Korean Journal Of Agricultural Science

pISSN : 2466-2402
eISSN : 2466-2410

ANIMAL

Effect of the supplementation of pig skin collagen on growth performance, organ weight, blood characteristics and intestinal microbiota in broilers

Ji Seon An[1], Won Yun[1], Ji Hwan Lee[1], Han Jin Oh[1], Young Gwang Kim[1], In Kyu Bae[1], Kwon Jung Kim[1], Ju Ho Lee[2], Gok Mi Kim[3], Yang Il Choi[1], Jin Ho Cho[1,*]

[1]Department of Food and Animal Science, Chungbuk National University, Cheongju 28644, Korea
[2]Hansalimfood, Goesan 28041, Korea
[3]Department of Beauty Art, Cheonan Yonam College, Cheonan 31005, Korea

*Corresponding author: jinhcho@chungbuk.ac.kr

Abstract

This experiment was conducted to investigate the effects of pig skin collagen supplementation on growth performance, organ weight, blood characteristics, and intestinal microbiota in broilers. A total of 50 Ross 308 broilers were used for 2 weeks. The five dietary treatments were as follows: NC) basal diet, PC) NC + fish collagen powder 0.1%, T1) NC + pig skin collagen 0.1%, T2) NC + pig skin collagen 0.5%, and T3) NC + pig skin collagen 1.0%. The body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were not affected ($p > 0.05$) by the dietary treatments in this experiment. Additionally, there were no significant differences ($p > 0.05$) in the organ weights among the treatments. Broilers fed T1, T2 and T3 diets had higher ($p < 0.05$) white blood cell (WBC) counts than the broilers fed the NC and PC diets. The *Lactobacillus* counts in the excreta were improved ($p < 0.05$) in the broilers fed the T1 and T2 diets. Moreover, the *Salmonella* counts in the excreta were decreased ($p < 0.05$) in the broilers fed the PC and T1 diets. In conclusion, supplementation of pig skin collagen in diets improved the white blood cells (WBCs) in the blood and *Lactobacillus* counts in the excreta, and reduced the *Salmonella* counts in the excreta. However, when pig skin collagen was increased in the diets, there were no significant differences ($p > 0.05$). Therefore, the addition of 0.1% pig skin collagen in the feed provided beneficial effects on the blood characteristics and the intestinal microbiota environment.

Keywords: blood characteristics, broiler, intestinal microbiota, pig skin collagen

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Citation: An JS, Yun W, Lee JH, Oh HJ, Kim YG, Bae IK, Kim KJ, Lee JH, Kim GM, Choi YI, Cho JH. 2019. Effect of the supplementation of pig skin collagen on growth performance, organ weight, blood characteristics and intestinal microbiota in broilers. Korean Journal of Agricultural Science. https://doi.org/10.7744/kjoas.20190040

DOI: https://doi.org/10.7744/kjoas.20190040

Received: April 09, 2019

Revised: July 05, 2019

Accepted: July 12, 2019

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Introduction

Collagen is a fibrous substrate protein that is high in animal skins and accounts for about 30% of the total weight of biocompatible proteins (Oikarinen et al., 1992). Collagen peptide consists of proline, glycine and hydroxyproline. Also, it has functions such as physiological

control function, superior biocompatibility, absorbability and low side effects (Iwai et al., 2005). Also, many studies have been conducted because collagen has safe biocompatibility due to its biological properties, such as biodegradability and low antigenicity (Maeda et al., 1999). Studies in diabetic rats showed the effect of preventing hyperlipidemia in blood lipid metabolism and lowering blood glucose (Kim et al., 2009b). And for collagen extract from shark skin, showed high antibacterial properties against S. enteritidis and E. coli (Kim et al., 2009d). The low-molecular enzyme decomposition extract from pig skin collagen also showed high antioxidant activity and the effect of protecting nerve cells against oxidative stress (Kim et al., 2013a). Like this, collagen has many characteristics such as arthritis treatment effects, lipid metabolism, antibacterial properties, and antioxidant effects, so many studies are conducted on humans and rats.

Collagen is especially high in fish, pigs and chicken. Among them, pig skin collagen has more stable properties and more year-round supply than any other collagen, but its molecular weight is about 300,000 Dalton. So, absorption in the body is relatively limited compared to other animal originated collagen (Jeon et al., 2016). For this reason, the demand for fish collagen, which is relatively small in molecular weight, has recently increased, and has been used for skin care, arthritis treatment (Yoo et al., 2008). But it is difficult to extract large amounts of fish collagen (Jeon et al., 2016). For the efficient use of pig skin collagen, a variety of processing methods are being studied, including enzyme treatment and radiation treatment for low molecularization (Cho et al., 2006; Yang and Shu, 2014). Accordingly, many studies have been conducted in humans and rats on skin anti-aging effects (Kim et al., 2009c), skin wrinkle betterment effects (Kang and Jeon, 2009), and skin barrier protection effects (Kim et al., 2011) using low-molecular pig skin collagen. However, research on livestock such as pigs and chickens is still scarce. This study was conducted to investigate the effects of pig skin collagen supplementation on growth performance, organ weight, blood characteristics, and intestinal microbiota in broilers.

Materials and Methods

The experimental protocol was approved and conducted under the guidelines of the Animal Care and Use Committee of Chungbuk National University.

Pig skin collagen

The experiment was conducted with pig skin collagen made by Chungbuk National University's Department of Animal Science. 6 Liter of distilled water and 3 kg of pig skin were put into an electronic pressure extractor (KS 220S, Kyungseo E&P, Korea) under 80℃ for 5 hours. After heating, insoluble collagen was ejected through the gauze. The collagen extract (CE) was adjusted to pH 3.0 by adding citric acid. CE was hydrolyzed at 35℃ for 5 hours using protease (Love me tender, H GROUP USA LLC, USA). CE was concentrated at 80℃ for 12 hours. CE was cooled down at room temperature for 20 minutes and was filtered to 9,450 Dalton using a 10,000 Dalton filter (Multi-Angle Light Scattering, Korea Basic Science Institute, Korea). Finalized extracted collagen from pig skin was stored at 4℃ for 24 hours and used immediately for feed additive.

Experimental design and animals

A total of 50 Ross 308 broilers (BW, body weight = 322.5 ± 0.3 g) were used in 2 weeks. Broilers were assigned to 5 treatments (2 replicate pens per treatment and 5 broilers per pen) in a randomized complete block. The experiment lasted for 2 weeks. The dietary treatments were as follows: (1) negative control, basal diet (NC), (2) positive control, basal diet + 0.1% fish collagen powder), (3) basal diet + 0.1% pig skin collagen (T1), (4) basal diet + 0.5% pig skin collagen (T2), (5) basal diet + 1.0% pig skin collagen (T3). The basal diets were formulated to meet or exceed the NRC (1994) requirements (Table 1). All broilers were allowed to consume feed and water *ad libitum*.

Sampling and measurements

The broilers were weighed individually, and body weight was recorded initially and end of the experimental period (2 weeks) to calculate body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). At the end of the experiment, five broilers per pen were bled via the wing vein using a sterilized syringe, and blood samples were collected in tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, USA) immediately. Samples were centrifuged at 3000 × g for 15 min at 4℃ and plasma was stored at - 20℃. The concentrations of white blood cell (WBC), and red blood cell (RBC) were analyzed by using an automatic blood analyzer (ADVID

Table 1. Compositions of the basal diets (as-fed basis).

Items	Contents
Ingredients (%)	
Corn (USA, No.3)	50.28
Soybean meal (44% CP)	16.50
Wheat	20.00
Wheat bran	4.00
Fish meal (local)	1.00
Animal fat	3.00
Rapeseed meal	2.00
Salt	0.23
Choline-HCl (50%)	0.01
DL-Methionine-99%	0.12
Lysine-HCl (78%)	0.66
Calcium carbonate	0.20
Tricalcium phosphate	1.60
Vitamin premix[y]	0.20
Mineral premix[z]	0.20
Analyzed composition (%)	
Crude protein	22.00
Ca	1.00
Lysine	1.20
Met + Cys	0.87

[y] Contained per kg of diet: vit A, 10,000 IU; vit D_3, 2,000 IU; vit E, 421 IU; vit K, 5 mg; riboflavin, 2,400 mg; vit B_2, 9.6 mg; vit B_6, 2.45 mg; vit B_{12}, 40 ug; niacin, 49 mg; pantothenic acid, 27 mg, biotin, 0.05 mg.
[z] Contained the mg per kg of diet: Cu 140 mg, Fe 145 mg, Zn 179 mg, Mn 12.5 mg, I 0.5 mg, Co 0.25 mg, Se 0.4 mg.

120, Bayer, USA). Immunoglobulin G (IgG), glucose, and blood urea nitrogen (BUN) were determined by using an automatic biochemistry analyzer (Hitachi 747, Tokyo, Japan). After blood collection, the same broilers were weighed individually and slaughtered by approved methods. The liver, spleen, and bursa of Fabricius removed and weighed. Organ weight was expressed as a percentage of BW.

Excreta samples were collected at the end of the experiment (2 weeks). The samples were stored at -20℃ until the experiment. Also, serial dilution (10-1 to 10-6) of samples was made using anaerobic diluents and placed on MacConkey agar plates (BD BBL, Maryland, USA), Lactobacilli MRS agar plates (Difco, Maryland, USA) and Salmonella shigella agar plates (BD BBL, Maryland, USA) to isolate the *Escherichia coli* (*E. coil*), *Lactobacillus* and *Salmonella*, respectively. The Lactobacilli MRS agar plates were then incubated for 48 h at 39℃ under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37℃ and the Salmonella shigella agar plates were incubated for 36 h at 37℃. The *E. coli*, *Lactobacillus* and *Salmonella* colonies were counted immediately after removal from the incubator.

Statistical analysis

Data were statistically analyzed by ANOVA using the GLM procedure SAS ver 9.4 (SAS Institute Inc., Cary, USA), with each pen being used as the experimental unit. Differences among all treatments were separated by Duncan's multiple range tests. Variability in the data is expressed as the standard error (SE) and a probability level of $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Growth performance

The effect of the supplementation of pig skin collagen in broiler feed on the growth performance is shown in Table 2. There were no significant differences ($p > 0.05$) among the NC and the other treatments at both in BWG, FI, and FCR.

The growth performance of broilers is always very important in the poultry industry (Liu et al., 2019). In an

Table 2. Effect of supplementation of pig skin collagen on growth performance in broilers.

Items	NC	PC	T1	T2	T3	SE	p-value		
							Linear	Quadratic	Cubic
BW (g)									
Initial	322	322	322	322	322	1	0.977	0.943	0.953
Final	1,238	1,258	1,270	1,218	1,243	87	0.798	0.971	0.887
BWG (g)	914	934	948	895	920	64	0.901	0.950	0.576
FI	1,415	1,474	1,474	1,525	1,598	80	0.111	0.936	0.932
FCR	1.542	1.590	1.562	1.706	1.728	0.102	0.321	0.643	0.593

NC, basal diet; PC, basal diet + 0.1% fish collagen powder; T1, basal diet + 0.1% pig skin collagen; T2, basal diet + 0.5% pig skin collagen; T3, basal diet + 1.0% pig skin collagen; SE, standard error; BW, body weight; BWG, body weight Gain; FI, feed intake; FCR, feed conversion ratio.

experiment by Park et al. (2012), the effect of collagen supplementation in 4-week-old rats showed significantly increased in BWG and FI compared to CON. However, in a study of SPF (special pathogen free) rats aged 8 weeks old, the results of direct oral administration of collagen showed no significant difference in the BWG and FI (Kim et al., 2009c). With a similar study, Matsuda et al. (2006) reported that the supplementation of pig skin collagen in weaning pigs was no significant difference in the BWG. This experiment has shown similar results in BWG, FI, and FCR. The collagen sample of this study is directly extracted and hydrolyzed collagen from pig skin, and since it is in liquid form, the size of the feed particles may increase due to lumps when adding collagen to feed. So, when the broilers fed diets, the feed intake may vary as the size of the larger particles makes it difficult to intake. As FI difference, the FCR may also increase the likelihood of error. Also, there may be differences follow as absorption levels of animals and the method of collagen supplementation such as oral administration or addition to feed.

Organ weight

The effect of the supplementation of pig skin collagen in broiler feed on the organ weight is shown in Table 3. There was no significant difference ($p > 0.05$) on organ weight among dietary treatments during the experiment.

The liver, spleen, and bursa of Fabricius are the major immune-related organs of broilers, and as the weight increases, the immune function improves (Rivas and Fabricant, 1988). Until 35 days after hatching, bursa of Fabricius is typically larger than the spleen. However, if the spleen is larger than the bursa of Fabricius, it usually indicates a complex vaccine response and an immune suppression condition and is prone to outbreaks such as respiratory infections (Kim et al., 2013b). The results of this study showed that there was a tendency to increase

Table 3. Effect of supplement of pig skin collagen on organ weight in broilers.

Items	NC	PC	T1	T2	T3	SE	p-value		
							Linear	Quadratic	Cubic
Liver	2.974	2.956	2.690	3.352	3.164	0.200	0.197	0.068	0.061
Spleen	0.116	0.094	0.092	0.138	0.096	0.018	0.403	0.132	0.066
Bursa of Fabricius	0.172	0.224	0.204	0.152	0.178	0.027	0.611	0.224	0.199

NC, basal diet; PC, basal diet + 0.1% fish collagen powder; T1, basal diet + 0.1% pig skin collagen; T2, basal diet + 0.5% pig skin collagen; T3, basal diet + 1.0% pig skin collagen; SE, standard error.

Table 4. Effect of supplementation of pig skin collagen on blood characteristics in broilers.

Items	NC	PC	T1	T2	T3	SE	p-value		
							Linear	Quadratic	Cubic
RBC	2.61	2.73	2.66	2.48	2.77	0.16	0.671	0.473	0.354
WBC	19.01b	24.08ab	30.99a	27.10a	30.15a	2.37	0.019	0.089	0.057
Glucose	314	218	274	237	237	35	0.120	0.580	0.829
BUN	1.33	1.00	1.00	1.00	1.66	0.33	0.517	0.164	0.827
IgG	4.33	5.00	5.33	4.33	4.33	0.59	0.715	0.421	0.286

NC, basal diet; PC, basal diet + 0.1% fish collagen powder; T1, basal diet + 0.1% pig skin collagen; T2, basal diet + 0.5% pig skin collagen; T3, basal diet + 1.0% pig skin collagen; SE, standard error; RBC, red blood cell; WBC, white blood cell; BUN, blood urea nitrogen; IgG, immunoglobin G.
a, b: Means in a row with different letters are significantly different ($p < 0.05$).

the weight of the liver as collagen was added, but there were no significant differences among dietary treatments. Moreover, the weight of the bursa of Fabricius is greater than the weight of the spleen, so the addition of the pig skin collagen doesn't appear to affect the organ weight and immune organs of the broilers.

Blood characteristics

Table 4. showed the effect of pig skin collagen on blood characteristics throughout the experiment. The concentration of WBC in the blood of the broilers was significantly higher ($p < 0.05$) in T1, T2, and T3 treatments than NC treatment. However, the results of the concentration of RBC, glucose, BUN, and IgG were unaffected by pig skin collagen supplementation in this study.

The WBC, which responds to pathogens or stress environments and is involved in innate immune responses according to non-specific and specific immune responses (Paul, 1998) showed significantly high levels at T1, T2, and T3 treatments in this study. This can be attributed to increased WBC in the broilers blood, resulting in an immune control effect, but due to a lack of prior research, a precise study is needed. In a study by Lee et al. (2018), oral administration of collagen in 7-week-old rats showed no significant difference in glucose, which was consistent with the results of this study. BUN (blood urea nitrogen) is the final product of protein metabolism and is released into the blood through the liver (Cho et al., 2010). In this study, the addition of collagen does not appear to affect BUN, given that there is no significant difference among the dietary treatments. IgG, produced at B-cells in chicken bone marrow, is the highest concentration of immune proteins and is mainly responsible for in vivo immunity (Kim et al., 2009a). There are reports that higher IgG levels in the blood improve growth performance (Cetin et al., 2005), but there were no significant differences on IgG in the present study.

Intestinal microbiota

The results of the intestinal microbiota are presented in Table 5. The *Lactobacillus* counts in the excreta of broilers were significantly higher in T1 and T2 treatments ($p < 0.05$) and the *Salmonella* counts were higher in PC and T1 treatments ($p < 0.05$). However, there were no significant differences in *E. coli* counts in excreta among treatments ($p > 0.05$).

Kim et al. (2009d) reported that as a result of studies using collagen extracted from shark shells to experiment with antibacterial properties for *S. enteritidis* and *E. coli*, the size of the clean zone increased as the concentration

Table 5. Effect of supplementation of pig skin collagen on intestinal microbiota in broilers.

Items	NC	PC	T1	T2	T3	SE	p-value		
							Linear	Quadratic	Cubic
Lactobacillus	6.514b	6.457b	6.836a	6.838a	6.407a	0.094	0.109	0.760	0.082
E. coli	4.980	4.925	4.991	5.009	4.959	0.063	0.995	0.984	0.996
Salmonella	2.988b	2.682b	2.625b	2.941a	2.936a	0.062	0.495	0.101	0.662

NC, basal diet; PC, basal diet + 0.1% fish collagen powder; T1, basal diet + 0.1% pig skin collagen; T2, basal diet + 0.5% pig skin collagen; T3, basal diet + 1.0% pig skin collagen; SE, standard error.
a, b: Means in a row with different letters are significantly different ($p < 0.05$).

of collagen increased and accordingly collagen causes to have the antimicrobial ability. In this study, *Salmonella* counts were significantly lower in the PC and T1 treatments, and this study agrees with precedent research. However, there was no significant difference in the concentration of *E. coli*, and it is considered to require a more precise study because it showed different results from the preceding study. For *Lactobacillus*, in the study of Kim et al. (2017), the mixed low-molecular collagen and *L. brevis* showed significantly higher levels in viable cell counts than the single-cultured treatment of *L. brevis* and were consistent with the results of this experiment.

However, the effect of T3 treatment with 1% collagen added in this study was not significant, which is estimated to be due to increased harmful bacteria resulting from the fermentation of high protein in the intestinal tract. Previous studies report that if protein content in feed increases above a certain level, intestinal pathogenic microorganisms (*E. coli, Clostridium* and *Enterobacteriaceae*) increase and viable cell counts of *Lactobacillus* decrease (Heo et al., 2013; Rist et al., 2014). In an experiment in broilers, the supplementation of high-level glycine, which a constituent amino acid of collagen, was significantly reduced the *Lactobacillus* counts of the extract and the *Clostridium* counts was significantly increased (Dahiya et al., 2005). This was similar to the results of this study, which 0.1% collagen supplementation in feed has been effective in improving the microbial environment, but 1% collagen addition has not been effective.

Conclusion

In this experiment, the addition of 0.1% pig skin collagen in the feed provided beneficial effects on blood characteristics and the intestinal microbiota environment. However, due to the lack of research on the appropriate level of addition of collagen, it is necessary to investigate the level of collagen addition in broiler feed.

Acknowledgements

This work (Grants No. C0505236) was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded Korea Small and Medium Business Administration in 2017.

Authors Information

Jin Ho Cho, https://orcid.org/0000-0001-7151-0778
Ji Seon An, https://orcid.org/0000-0002-9205-8095
Won Yun, https://orcid.org/0000-0002-1835-2640
Ji Hwan Lee, https://orcid.org/0000-0001-8161-4853
Han Jin Oh, https://orcid.org/0000-0002-3396-483X
Young Gwang Kim, https://orcid.org/0000-0003-2093-695X
In Kyu Bae, https://orcid.org/0000-0001-6088-3624
KwonJung Kim, https://orcid.org/0000-0001-7873-3747
Ju Ho Lee, https://orcid.org/0000-0003-2222-5561

Gok Mi Kim, https://orcid.org/0000-0003-1053-4535
Yang Il Choi, https://orcid.org/0000-0002-3423-525X

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