TITLE PAGE
- Korean Journal for Food Science of Animal Resources -
Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Effects of pig skin collagen supplementation on broiler breast meat
Running Title (within 10 words)	Pig skin collagen supplementation for improved broiler breast meat
Author	Sanghun Park, Yun-a Kim, Sanghun Lee, Yunhwan Park, Nahee Kim, Jungseok Choi
Affiliation	1 Department of Animal Science, Chungbuk National University, Cheongju 28644, Korea, Republic of
Special remarks – if authors have additional information to inform the editorial office	
ORCID (All authors must have ORCID) https://orcid.org	Sanghun Park (https://orcid.org/0000-0003-4804-0848) Yun-a Kim (https://orcid.org/0000-0002-5505-030X) Sanghun Lee (https://orcid.org/0000-0001-5632-5064) Yunhwan Park (https://orcid.org/0000-0002-2239-6697) Nahee Kim (https://orcid.org/0000-0002-2041-6893) Jungseok Choi (https://orcid.org/0000-0001-8033-0410)
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This work was supported by a grant (715003-07) from the Research Center for Production Management and Technical Development for High Quality Livestock Products through Agriculture, Food and Rural Affairs Convergence Technologies Program for Educating Creative Global Leader, Ministry of Agriculture, Food and Rural Affairs. This work also was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education (No. 2020R1A4A1017552, 2020R1G1A1006498).
Author contributions (This field may be published.)	Conceptualization: Sanghun Park, Jungseok Choi Data curation: Sanghun Park, Yun-A Kim Formal analysis: Sanghun Park, Sanghun Lee, Nahee Kim Methodology: Sanghun Park Software: Yunhwan Park Validation: Nahee Kim Investigation: Jungseok Choi Writing - original draft: Sanghun Park, Yun-A Kim, Jungseok Choi Writing - review & editing: Sanghun Park, Yun-a Kim, Sanghun Lee, Yunhwan Park, Nahee Kim, Jungseok Choi
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Choi, Jungseok
Email address – this is where your proofs will be sent	jchoi@chungbuk.ac.kr
Secondary Email address	

Postal address	Department of Animal Science, Chungbuk National University, Cheonju, Chungcheongbuk-do Korea, 28644
Cell phone number	+82-10-3235-2127
Office phone number	+82-43-261-2551
Fax number	+82-43-261-2773

Abstract

This study aimed to enhance the quality of broiler breast meat by adding pig skin collagen to feed. A total of 50 Ross 308 broilers were classified according to the following feeding regime for two weeks: basal diet (NC), basal diet + 0.1% fish collagen (PC), basal diet + 0.1% pig skin collagen (T1), basal diet + 0.5% pig skin collagen (T2), and basal diet + 1.0% pig skin collagen (T3). The moisture content was the highest in the PC group, and the protein content was the lowest in the T1 group ($p < 0.05$). The fat content was higher in the T1 and PC groups, whereas the ash content was higher in the T3 group ($p < 0.05$). Drip loss was the highest in the NC group and the lowest in the T2 group ($p < 0.05$). Lightness was low in groups T2 and T3, redness was low in groups T2 and PC, and yellowness was low in groups T1, T2, and PC ($p < 0.05$). The collagen content of the chicken breast was the highest in the T3 group, and that of the skin was the highest in the T1 group ($p < 0.05$). The tissue characteristics of springiness, cohesiveness, chewiness, and hardness were the highest in the T3 group ($p < 0.05$). In conclusion, the supplementation of a broiler diet with pig skin collagen was found to increase the collagen content of the breast meat, indicating the improved quality of the broiler breast meat.

Keywords: broiler meat, pig skin, collagen, drip loss, tissue characteristics

Introduction

Meat and meat products play an important role in the human diet as they provide proteins and essential amino acids that are difficult to obtain from vegetables and fruits (Kushi et al., 2006). In particular, poultry meat is preferred by consumers owing to its nutritional value as well as cost (Stanciu, 2020). The average poultry meat consumption of the Organization for Economic Cooperation and Development (OECD) member countries was 31.7 kg/capita in 2020, which reflects an increase of approximately 5 kg from 26.57 kg/capita in 2010. In contrast, the world's average poultry meat consumption was 14.88 kg/capita in 2020, compared to approximately 12.73 kg/capita in 2010. The consumption of poultry meat in the United States was 50.1 kg/capita in 2020, whereas it was 14 kg/capita in China, and 18.7 kg/capita in Korea (OECD meat consumption, 2020). Poultry production and consumption have increased significantly over the past 40 – 50 years, and are expected to increase further, especially in developing countries, making chicken the most valuable source of meat protein for the growing world population (Baldi et al., 2020; OECD-FAO, 2020). In a survey of 1,100 adult men and women aged between 20 and 69 years old in Korea, the annual consumption of chicken was found to have increased due to easy delivery and online purchases, despite the challenges introduced by COVID-19. When purchasing chicken meat at home, the first criterion was freshness (63.6%), followed by price (39.9%), meat quality (36.9%), and expiration date (29.1%). In addition, the most common improvement for promoting chicken consumption was providing grade-based information (83.7%) and soft-textured meat (93.5%) (Rural Development Administration, 2020). The livestock industry focuses on health-oriented livestock products. In particular, meat processing products were developed to meet the requirements of modern consumers who prioritize improved living standards, diet, and food safety (Hafez and Attia, 2020). Therefore,

chicken products have been improved in various ways, such as changing the cooking methods and developing partial meat products (Kook et al., 2020).

Most waste in the meat industry is generated during slaughtering, which raises concerns for environmental protection and sustainability (Russ and Meyer-Pittroff, 2004). The slaughter by-products include bones, tendons, skin, gastrointestinal contents, blood, and internal organs (Grosse, 1984). These can be generally utilized as food or reprocessed as secondary by-products in agriculture and industry (Liu, 2002). Many studies have been conducted to utilize the slaughter by-products (Jayathilakan et al., 2012; Min et al., 2017; Mirzapour-Kouhdasht et al., 2020). Collagen is an animal protein that accounts for approximately 30% of the animal protein and can be easily obtained from livestock, including fish, pigs, and chicken (Aberoumand, 2012; Cheng et al., 2009; Huo and Zheng, 2009; Muyonga et al., 2004; Pataridis et al., 2008). It provides strength and support to the skin, bone, cartilage, tendon, and blood vessels, and plays a major role in the extracellular matrix (Erdmann et al., 2008; Mendis et al., 2005; Ngo et al., 2011). Collagen forms a scaffold for tissue treatment and wound healing and is used in applications related to drug delivery systems in the urology, biotechnology, and medical fields (Nune et al., 2017). In the food industry, collagen is used in protein supplements, fragrances, gelling agents, emulsifiers, and additives aimed at improving texture (Nazeer and Kumar, 2012). The ingredients in poultry feed are high in methionine and lysine and should be a source of energy and minerals. Collagen is high in methionine (6%) and lysine (19%), so it plays an important role as a feed supplement in the poultry industry (Nazeer et al., 2011). Proper protein feed is important for broiler growth and meat quality (Beski et al., 2015). Many studies have focused on improving the meat quality of chicken through feed supplementation using natural products and by-products, such as collagen (Choi, 2005; Park et al., 2005; Woo et al., 2007). Supplementation with hydrolyzed collagen is known to have a positive impact on tibial dimensions, strength, and the mineral content of broilers (Güz et al., 2019). Collagen

feeding can play an important role in increasing the muscle content of the broiler, and the collagen component proline can provide a higher initial growth rate than other amino acids, and thereby is a necessary ingredient. Therefore, collagen-related studies have focused on extracting collagen from by-products produced by the livestock and fish industries and using it as an effective supplement for alternative feed (Nurubhasha et al., 2019). The current study aimed to improve the availability of pig skin, which is a by-product of the pig industry, to enhance the quality of broiler breast meat.

Materials and Methods

Pig skin collagen extract

Six liters of distilled water and 3 kg of pig skin were put into an electronic pressure extractor (KS 220S, Kyungseo E&P, Korea) at 80 ℃ for 5 h. After heating, the insoluble collagen was strained through the gauze. The collagen extract was hydrolyzed at 60 ℃ for 5 h using protease (Love Me Tender, H GROUP USA LLC, USA) and concentrated at 80 ℃ for 12 h. The collagen extract was cooled at room temperature for 20 minutes and filtered to 9,450 daltons using a 10,000-dalton filter (Multi-Angle Light Scattering, Korea Basic Science Institute, Korea). The final collagen extracted from pig skin was stored at 4 ℃ for 24 hours and used for feed additive.

Experimental design and animals

Fifty Ross 308 broilers, two weeks old with an average weight of 322 ± 0.3 g, were housed for two weeks before the experiment. The experiment was conducted with five random, completely blocked treatments, classified into the following groups: the negative control (basal diet, NC), positive control (basal diet + 0.1% fish collagen (fish collagen premium power, Graviola House,

Korea), PC), T1 (basal diet + 0.1% pig skin collagen), T2 (basal diet + 0.5% pig skin collagen), and T3 (basal diet + 1.0% pig skin collagen) groups. The basal diet met or exceeded the National Research Council (NRC) (1994) requirements. All broilers were allowed to consume both feed and water ad libitum. After completing the two-week feeding regime, the tibial bone size, weight and skin thickness of the broilers were measured and the left breast was harvested for meat quality analysis.

Analysis method

In this study, five replicates of the same treatment were prepared and analyzed. The average value was considered the result.

General components

Moisture, fat, protein, and ash (%) were measured in accordance with the Association of Official Agricultural Chemists method (AOAC) (2012).

pH

pH was measured after adding 50 ml of distilled water to 5 g of breast meat sample. All samples were homogenized at 230 rpm for 30 s using a homogenizer (Stomacher®400 Circulator, Seward, UK) and the pH was measured using a pH meter (Mettler Toledo Delta 340, Mettler-Toledo, Ltd, UK).

Water-holding capacity

The water-holding capacity was estimated by the centrifugation method as reported by Lakkonen et al. (1970). The crushed sample (0.5 ± 0.05 g) was placed in the upper filter tube of the centrifuge tube, heated in an 80 ℃ water bath for 20 min, and cooled thereafter for 10

min. The centrifuge tube centrifuged for 10 min at 2000 rpm. It is displayed as the ratio of the remaining sample weight / the sample weight before heating

Tissue characteristics and shear force

To measure the texture characteristics of the sample, the mastication test, shear, and cutting tests were performed using a rheometer (COMPAC-100, Sun Scientific Co., Japan), and the sample was placed at right angles to the direction of the muscle fibers in a 3cm-thick steak shape. The muscle was sheared and heated to an internal temperature of 70 ℃, and then allowed to cool under running water for 30 min. From the cooled sample, a 1-cm-diameter core was drilled in a cylindrical shape along the muscle fiber direction to collect the sample and then cut in the direction perpendicular to the muscle fiber using a rheometer (Compac-100, Sun Scientific Co., Japan) to measure the shear force. The measurement was repeated three or more times.

Drip loss

After shaping a 2-cm-thick sample into a circle (100 ± 5 g), it was put in a polypropylene bag and vacuum packed. The amount of drip loss generated during storage in a refrigerator at 4 ℃ for 24 h was measured as the weight ratio (%) of the initial sample.

Cooking loss

After shaping a 3-cm-thick sample into a circle (150 ± 5 g), it was put into a polypropylene bag, vacuum packed, and heated in a 70 ℃ water bath for 40 min, followed by cooling for 30 min. The weight lost after heating was measured as the weight ratio (%) of the first sample.

Hunter color measurement

The surface color of the breast meat was measured using a spectrophotometer (Model JX-777, Color Techno. System Co., Japan) standardized with a white plate (CIE L*, 94.04; CIE a*, 0.13; CIE b*, -0.51), with the CIE L* value on a HunterLab color system representing lightness, the CIE a* value representing redness, and the CIE b* value representing yellowness using a white fluorescent lamp (D65).

Collagen measurement

Approximately 4 g of sample was placed in an Erlenmeyer flask, and 30 ml of sulfuric acid solution was added to the sample. The flask was covered with a lid and heated in a dry oven at 105 ℃ for 16 h. The contents of the flask were transferred to a 500 ml volumetric flask, diluted with tertiary distilled water, and homogenized. A Whatman No. 2 150 mm filter was used to filter the sample. The filtrate (5 ml) was diluted to 100 ml, and 2 ml of the diluted sample was added to a test tube, to which 1 ml of oxidant solution was added, shaken, and finally left at 20 ℃ to 25 ℃ for 20 min. Next, 1 ml of a color reagent was added to each test tube, mixed, and these samples were incubated in a 60 ℃ water bath for 15 min, cooled under flowing water for 3 min or more, and finally, the absorbance was measured at a fixed wavelength of 558 nm using a spectrophotometer. For preparation of the standard curve, 2 ml of the working standard solution was taken and its absorbance measured based on color development. The collagen content (g/100 g) was analyzed by substituting the sample absorbance into the regression equation (Kolar, 1990).

Measurement of tibial bone size, weight, and skin thickness

The left tibia bones of each broiler were removed as drumsticks with the flesh intact. The drumsticks were immersed in boiling water (100 ℃) for 10 min. After cooling to room temperature, the drumsticks were defleshed by hand and the patella was removed. Then, they

were dried for 24 h at room temperature. The tibial length and bone weight were measured. Skin thickness was measured immediately after slaughter.

Fatty acid analysis

The sample (50 g) was homogenized in 250mL of chloroform:methanol (2:1) solution at 3000 ppm according to the method of Folch et al. (1957). The lipids were extracted from the homogeneous solution and sodium anhydrous sulfate was used to remove moisture from the liquid from which the lipids were extracted and the filtrate was concentrated at 50 – 55 ℃. One milliliter of tricosanic acid was added first, followed by 1 ml of 0.5 N NaOH. The sample was heated at 100 ℃ for 20 min, cooled for 30 min, and 2 ml of BF3 was added, heated for 20 min, and finally cooled for 30 min. After adding heptane and 4 ml of NaCl, the supernatant was removed and injected into a gas chromatograph to measure the fatty acid content.

Analysis of free amino acids

Amino acid analysis was conducted according to the standard analysis method proposed by the Livestock Technology Research Institute in 2000 using an amino acid analyzer. Briefly, 100 mg of sample (approximately 30 mg of crude protein) was placed in a decomposition bottle, and 40 ml of 6 N HCl was added, followed by the injection of nitrogen gas. It was placed in an evaporating flask, connected to a rotating evaporator, and the hydrochloric acid was removed at 50 ℃. When the evaporation was completed, the distilled water bottle was washed with distilled water, the contents transferred to the evaporative flask, and evaporation was conducted thrice. A small amount of buffer solution (pH 2.2) or distilled water was added to the final evaporative flask to dissolve the amino acids, and the sample was then filtered through No. 5B filter paper, and the volume was made up to 50 ml. This sample was used as the amino acid analyzer specimen and its absorbance was measured at 570 nm.

Statistical analysis

Statistical analysis was conducted using the General Linear Model (GLM) procedure of the SAS program (Statistical Analysis System 2002, USA), and the significance of the comparisons between the means of the treatment groups was verified ($p < 0.05$) using Duncan's multiple range test.

Results and Discussion

General composition of breast meat from pig skin collagen-fed broilers

Table 1 shows the general composition of the breast meat from pig skin collagen-fed broilers. While the moisture content was the highest in the PC (74.33%) group, it was the lowest in the T3 group (72.94%). There was no significant difference between the NC, T1, and T2 groups in terms of moisture content. The protein content was significantly lower in the T1 group than in the NC group, and the PC group showed significantly low protein content. Previous studies reported that the growth of salmon was reduced upon replacing much of the fish protein concentrate fed to the fish with gelatin (Mundheim et al., 2004; Opstvedt et al., 2003). Studies have also shown that increasing the supply of bovine skin gelatin reduced the rate of protein digested from the meat and that the protein source affected the flow of endogenous amino acids into the intestines (Rutherfurd et al., 2015). According to research on the performance of broilers—which were provided feed in which a portion of soymeal was replaced by cow skin gelatin—the weight of the broilers decreased as the supply of cow skin gelatin gradually increased (Rutherfurd et al., 2015). These studies suggested that collagen supplementation may reduce the amino acid digestion rate of broilers, thus reducing the protein content in broiler

breast meat. As shown in Table 1, groups T1 and PC had significantly higher levels of fat than the groups given other treatments. The fatty acid biosynthesis of algae depends upon the supply of dietary carbohydrates for acetyl-CoA production, and when such carbohydrates are ingested, insulin stimulation results in the increased activity of fatty acid synthase (FAS) and malate dehydrogenase (MD) (Hillgartner et al., 1995). Most of the fat present in poultry comes from carbohydrates, and glycolysis and NADPH are essential for the synthesis of fatty acids in the cytoplasm. Therefore, fatty acid synthesis depends largely upon the supply of carbohydrates from feed and the activity of the glycolytic system (Moon, 2018). The significantly higher fat content in the PC group can be attributed to the carbohydrate content of the feed and is not thought to be affected by collagen. The PC group also has a relatively high fat content owing to its low protein content. Table 2 shows that the ash content significantly increased with increases in the collagen levels. Feeding calf bone-extracted gelatin to early breeding chicks, with high bone hemostatic capability (in 14 days) significantly improved the tibial ash, calcium, and phosphorus content in the chicks. Considering that gelatin can improve bone strength by promoting the mineralization of cartilage sheets (Kim et al., 2017), the feeding of pig skin collagen can be assumed to increase the ash content in broiler breast meat.

Quality characteristics of breast meat from pig skin collagen-fed broilers

Table 2 shows the quality characteristics of the broiler breast meat. The NC group showed significantly higher drip loss than the other treatments, whereas T2 showed the lowest. There was no significant difference between cooking loss and the CIE L* of Hunter color. However, the CIE a* of the T2 and PC groups was significantly lower than that of the other treatments, whereas the CIE b* of the T1 group was the lowest. The collagen content of the skin was significantly higher in the T1 group than in other treatment groups and was the lowest in the T3 group. The drip loss, cooking loss, water-holding capacity, and color of the breast meat are

known to be related to pH (Berri et al., 2008; Fletcher, 1995; Mir et al., 2017). However, since there was no significant difference in pH, an association between drip loss, cooking loss, water-holding capacity, and color due to the difference in pH could not be determined. Increases in the water-holding capacity were reported to improve tenderness, juiciness, firmness, and appearance (Offer, 1988). Therefore, since there was no significant difference in the water-holding capacity of the broiler breast meat, it is judged that there was no significant difference in the shear force. A previous study reported a positive correlation between drip loss and time in storage, suggesting that the oxidative processes occurring in both the lipid and protein fractions during storage may alter the water-holding capacity (Lonergan et al., 2001). However, since there was no significant difference in drip loss and water-holding capacity in this study, the relationship between drip loss and water-holding capacity could not be determined. In this study, the collagen content of the broiler breast meat was found to increase with the collagen content of the feed, and the T3 group showed a significantly higher content than the NC group. Preceding studies reported that the human consumption of collagen extracted from pig skin and chicken feet was likely to affect the proliferation of fibroblasts and the formation of collagen fibroblasts in collagen-specific ways (Iwai et al., 2005). The results of the previous study may be related to the difference in the collagen content of the breast meat of broilers fed pig skin collagen in this study.

Tissue characteristics of breast meat from pig skin collagen-fed broilers

Table 3 shows the tissue characteristics of broiler breast meat. Springiness and cohesiveness were significantly higher in all treatment groups relative to those in the NC group, and chewiness was significantly higher in the T3 group than in the other groups. Hardness was significantly higher in the following order: T3, PC, T2, and T1. There was no significant difference in shear force between the treatments. In a previous study, pigs that orally ingested

feed with collagen peptides were shown to have a higher fibroblast density in the dermis, larger diameter and density of collagen fibrils, and a larger area of collagen than those that ingested the basic diet and feed with lactalbumin (Matsuda et al., 2006). Small-diameter collagen fibrils are mechanically weaker than thicker ones (Parry et al., 1978), and collagen fibrils are denser due to the proliferation of fibroblasts in the dermis (Yamamoto et al., 2002). Collagen has been proven to play a key role in determining the meat strength in various livestock, including birds (Sirri et al., 2016). Broilers that ingested pig skin may be expected to have a greater diameter and higher density of collagen fibrils in the dermis than those in the NC group. As the pig skin-fed broilers had harder meat due to the high density of fibroblasts in the dermis, springiness, cohesiveness, and chewiness of the meat may also be affected.

Bone length, weight, and skin thickness of the breast meat from pig skin collagen-fed broilers

Table 4 shows the bone length, weight, and skin thickness of broiler breast meat. No significant difference between the treatments was seen (Table 4). The bone weight was the heaviest in the T1 group and tended to decrease with the addition of collagen extract. Although there was no significant difference in the four treatment groups, the bone length tended to increase in the T1 and T2 groups compared to the NC group and tended to decrease in the T3 group. The addition of gelatin extracted from calf bones to broiler feed is known to result in longer broiler tibiae than those in the NC group (Beyranvand et al., 2019). In animal experiments, the intake of hydrolyzed collagen by livestock was reported to improve bone density and bone mineral content (Wu et al., 2004). The amount of type I collagen in bone substrate increased when mice consumed hydrolyzed collagen (Nomura et al., 2005). Research has shown that the consumption of alkali and acid-treated bone gelatin enhanced the surface area of the small intestine, and consequently improved bone properties and the intestinal absorption of

phosphorus (Van Harn et al., 2017). There are report that gelatin intake promotes bone-forming cell differentiation and bone regeneration in broilers and that gelatin can improve bone strength by promoting the mineralization of cartilage sheets (Kim et al., 2017). The results of previous studies show that collagen intake and bone are related, but no significant difference was observed in this research. Compared to the NC group, there was no significant difference in skin thickness between the T1 and T2 groups, although it was significantly lower in the T3 group. The results of a previous experiment showed that the thickness of the dermis in pigs fed collagen peptide was similar to that of pigs fed lactalbumin group and the NC group (Matsuda et al., 2006).

Analysis of fatty acids and amino acids in breast meat from pig skin collagen-fed broilers

Table 5 shows the fatty acid analysis of the broiler breasts. No significant difference was observed overall. The γ-linoleic acid (C18:3n6) content was the highest in the NC group (at 16.38%) and tended to decrease as the extract content in the treatments increased. The γ-linoleic acid (C18:3n6) content in the PC group was 14.80% and it was the lowest level in the T3 group at 13.59%. Feeding pigs with diets containing conjugated linoleic acid has been reported to significantly increase the L* and a* values of pig tenderloin, although it did not increase the b* value (Dunshea et al., 2005). As shown in Table 5, the γ-linoleic acid (C18:3n6) content of NC and T1 was high, and as shown in Table 2, the L* and a* values of the NC and T1 groups were high. The results of this study are similar to those reported in previous studies. The L* and a* values were attributed to the γ-linoleic acid content (C18:3n6). Table 6 shows the amino acid analysis of broiler breast meat. While there was no significant difference in the overall amino acid content, lysine, histidine, and arginine showed lower levels in the T1, T2, and T3 groups than in the NC group.

Conclusions

This study was conducted to investigate the quality of broiler breast meat after feeding broilers pig skin collagen, a by-product of the pig farming industry, and to upgrade broiler breast meat to meet the consumer requirements. The protein content was highest in the NC and T3 groups ($p < 0.05$). Drip loss was the highest in the negative control ($p < 0.05$). As the pig skin collagen content increased in broiler feed, the collagen content, springiness, cohesiveness, chewiness, and hardness also gradually increased ($p < 0.05$). In terms of Hunter color, CIE L* and the CIE a* value were high in the NC and T1 groups ($p < 0.05$), and the CIE b* was low in the T1 group ($p < 0.05$). Bone weight was the highest in the T1 group, bone length was the shortest in the T3 group, and the skin also was the thinnest in the T3 group ($p < 0.05$). Therefore, the breast meat of pig skin collagen-fed broilers was of better quality than that of the NC group. Among them, T3 was considered to have the most improved broiler breast meat quality because it had the highest collagen content, best tissue characteristics, and the lowest drip loss, despite the disadvantage of poor skin thickness. Research is needed to offset the disadvantages of poor skin thickness for higher meat quality.

Acknowledgements

References

Aberoumand A. 2012. Comparative study between different methods of collagen extraction from fish and its properties. World Appl Sci J, 16(3), 316-319.

AOAC. 2012. Official Methods of Analysis of AOAC International. 19th ed. AOAC Internationl. Gaithersburg, MD, USA.

Baldi G, Soglia F, Petracci M. 2020. Current status of poultry meat abnormalities. Meat Muscle Biolog, 4(2).

Berri C, Besnard J, Relandeau C. 2008. Increasing dietary lysine increases final pH and decreases drip loss of broiler breast meat. Poult Sci, 87(3), 480-484.

Beski SS, Swick RA, Iji PA. 2015. Specialized protein products in broiler chicken nutrition: A review. Anim Nutr, 1(2), 47-53.

Beyranvand F, Khalaji S, Zamani A, Manafi M. 2019. Effects of gelatin prepared from calf bones rich in phosphorus on broiler performance, bone characteristics and digestive enzymes activity. Br Poult Sci, 60(1), 31-38.

Cheng FY, Hsu FW, Chang HS, Lin LC, Sakata R. 2009. Effect of different acids on the extraction of pepsin-solubilised collagen containing melanin from silky fowl feet. Food Chem, 113(2), 563-567.

Choi I. 2005. Effects of dietary supplementation of loess on the performance and meat quality of broiler chicks. Korean J Poult Sci, 32(1), 1-7.

Dunshea F, Dsouza D, Pethick D, Harper G, Warner R. 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci, 71(1), 8-38.

Erdmann K, Cheung BW, Schröder H. 2008. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. J Nutr Biochem, 19(10), 643-654.

Fletcher D. 1995. Relationship of breast meat color variation to muscle pH and texture. Poult Sci, 74(Suppl 1), 120.

Folch J, Lees M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226, 497-509.

Grosse C. 1984. Sales and marketing opportunities for slaughter by-products and slaughterhouse waste in the Federal Republic of Germany. Diss, Univ Bonn.

Güz B, Molenaar R, De Jong IC, Kemp B, Van Den Brand H, Van Krimpen M. 2019. Effects of dietary organic minerals, fish oil, and hydrolyzed collagen on growth performance and tibia characteristics of broiler chickens. Poult Sci, 98(12), 6552-6563.

Hafez HM, Attia YA. 2020. Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. Front Vet Sci, 7.

Hillgartner FB, Salati LM, Goodridge AG. 1995. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. Physiol rev, 75(1), 47-76.

Huo JX, Zheng Z. 2009. Study on enzymatic hydrolysis of Gadus morrhua skin collagen and molecular weight distribution of hydrolysates. Agric Sci China, 8(6), 723-729.

Iwai K, Hasegawa T, Taguchi Y, Morimatsu F, Sato K, Nakamura Y, Higashi A, Kido Y, Nakabo Y, Ohtsuki K. 2005. Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. J Agric Food Chem, 53(16), 6531-6536.

Jayathilakan K, Sultana K, Radhakrishna K, Bawa AS. 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. J Food Sci Technol, 49(3), 278-293.

Kim AY, Kim Y, Lee SH, Yoon Y, Kim WH, Kweon OK. 2017. Effect of gelatin on osteogenic

cell sheet formation using canine adipose-derived mesenchymal stem cells. Cell Transplant, 26(1), 115-123.
Kolar K. 1990. Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. J Assoc Offic Anal Chem, 73.1, 54-57.
Kook K, Kim M, Chung H. 2020. A Literature Review on the Dry-Heat Cooking Recipes of Chicken: Focus on the Recipe Books from 1945 to 1999. Culin Sci Hosp Res, 26(2), 90-98.
Kushi LH, Byers T, Doyle C, Bandera EV, McCullough M, Gansler T, Andrews KS, Thun MJ. 2006. American Cancer Society Guidelines on Nutrition and Physical Activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA: Cancer J Clin, 56(5), 254-281.
Liu DC. 2002. Better utilization of by-products from the meat industry. Food Fertil Technol Cent.
Lonergan SM, Huff-Lonergan E, Rowe LJ, Kuhlers DL, Jungst SB. 2001. Selection for lean growth efficiency in Duroc pigs influences pork quality. J anim sci, 79, 2075-2085.
Matsuda N, Koyama Yi, Hosaka Y, Ueda H, Watanabe T, Araya T, Irie S, Takehana K. 2006. effects of ingestion of collagen peptide on collagen fibrils and glycosaminoglycans in the dermis. J Nutr Sci Vitaminol, 52(3), 211-215.
Mendis E, Rajapakse N, Byun HG, Kim SK. 2005. Investigation of jumbo squid (Dosidicus gigas) skin gelatin peptides for their in vitro antioxidant effects. Life Sci, 77(17), 2166-2178.
Min SG, Jo YJ, Park SH. 2017. Potential application of static hydrothermal processing to produce the protein hydrolysates from porcine skin by-products. LWT-Food Sci Technol, 83, 18-25.
Mir NA, Rafiq A, Kumar F, Singh V, Shukla V. 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J Food Sci Technol, 54(10), 2997-3009.
Mirzapour-Kouhdasht A, Moosavi-Nasab M, Krishnaswamy K, Khalesi M. 2020. Optimization of gelatin production from Barred mackerel by-products: Characterization and hydrolysis using native and commercial proteases. Food Hydrocoll, 108, 105970.
Moon YS. 2018. Lipid Metabolism and Fatty Liver in Poultry. Korean J Poult Sci, 45(2), 109-118.
Mundheim H, Aksnes A, Hope B. 2004. Growth, feed efficiency and digestibility in salmon (Salmo salar L.) fed different dietary proportions of vegetable protein sources in combination with two fish meal qualities. Aquac, 237(1-4), 315-331.
Muyonga JH, Cole CGB, Duodu KG. 2004. Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (Lates niloticus). Food Chem, 86(3), 325-332.
Nazeer RA, Deeptha R, Jaiganesh R, Sampathkumar NS, Naqash SY. 2011. Radical Scavenging Activity of Seela (Sphyraena barracuda) and Ribbon Fish (Lepturacanthus savala) Backbone Protein Hydrolysates. Int J Pept Res Ther, 17(3), 209-216.
Nazeer RA, Kumar NSS. 2012. Fatty acid composition of horse mackerel (Magalaspis cordyla) and croaker (Otolithes ruber). Asian Pac J Trop Dis, 2, S933-S936.
Ngo DH, Ryu B, Vo TS, Himaya S, Wijesekara I, Kim SK. 2011. Free radical scavenging and angiotensin-I converting enzyme inhibitory peptides from Pacific cod (Gadus macrocephalus) skin gelatin. Int J Biol Macromol, 49(5), 1110-1116.
Nomura Y, Oohashi K, Watanabe M, Kasugai S. 2005. Increase in bone mineral density through oral administration of shark gelatin to ovariectomized rats. Nutr, 21(11-12), 1120-1126.

NRC. 1994. Nutrient requirements of poultry, 9th. ed. National Academy Press, Washingtion D.C., USA.
Nune SK, Rama KS, Dirisala VR, Chavali MY. 2017. Electrospinning of collagen nanofiber scaffolds for tissue repair and regeneration. Nanostruct Nov Ther, 281-311.
Nurubhasha R, Dirisala VR, Thirumalasetti S, Konatham HR, Simhachalam G, Ingilala S, Kumar S. 2019. Effect of Collagen Supplementation in Broiler Feed: Influence on Productive Performance and Muscle Quality in Broiler Chicken. Karbala Int J Mod Sci, 5(3).
OECD-FAO. 2020. Agricultural Outlook. Available from: https://www.oecd-ilibrary.org/sites/1112c23b-en/index.html?itemId=/content/publication/1112c23b-en
OECD. 2020. Meat consumption. Available from: https://data.oecd.org/agroutput/meat-consumption.htm
Offer G. 1988. The structural basis of water-holding in meat. Part I : General principles and water uptake in meat processing. Dev Meat Sci.
Opstvedt J, Aksnes A, Hope B, Pike IH. 2003. Efficiency of feed utilization in Atlantic salmon (Salmo salar L.) fed diets with increasing substitution of fish meal with vegetable proteins. Aquac, 221(1-4), 365-379.
Park MR, Han DY, Shin YS, Won C, Yeon SC, Jung TS, Kim JS, Lee H, Kim YH, Kim EH, Kim GS. 2005. Effects of Saururus chnensis B. and Carthamus tinctorius L. on physico-chemical properties of broiler chicks. J Vet Clin, 22(2), 125-129.
Parry D, Craig A, Barnes G. 1978. Tendon and ligament from the horse: an ultrastructural study of collagen fibrils and elastic fibres as a function of age. Proceedings of the Royal Society of London. Series B. Biol Sci, 203(1152), 293-303.
Pataridis S, Eckhardt A, Mikulikova K, Sedlakova P, Miksik I. 2008. Identification of collagen types in tissues using HPLC-MS/MS. J Sep Sci, 31(20), 3483-3488.
Rural Development Administration. (2020). Available from: http://www.rda.go.kr/main/mainPage.do
Russ W, Meyer-Pittroff R. 2004. Utilizing waste products from the food production and processing industries. Crit Rev Food Sci Nutr, 44(1), 57-62.
Rutherfurd SM, Cui J, Goroncy AK, Moughan PJ. 2015. Dietary protein structure affects endogenous ileal amino acids but not true ileal amino acid digestibility in growing male rats. J Nutr, 145(2), 193-198.
Sirri F, Maiorano G, Tavaniello S, Chen J, Petracci M, Meluzzi A. 2016. Effect of different levels of dietary zinc, manganese, and copper from organic or inorganic sources on performance, bacterial chondronecrosis, intramuscular collagen characteristics, and occurrence of meat quality defects of broiler chickens. Poult Sci, 95(8), 1813-1824.
Stanciu M. 2020. Research regarding consumers attitude, in relation with poultry meat purchase and consumption. Case study Sibiu, Romania. Sci Pap. Ser " Manag, Econom Eng Agric Rural Dev", 20(1), 129.
Van Harn J, Spek J, Van Vuure C, Van Krimpen M. 2017. Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers. Poult Sci, 96(5), 1334-1340.
Woo KC, Kim CH, NamGung Y, Paik IK. 2007. Effects of Supplementary Herbs and Plant Extracts on the Performance of Broiler Chicks. Korean J Poult Sci, 34(1), 43-52.
Wu J, Fujioka M, Sugimoto K, Mu G, Ishimi Y. 2004. Assessment of effectiveness of oral administration of collagen peptide on bone metabolism in growing and mature rats. J Bone Miner Metab, 22(6), 547-553.
Yamamoto E, Hata D, Kobayashi A, Ueda H, Tangkawattana P, Oikawa M, Takehana K. 2002. Effect of beta-aminopropionitrile and hyaluronic acid on repair of collagenase-induced

injury of the rabbit Achilles tendon. J Comp Pathol, 126(2-3), 161-170.

Tables

Table 1 General components of breast meat from pig skin collagen-fed broilers

Treatments	NC	PC	T1	T2	T3
Moisture	73.05±0.36[bc]	74.33±0.04[a]	73.33±0.09[bc]	73.57±0.30[b]	72.94±0.01[c]
Protein	24.42±0.12[a]	21.80±0.39[c]	23.35±0.27[b]	23.78±0.18[ab]	24.13±0.35[a]
Fat	1.11±0.21[b]	2.00±0.26[a]	2.20±0.18[a]	1.16±0.25[b]	1.29±0.08[b]
Ash	1.10±0.04[b]	1.10±0.00[b]	1.06±0.02[b]	1.12±0.02[ab]	1.18±0.01[a]

NC: basal diet, PC: basal diet + 0.1% fish collagen powder T1: basal diet +0.1% collagen, T2: basal diet + 0.5% collagen, T3: basal diet + 1.0% collagen,
[a, b]Means in the same row with different superscripts differ ($p < 0.05$).

Table 2 Quality characteristics of breast meat from pig skin collagen-fed broilers

Treatments		NC	PC	T1	T2	T3
Drip loss (%)		4.41±0.19[a]	3.26±0.47[b]	2.77±0.15[bc]	2.37±0.32[c]	3.38±0.84[b]
Cooking loss (%)		20.2±2.32	20.86±1.81	21.37±1.04	19.25±2.16	21.67±1.11
WHC[1)] (%)		66.6±5.24	61.64±8.29	59.05±4.67	63.46±0.15	65.16±1.24
pH		6.19±0.14	6.22±0.14	6.11±0.15	6.26±0.13	6.15±0.21
Hunter Color[2)]	**L***	58.82±2.72[a]	55.84±1.06[ab]	56.88±1.2[ab]	54.08±1.02[b]	54.16±1.91[b]
	a*	18.57±1.04[a]	12.78±0.66[b]	18.26±0.4[a]	12.58±0.62[b]	18.73±1.23[a]
	b*	14.61±2.01[ab]	12.11±3.09[bc]	9.56±0.44[c]	11.88±0.61[bc]	17.52±0.39[a]
Collagen in breast meat (mg/g)		4.69 ±1.58[e]	29.42 ±1.58[d]	50.90 ±1.55[c]	79.88 ±4.96[b]	226.14 ±6.29[a]
Collagen in skin (g/100g)		0.34 ±0.02[b]	0.26 ±0.02[b]	0.50 ±0.06[a]	0.31 ±0.00[b]	0.16 ±0.01[c]

NC: basal diet, PC: basal diet + 0.1% fish collagen powder T1: basal diet +0.1% collagen, T2: basal diet + 0.5% collagen, T3: basal diet + 1.0% collagen,
[a, b]Means in the same row with different superscripts differ ($p < 0.05$).
1) WHC: water holding capacity
2) L*: lightness, a*: redness, b*: yellowness.

Table 3 Tissue characteristics of breast meat from pig skin collagen-fed broilers

Treatments	NC	PC	T1	T2	T3
Springiness (%)	32.13 ±2.72[c]	51.95 ±2.13[a]	47.16 ±2.76[ab]	46.36 ±2.6[b]	50.13 ±2.95[ab]
Cohesiveness (%)	43.74 ±3.82[c]	55.44 ±1.86[ab]	55.54 ±2.18[ab]	49.29 ±7.26[bc]	57.26 ±2.46[a]
Chewiness (%)	35.52 ±17.97[c]	98.89 ±6.44[b]	58.57 ±11.08[c]	85.14 ±13.97[b]	166.91 ±16.18[a]
Hardness (g)	1174.26 ±702.75[e]	5190.88 ±251.98[b]	2776.36 ±659.36[d]	3951.58 ±713.41[c]	8339.29 ±404.09[a]
Shear force (g)	1156.12 ±201.07	1206.75 ±325.27	1755.27 ±721.66	1518.99 ±277.61	1953.59 ±912.12

NC: basal diet, PC: basal diet + 0.1% fish collagen powder T1: basal diet +0.1% collagen, T2: basal diet + 0.5% collagen, T3: basal diet + 1.0% collagen,
[a, b]Means in the same row with different superscripts differ ($p < 0.05$).

Table 4 Bone length, weight, and skin thickness of breast meat from pig skin collagen-fed broilers

Treatments	NC	PC	T1	T2	T3
Bone weight (g)	8.92±2.77	8.98±3.35	9.44±2.94	8.90±1.47	7.00±0.19
Bone length (cm)	7.46±1.46	7.66±0.70	7.72±0.56	7.98±0.52	7.00±0.24
Skin thickness (cm)	0.37±0.04[a]	0.31±0.03[ab]	0.38±0.03[a]	0.38±0.04[a]	0.26±0.06[b]

NC: basal diet, PC: basal diet + 0.1% fish collagen powder T1: basal diet +0.1% collagen, T2: basal diet + 0.5% collagen, T3: basal diet + 1.0% collagen,
[a, b]Means in the same row with different superscripts differ ($p < 0.05$).

Table 5 Analysis of fatty acids in breast meat from pig skin collagen-fed broilers (%)

Fatty acids	NC	PC	T1	T2	T3
Myristic acid (C14:0)	0.84	0.84	0.82	0.84	0.87
Palmitic acid (C16:0)	20.76	22.95	22.19	23.76	23.81
Palmitoleic acid (C16:ln7)	5.08	5.40	5.08	8.42	6.78
Stearic acid (C18:0)	7.58	7.94	7.54	5.45	7.18
Oleic acid (C18:ln9)	45.04	44.23	45.15	44.52	44.35
Linoleic acid (C18:2n6)	0.00	0.00	0.00	0.00	0.00
γ-Linoleic acid (C18:3n6)	16.38	14.80	15.60	13.74	13.59
Linolenic acid (C18:3n3)	0.10	0.17	0.15	0.14	0.11
Eicosenoic acid (C20:ln9)	2.38	1.89	2.12	2.03	2.17
Arachidonic acid (C20:4n6)	0.58	0.69	0.66	0.56	0.61
Total	1.26	1.09	0.70	0.54	0.52
SFA[1)]	29.18	31.73	30.55	30.05	31.86
USFA[2)]	70.82	68.27	69.45	69.95	68.14
MUFA[3)]	50.12	49.63	50.23	52.94	51.13
PUFA[4)]	49.88	50.37	49.77	47.06	48.87
n-3 fatty acids	29.18	31.73	30.55	30.05	31.85
n-6 fatty acids	70.82	68.27	69.45	69.95	68.15

NC: basal diet, PC: basal diet + 0.1% fish collagen powder T1: basal diet +0.1% collagen, T2: basal diet + 0.5% collagen, T3: basal diet + 1.0% collagen,
1) SFA: saturated fatty acid
2) USFA: unsaturated fatty acid
3) MUFA: monounsaturated fat
4) PUFA: polyunsaturated fat

Table 6 Analysis of free amino acids in breast meat from pig skin collagen-fed broilers (%)

Treatments	NC	PC	T1	T2	T3
Cysteine	0.266	0.249	0.251	0.263	0.265
Methionyl	0.575	0.509	0.536	0.553	0.557
Aspartic acid	2.115	1.957	2.021	2.107	2.078
Threonine	1.027	0.957	0.983	1.027	1.011
Serine	0.899	0.862	0.864	0.909	0.893
Glutamic acid	3.322	3.108	3.140	3.323	3.281
Glycine	0.952	0.907	0.925	0.955	0.949
Alanine	1.301	1.196	1.245	1.287	1.276
Valine	1.059	0.954	1.004	1.039	1.031
Isoleucine	1.026	0.918	0.956	1.004	0.998
Leucine	1.905	1.735	1.805	1.879	1.859
Tyrosine	0.724	0.657	0.684	0.712	0.704
Phenylalanine	0.900	0.845	0.859	0.896	0.891
Lysine	2.123	1.883	1.988	2.049	2.026
Histidine	0.837	0.726	0.693	0.707	0.697
Arginine	1.500	1.339	1.404	1.474	1.457
Proline	0.778	0.713	0.739	0.750	0.749

NC: basal diet, PC: basal diet + 0.1% fish collagen powder T1: basal diet +0.1% collagen, T2: basal diet + 0.5% collagen, T3: basal diet + 1.0% collagen