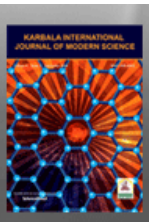

Karbala International Journal of Modern Science

Volume 5 | Issue 3 Article 5

Effect of Collagen Supplementation in Broiler Feed: Influence on Productive Performance and Muscle Quality in Broiler Chicken

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Recommended Citation

Nurubhasha, Ramesh; Dirisala, Vijaya R.; Thirumalasetti, Satish; Konatham, Hanumohan Reddy; G, Simhachalam; Ingilala, Satheesh; and Kumar, Sampath (2019) "Effect of Collagen Supplementation in Broiler Feed: Influence on Productive Performance and Muscle Quality in Broiler Chicken," *Karbala International Journal of Modern Science*: Vol. 5 : Iss. 3 , Article 5.
Available at: https://doi.org/10.33640/2405-609X.1158

Effect of Collagen Supplementation in Broiler Feed: Influence on Productive Performance and Muscle Quality in Broiler Chicken

Abstract

Present study was conducted to evaluate different strategies for feed formulation in commercial broiler production. A total of 16 male newly hatched broiler chickens (*Gallus gallus domesticus*) were fed with different formulated diets in 3 feeding phases, starter (1–11 d), grower (12-22 d) and finisher (23–33 d). Four strategies of feed formulation (F1, F2, F3 and F4) were compared; where in F1 group was fed with traditional diet, F2 and F3 were fed with traditional diet after supplementation with 10% & 20 % paneer respectively. The last group (F4) was fed with traditional diet supplemented with 10% collagen and is incorporated into paneer to provide extra protein. The collagen used in the study was analyzed and characterized by UV-Vis spectroscopy, SDS-PAGE and FTIR which was confirmed type-I collagen. All the experimental birds had shown significantly high body weight (BW) for F4 birds compared with other groups. Feed conversion ratio was significantly lower for F3 birds compared to the birds in other groups. No differences were detected for feed intake and mortality. At 33 d, F4 birds (2.227) were significantly heavier than other groups and a remarkable difference was noticed in the decreasing order F3>F2>F1 (2.004 kg, 1.998 kg and 1.749 kg respectively). Notable differences were detected for carcass yield (%), breast yield (%) and breast-to-carcass ratio at 33 d. In conclusion, our results demonstrated that the enhancement in growth rate of birds was observed by providing them with collagen along with the regular diet.

Keywords

Broiler; Collagen; Paneer; Feed formulation; Growth rate

Cover Page Footnote

The authors Vignan's University (VU/18/007) for providing necessary facilities for successful execution of this work

Authors

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1. Introduction

Collagen is one of the most abundantly available fibrous protein present in the extra cellular matrix and connective tissues of animals [1]. It has various biological functions and broadly classified into 29 different types, among which type I, II and III are predominant. It is composed of three polypeptide chains which include two α1 chains and one α2 chain [2]. These chains contain approximately 1050 amino acids with Glycine (Gly), Proline (Pro) and Hydroxyproline (Hyx) as major amino acids. Collagen molecules are coded by 25 different genes. It acts as a scaffold to give strength and support to the body [3]. The collagen synthesized by our body is referred to as endogenous collagen whereas exogenous collagen is supplemented through diet. Collagen has enormous applications in medical field for wound dressing, tissue engineering, treatment of osteoarthritis, drug delivery system in ophthalmology, urology, biomedical engineering etc [4]. In food industries, it is used as protein supplement, food texture and flavor agent, gelling agent, emulsifying agent etc [5]. Collagen can be classified into two groups based on its varied properties. Firstly, its gelling nature allows the food products to enhance or engineer their texture, thickening, gel formation, and water binding capacity. Second, its nature related to surface behaviour, allow forming emulsion, foam formation, stabilization, adhesion and cohesion, protective colloid function and film-forming capacity [6]. Mostly, industrial level collagen is procured from mammalian sources and there is a current interest in alternative sources such as fresh and marine water fishes due to their abundance and free of transmitting contagious diseases.

For maintaining various metabolic activities, animals require a balanced nutrition that provides adequent energy [7,8]. The major components of animal feed are water, carbohydrates, fats, proteins, minerals and vitamins out of these, proteins play a significant role for the erection of body tissues such as muscles, skin, feathers, beak etc. Generally, the major amino acid constituents in poultry diet are methionine and lysine which are good sources of energy and minerals. As collagen is rich source of methionine (6%) and lysine (19%) it plays a key role as feed supplement in poultry industry [9]. Besides, glycine that is considered as another important amino acid of collagen acts as anti-inflammation agent and renders protection against various diseases [10]. Proline act as a dispensable amino acid as it gives a high initial growth rate compare to other amino acids. Thus, collagen can act a important role in enhancing muscle content; improving the health of poultry birds when provided in diet in a short span. Owing to the importance of collagen, the present work was focused upon extraction of collagen

Table 1
Feed formulations for starter, grower and finisher batches.

Ingredient (%)	Starter (1−11d)				Grower (12−22 d)				Finisher (23−33 days)			
	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
Corn	50.550	40.250	30.100	30.100	50.550	40.250	30.100	30.100	50.550	40.250	30.100	30.100
SBM 48%	33.100	33.100	33.100	33.100	29.320	29.320	29.320	29.320	27.450	27.450	27.450	27.450
DDGS-corn	8.000	8.000	8.000	8.000	8.000	8.000	8.000	8.000	8.000	8.000	8.000	8.000
Soyabean oil	3.500	2.952	2.670	2.439	3.500	2.952	2.670	2.439	3.500	2.952	2.670	2.439
Limestone	1.400	1.300	1.300	1.300	1.500	1.450	1.450	1.400	1.500	1.450	1.450	1.400
$Ca(H_2PO_4)_2$	1.500	1.500	1.500	1.500	1.300	1.300	1.300	1.300	1.040	1.040	1.040	1.040
Natural pigment	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.400	0.400	0.400	0.400
Sodium chloride	0.350	0.410	0.420	0.410	0.350	0.410	0.420	0.410	0.350	0.410	0.420	0.410
$_{DL}$-Met	0.186	0.250	0.469	0.528	0.186	0.250	0.469	0.528	0.186	0.250	0.469	0.528
$_{L}$-Lys HCl	0.036	0.478	0.662	0.619	0.036	0.478	0.662	0.619	0.036	0.478	0.662	0.619
$_{L}$-Thr HCl	0.000	0.320	0.412	0.390	0.000	0.320	0.412	0.390	0.000	0.320	0.412	0.390
Choline chloride	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096
Mineral premix	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096
Vitamin premix	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096	0.096
Growth promoter	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Coccidiostat	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040
Paneer	0.000	9.750	19.90	9.750	0.000	9.750	19.90	9.750	0.000	9.750	19.90	9.750
Collagen	0.000	0.000	0.000	10.000	0.000	0.000	0.000	10.000	0.000	0.000	0.000	10.000

https://doi.org/10.33640/2405-609X.1158

and its application as feed supplement in broiler chicken with an expectation of enhancement in the muscle growth and nutrition value.

2. Materials and methods

Sixteen *Gallus gallus domesticus* broilers were kept in nootie steel wire enclosures and separated into four experimental batches of 4 birds each. Experimental birds were maintained under the same managerial, hygienic, and environmental temperature ranged in between 24 and 37 °C; 42–62% humidity and 16:8 h dark:light schedule for 5 weeks.

2.1. Treatment feed formulation

Experimental birds were fed with mash diet but difference in the composition between four groups (Table 1). Traditional diet (**F1**) was prepared using standard total amino acids [11] to meet the requirements of broilers [12]. Feed for groups 2 & 3 (**F2 & F3**) were prepared with the same diet specifications as F1 with an extra supplementation of 10 & 20% paneer respectively. Group 4 (**F4**) diet was prepared in a similar fashion to the F2 diet with an additional content of 10% collagen extracted from fish skin supplemented with paneer [13]. Feed and water were provided for ad libitum consumption (see Table 2).

2.2. Productive performance and slaughtering measurements

All the experimental birds were maintained in enclosures with proper labelling, feed intake, body weight and mortality rate was studied on a daily basis and cumulative reading were taken on 11th, 22nd and 33rd day. Productive performance was recorded based on the calculations of feed conversion ratio and cumulative feed conversion for all the batches during experimental period [14]. Dead birds were weighed, necropsied, and recorded to calculate the mortality percentage. On the last day of experimentation (33 d), all the birds were slaughtered according to the legislation in force using water-bath electrical stunning (200–220 mA, 1500 Hz). Carcasses, breast muscle and other body parts of the all the experimental groups were collected separately and readings were taken carefully. The overall carcass weight of four batches was recorded after air-chilling and calculated as percentage of carcass yield of live body weight. In same way, skin and bone was removed mechanically from breast after separating from the carcass and yield calculated on a batch basis as percentage of carcass weight.

3. Results and discussion

As reported by Ramesh et al. [13], collagen used in the formulated feed was obtained and purified from the hard skin of *Pterygoplichthys pardalis* using the pepsin to breakdown the excessive bond holding the tissue. Extracted protein was identified as collagen (type I) by UV–Vis spectroscopy, FTIR spectroscopy and SDS–PAGE. Presence of characteristic peak at 230 nm [5] and absence of elevation at 280 nm has proven that the extracted protein is collagen (Fig. 1a). Further, FTIR spectra has confirmed the molecule as collagen based on the clear presence of 5 characteristic peaks [3] namely Amide I, II, III, A and B at 1659, 1550, 1274, 2889 and 3321 cm^{-1} respectively (Fig. 1b). Upon loading of sample along with protein marker and standard type I collagen in three different lanes of SDS PAGE gel run at 100 v using electrophoresis unit illustrated three clear bands in lane 1 and 2 pertaining to the sample and confirming the molecule as collagen type I (Fig. 2).

Analysis of amino-acids confirmed the presence and percentage of various useful amino acid such as glycine (27.9%), proline (16.3%) and hydroxylproline (16.8) [13]. Especially presence of methionine (1.4%) and lysine (2.6%) is a very interesting and had definite impact upon feeding to the broiler hen. Taking this into account a feed was formulated for broiler chicken by incorporating collagen with paneer and provided for broilers as supplementation and studied its influence on the enhancement of weight and quality of muscle.

Table 2
Performance results as on 11, 22 and 33 days of age.

Item	11 day of age				22 day of age				33 day of age			
	BW (kg)	Feed Intake (KG)	FCR	Mortality (%)	BW (kg)	Feed Intake (KG)	FCR	Mortality (%)	BW (kg)	Feed Intake (KG)	FCR	Mortality (%)
F1	0.256	0.451	1.761	9.3%	0.513	0.966	1.883	3.9%	1.749	3.501	2.001	7.3%
F2	0.313	0.512	1.635	8.6%	0.569	1.006	1.768	3.8%	1.998	3.509	1.756	6.9%
F3	0.323	0.521	1.613	8.6%	0.586	1.006	1.716	3.8%	2.004	3.509	1.750	6.9%
F4	0.346	0.444	1.283	8.1%	0.626	0.854	1.364	3.1%	2.227	3.312	1.487	5.1%

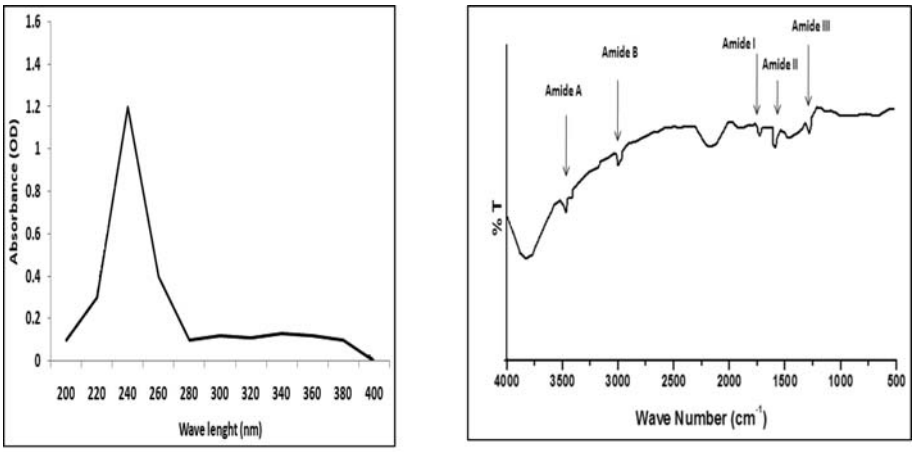

Fig. 1. Characterization of extracted protein as collagen by (a) UV−Vis and (b) FTIR spectroscopy.

Collagen and paneer being a nutritious staple food with all essential amino acids were mixed with the regular feed and tested on broiler chicken for a period of 33 days in three different stages ie., starter stage (1−11 d), grower stage (12−22 d) and finisher stage (23−33 d). On 33 day, body weight of experimental birds were significantly greater for broilers fed with F4 diet formulated with collagen and paneer (Table 1) compared with those fed with F1, F2 & F3 as shown in Table 2. Enhancement in the growth is purely due to the intake and supplementation of extra protein via diet because growth of birds is directly proportional to the ingredients of feed, species, and the age of the bird [14].

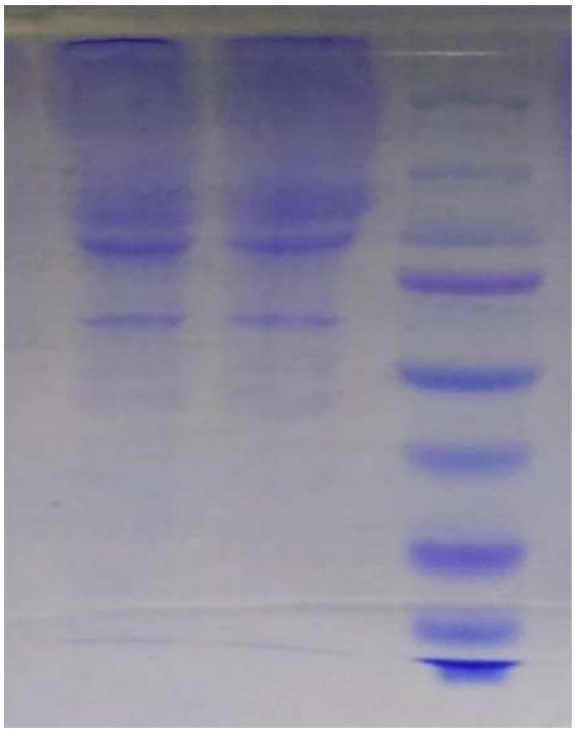

Fig. 2. Characterization of extracted protein as collagen by SDS PAGE. Lane 1- extracted collagen, Lane 2- Standard type I collagen, Lane 3- protein marker (Left to right).

As shown in Table 2, body weight and feed conversion ratio (FCR) were significantly better in birds consuming feeds formulated with collagen and paneer (F4) in all the three stages compared to the remaining groups. Low FCR is a positive sign for poultry industry because maximum utilization of available nutrients and converting into useful energy by the birds through their regular metabolism may avoid the wastage of feed and in turn reduces the investment cost. Usually birds fed with total amino acids/digestible amino acids without extra supplementation of lysine shows moderate FCR ratio [15] and reported to gain extra weight in short time, when fed with bone and meat meal [16]. Moreover, the total methionine, lysine and threonine contents were comparatively more in F4 than the remaining groups and must have provided a sufficient responsibility in the development of birds. Lysine (0.6%) was found to be sufficient for the growth, whereas 1.1% requirement was reported by Dozier et al. [17] for proper body weight. Formulated diet has no significant effect on mortality but as a general observation the rate of mortality was slightly less in the groups fed with collagen and paneer. Meal type is one of the factors that influence mortality besides replacement level and sex in between 15 and 33 d [18].

Table 3
Broiler cumulative performance at 33 d of age.

Item	BW (kg)	Feed Intake (KG)	FCR	Mortality (%)	Carcass yield (%)	Breast yield (%)	Breast-to-carcass ratio
F1	1.749	3.501	2.001	7.3%	69.4	18.6	26.8
F2	1.998	3.509	1.756	6.9%	68.7	18.1	26.3
F3	2.004	3.509	1.750	6.9%	67.7	18.3	27.0
F4	2.227	3.312	1.487	5.1%	66.9	19.9	29.7

Presence of good percentage of glycine might be one of the reasons for reduction of mortality, because of its anti-inflammatory and boosting immunity properties.

All the experimental animals were healthy and showed no significant differences for FCR and mortality at 33 d (Table 3). On 33 d, fourth group (F4) showed better values in all the recorded parameters among other treated groups. The improved performance of experimental birds indicates the nutritional impact of collagen and paneer, this may be due to the availability of nutrients like essential amino acids, unsaturated and polyunsaturated fatty acids along with necessary vitamins and minerals. A direct comparison of amino acid profile from fish collagen [13] showed that, collagen protein is providing quality amino acids than that of traditional feed [19]. Mortality rate was significantly reduced in the test group (F4), because, collagen being a potential antioxidant which can inhibit oxidative reaction of free radicals viz., reactive oxygen/nitrogen species which can cause cells death [20]. Seyyed and Hasan [21] reported reduction in mortality by inclusion of omega-3 and omega-6 fatty acids (especially 2% Flax seed oil) in broiler; similarly inclusion of lecithin with choline also had improved the rate of mortality [22]. On an overall comparison, the improvement in performance of broiler chicken muscle growth with comparatively less feed consumption.

4. Conclusion

Based on the present study, the supplementation of diet with paneer or in combination with collagen in broiler chicken has improved the growth performance, body weight and yield of muscle. It has no or low effect on mortality but definitely increased the immunity of experimental birds. Hence fish collagen can be used as an alternative and effective meal in broiler chicken industry.

References

[1] T. Maurer, M.H. Stoffel, Y. Belyaev, N.G. Stiefel, B. Vidondo, S. Küker, H. Mogel, B. Schäfer, J. Balmer, Structural characterization of four different naturally occurring porcine collagen membranes suitable for medical applications, PLoS One 13 (2018), e0205027.

[2] R.A. Nazeer, R. Kavitha, R.J. Ganesh, S.Y. Naqash, N.S.S. Kumar, R. Ranjith, Detection of collagen through FTIR and HPLC from the body and foot of *Donax cuneatus* Linnaeus, 1758, J. Food Sci. Technol. 51 (2014) 750–755.

[3] N.S. Sampath Kumar, R.A. Nazeer, Characterization of acid and pepsin soluble collagen from the skin of horse mackerels (*Magalaspis cordyla*) and croaker (*Otolithes ruber*), Int. J. Food Prop. 16 (2013) 613–621.

[4] S.S.K. Nune, K.S. Rama, V.R. Dirisala, M.Y. Chavali, Electrospinning of collagen nanofiber scaffolds for tissue repair and regeneration, in: Nanostructures for Novel Therapy, Elsevier, 2017, pp. 281–311.

[5] R.A. Nazeer, N.S. Sampath Kumar, Fatty acid composition of horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*), Asian Pac. J. Trop. Dis. S9 (2012) 303–S936.

[6] C.H. Lee, A. Singla, Y. Lee, Biomedical applications of collagen, Int. J. Pharm. 221 (2001) 1–22.

[7] N.S. Sampath Kumar, R.A. Nazeer, *In vivo* antioxidant activity of peptide purified from viscera protein hydrolysate of horse mackerel (*Magalaspis cordyla*), Int. J. Food Sci. Technol. 47 (2012) 1558–1562.

[8] N.S.S. Kumar, R.A. Nazeer, R.J. Ganesh, Functional properties of protein hydrolysates from different body parts of horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*), Med. J. Nutrition. Metab. 5 (2012) 105–110.

[9] R.A. Nazeer, R. Deeptha, R. Jaiganesh, N.S. Sampathkumar, S.Y. Naqash, Radical scavenging activity of seela (*Sphyraena barracuda*) and ribbon fish (*Lepturacanthus savala*) backbone protein hydrolysates, Int. J. Pept. Res. Ther. 17 (2011) 209.

[10] W. Wang, Z. Wu, Z. Dai, Y. Yang, J. Wang, G. Wu, Glycine metabolism in animals and humans: implications for nutrition and health, Amino Acids 45 (2013) 463–477.

[11] C. Soto, E. Avila, J. Arce, F. Rosas, D. McIntyre, Evaluation of different strategies for broiler feed formulation using near infrared reflectance spectroscopy as a source of information for determination of amino acids and metabolizable energy, J. Appl. Poult. Res. 22 (2013) 730–737.

[12] Nutrient Requirements of Poultry, Natl. Acad. Press, Washington, DC, 1994.

[13] R. Nurubhasha, N.S. Sampath Kumar, S.K. Thirumalasetti, G. Simhachalam, V.R. Dirisala, Extraction and characterization of collagen from the skin of *Pterygoplichthys pardalis* and its potential application in food industries, Food Sci. Biotechnol. (2019), https://doi.org/10.1007/s10068-019-00601-z.

[14] M. Zampiga, L. Laghi, M. Petracci, C. Zhu, A. Meluzzi, S. Dridi, F. Sirri, Effect of dietary arginine to lysine ratios on productive performance, meat quality, plasma and muscle metabolomics profile in fast-growing broiler chickens, J. Anim. Sci. Biotechnol. 9 (2018) 79.

[15] H.S. Rostagno, J.M.R. Pupa, Diet formulation for broilers based on total versus digestible amino acids, J. Appl. Poult. Res. 4 (1995) 293−299.

[16] X. Wang, C.M. Parsons, Dietary formulation with meat and bone meal on a total versus a digestible or bioavailable amino acid basis, Poult. Sci. 77 (1998) 1010−1015.

[17] W.A. Dozier, A. Corzo, M.T. Kidd, P.B. Tillman, S.L. Branton, Digestible lysine requirements of male and female broilers from fourteen to twenty-eight days of age, Poult. Sci. 88 (2009) 1676−1682.

[18] R. Jahanian, M. Khalifeh-Gholi, Marginal deficiencies of dietary arginine and methionine could suppress growth performance and immunological responses in broiler chickens, J. Anim. Physiol. Anim. Nutr. 102 (2018) e11−e20.

[19] M.A. Oguntoye, A.R. Akintunde, U.M. Mafindi, F. Adamu, B. Daniel, Effect of dietary supplementation of dl-methionine and sodium sulphate on growth performance carcass characteristics and cost benefit analysis of broiler chickens fed maize-cassava based diets, J. Anim. Prod. Res. 30 (2018) 212−226.

[20] A.D. Sarma, A.R. Mallick, A.K. Ghosh, Over Review: free radicals and their role in different clinical conditions, Int. J. Pharm. Sci. Res. 1 (2010) 185−192.

[21] S.N. Saber, H.R. Kutlu, Effect of including n-3/n-6 fatty acid feed sources in diet on fertility and hatchability of broiler breeders and post-hatch performance and carcass parameters of progeny, Asian-Australas. J. Anim. Sci. (2019), https://doi.org/10.5713/ajas.19.0055.

[22] R. Mostafa, M. Bouyeh, I. Kadim, A. Seidavi, M.M.M.Y. Elghandour, P.R.K. Reddy, J.C. Monroy, A.Z.M. Salem, Effect of dietary inclusion of lecithin with choline on physiological stress of serum cholesterol fractions and enzymes, abdominal fat, growth performance, and mortality parameters of broiler chickens, Anim. Biotechnol. (2019), https://doi.org/10.1080/10495398.2019.1622557.