggests that the wound healing process is likely still in the tissue proliferation phase. This is further explained by Kumar *et al.* (2016), which states that the condition of injured skin will result in damage to skin tissue, especially to the structure of collagen. This causes collagen to release free hydroxyproline, which can then be used as one of the parameters to measure the healing process in the skin. When hydroxyproline levels increase, the condition of the tissue is still affected, but the amount will tend to decrease along with the process of repairing the collagen structure during the *remodeling* and maturation stages. In this case, hydroxyproline levels in mice in groups P1, K+1, and K+2 reflect the wound healing process that may be in the transition phase towards the formation of a *collagen triple-helix* structure in post-wound tissue. The comparatively low hydroxyproline level in the K+1 (bioplacenton) group is consistent with this interpretation: because bioplacenton is a fast-acting commercial wound agent, healing in this group may have progressed further into the remodeling stage by day 12, at which point collagen turnover – and therefore free hydroxyproline release – has already begun to subside, rather than indicating a failure of the positive control to support healing. The administration of 5% chicken feet collagen ointment, bioplacenton, and ointment base was proven to have a relatively similar effect on the hydroxyproline levels of mouse skin tissue.

## Conclusion

Based on the research that has been conducted, the yield value of the extraction can be obtained, namely a wet yield of 10% (100 g) and a dry yield of 4.6% (19 g). In addition, the results of FTIR analysis of chicken feet extract showed the presence of a typical collagen functional group characterized by the appearance of amide bands A, B, I, II, and III. The results of the analysis can be concluded that the administration of chicken feet collagen ointment with concentrations of 5%, 10%, and 15% does not provide a noticeable effect macroscopically. However, based on protein and hydroxyproline levels, the P1 group (5%) showed the lowest tissue protein level together with a hydroxyproline level comparable to the positive controls, consistent with a healing process that had progressed further toward the remodeling and maturation phase. In contrast, the P2 and P3 groups (10% and 15%) showed protein levels not significantly different from the ointment-base control, together with hydroxyproline levels comparable to the untreated negative control, suggesting that healing in these groups remained in a more active, collagen-synthesising phase at the time of sampling. This indicates that ointment concentration influenced the timing of the transition between the proliferative and remodeling phases of wound healing rather than producing a uniform, dose-dependent effect on tissue protein and hydroxyproline levels.

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**Generative AI:** The author uses generative artificial intelligence tools (*ChatGPT* and *GitHub Copilot*) to help improve language quality. All content has been reviewed and re-adjusted by the author to ensure scientific accuracy. The author is solely responsible for the final version of the manuscript.

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