Korean Journal Of Agricultural Science

pISSN : 2466-2402
eISSN : 2466-2410

ANIMAL

Effect of *Cordyceps militaris* with probiotics supplement on growth performance, meat quality characteristics, storage characteristics and cordycepin content of the breast meat in broilers

Jae Woo An, Ji Hwan Lee, Han Jin Oh, Yong Ju Kim, Se Yeon Chang, Young Bin Go, Dong Cheol Song, Hyun Ah Cho, Jin Ho Cho*

Division of Food and Animal Science, Chungbuk National University, Cheongju 28644, Korea

*Corresponding author: jinhcho@chungbuk.ac.kr

Abstract

This experiment was conducted to investigate the effect of *Cordyceps militaris* with probiotics (CMP) supplementation on the growth performance, meat quality and storage characteristics, and cordycepin content in the meat. Sixty one-day-old broilers (Ross 308) were allotted to two treatment groups of 30 each. In addition, six broilers were randomly assigned to a cage in the two treatment groups. The two dietary treatments were as follows: Control (CON) and basal diet + 0.5% of CMP. Body weight and feed intake were measured on the 1st, 14th, and 28th days from the start of the experiment. On days 1 - 14, the supplementation of CMP improved ($p < 0.05$) the body weight gain (BWG) and feed conversion ratio (FCR). Additionally, the feed intake (FI) and FCR scores in the CMP groups improved ($p < 0.01$) compared to the CON during the entire period. For the meat quality characteristics, water holding capacity (WHC), cooking loss (CL), redness (a*) in meat color value, and shearing force (SF) for the CMP group were improved ($p < 0.01$) compared to the CON group. For the meat storage characteristics, pH and thiobarbituric acid reactive substances (TBARS) were improved ($p < 0.01$) when the broilers were fed CMP compared to the CON group. Broilers fed CMP had a higher ($p < 0.01$) cordycepin content in the meat compared to the CON group. In conclusion, CMP improves the growth performance and meat quality of broilers.

Keywords: broiler, cordycepin, *cordyceps militaris* with probiotics

Check for updates

OPEN ACCESS

Citation: An JW, Lee JH, Oh HJ, Kim YJ, Chang SY, Go YB, Song DC, Cho HA, Cho JH. Effect of *Cordyceps militaris* with probiotics supplement on growth performance, meat quality characteristics, storage characteristics and cordycepin content of the breast meat in broilers. Korean Journal of Agricultural Science 48:423-432. https://doi.org/10.7744/kjoas.20210032

Received: May 11, 2021

Revised: June 18, 2021

Accepted: June 23, 2021

Introduction

Herbal feed additives in broilers feed have been reported to have beneficial non-nutrient roles such as pH, growth, and metabolic modifier (Hashemi and Davoodi, 2010). *Cordyceps militarys* is a type of mushroom that grows parasitically in the body of insects in winter and grows like grass as a host in summer. It contains carbohydrates, protein, essential fats, iron, vitamin A, vitamin C, and B_{12}, cordycepin, and ergosterol, a precursor of vitamin D (Ohmori et al., 1989; Shimizu, 1997;

Shim et al., 2000; Choi et al., 2004; Koo and Lee, 2004). *Cordyceps militaris* is known to have numerous pharmacological and therapeutic effects, and has been prescribed for a long time in oriental medicine for the treatment of weakness, chronic bronchitis, asthma, tuberculosis, and anemia (Zhu and Liu, 1992; Yong-Lu et al., 1997; Jeong and Choi, 2015). It has also been reported to have antibacterial, immune function enhancement, and anticancer effects (Ying, 1987; Kuo et al., 1996; Lin et al., 1999). For instance, Joshi et al. (2019) reported that *Cordyceps militaris* extract had antibacterial effects against *E.coli* and *S.aureus*. Cordycepin (3'-deoxyadenosine) which is isolated from *Cordyceps militaris*, is a novel metabolite (Park et al., 2005; Cheng et al., 2016) that have been reported to inhibit cancer cells such as prostate cancer, colon cancer, and liver cancer by activating reduced immune function while being involved in the genetic information of cells (Wang et al., 2005; Lee et al., 2013a; 2013b), have antibiotic, anti-inflammatory and antioxidant effects (Won and Park, 2005; Lee et al., 2006). These outstanding biological and pharmacological has seen its listing as a physiologically active substance in the second class by the Ministry of Food and Drug Safety, Korea. Interestingly, *Cordyceps militaris* has been reported to have a similar positive effect on poultry, pigs, and cattle. The addition of *Cordyceps militaris* to the weaning piglets feeds improved growth performance and intestinal environment (Boontiam et al., 2020). Han et al. (2015) reported that fermentation products of *Cordyceps militaris* had a positive effect on growth in a broiler. In addition, *Cordyceps militaris* has shown positive effects in Nile tilapia (Van Doan et al., 2017). However, there are limited studies on the antioxidant and antibacterial effects of Cordyceps in animals. Therefore, this study was conducted to investigate the effect of *Cordyceps militaris,* as a feed additive, on broiler growth performance, meat quality, and storage characteristics. In addition, we investigated cordycepin content in the broilers' breast meat and to confirm the possibility of producing functional chicken meat containing cordycepin.

Materials and Methods

The experimental protocol of this study was approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (CBNUA-1477-20-02).

Cordyceps militaris with probiotics (multi-species probiotics, *cordyceps militaris*)

The manufacturing method of *Cordyceps militaris* with probiotics (CMP), probiotics are inoculated into the sterilized medium and red ginseng meal raw materials by expanding soybean meal and corn at 130℃, and the whole is secondarily fermented for more than 50 hours. After that, it was dried at low temperature to add *cordyceps militaris*. CMP contains multi-species probiotics of *Bacillus subtilis*, *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Lactobacillus acidophillus*, and *Streptococcus thermophilus*.

Animals and experimental design

At the start of the test, the one-day-old broilers body weight was 42.1 ± 0.1 g, and it was performed for 4 weeks. The test design was divided into 1) CON (basal diet), 2) CMP (CON + *cordyceps militaris* with probiotics). Based on the initial body weight, and a total of 60 broilers (Ross 308) were randomly placed at 5 per treatment group with 6 per cage. The basal diet was formulated based on the NRC (1994) requirements for poultry. Compositions of basal diets are shown in Table 1 that was based on the basal diet composition table of Oh et al. (2020). The feed was fed freely, and the water was adjusted so that it could be eaten freely using automatic water supply.

Table 1. Compositions of basal diets (as-fed-basis)[x].

Ingredient (%)	Content	
	Phase 1 (0 - 2 week)	Phase 2 (2 - 4 week)
Corn	46.16	57.75
Soybean meal, 48% CP	36.04	28.00
Wheat	10.00	10.00
Wheat bran	4.50	-
Corn gluten meal, 60% CP	-	0.80
L-lysine	-	0.140
DL-methionine	0.20	0.21
Choline chloride	0.20	0.20
Limestone	1.00	1.00
Dicalcium phosphate	1.50	1.50
Salt	0.20	0.20
Vitamin premix[y]	0.10	0.10
Mineral premix[z]	0.10	0.10
Calculated composition		
ME ($Kcal \cdot kg^{-1}$)	3,220	3,283
CP (%)	23.00	20.04
Lysine (%)	1.275	1.151
Methionine (%)	0.55	0.527
Ca (%)	0.80	0.80
P (%)	0.64	0.64

CP, crude protein; ME, metabolizable energy.

[x] Based on the basal diet composition table of Oh et al. (2020).

[y] Contained per kg of diet: Vit A, 12,000 IU; vit D_3, 2,500 IU; vit E, 25 mg; vit K_3, 0.7 mg; thiamine, 1 mg; riboflavin, 12 mg; pyridoxine, 2 mg; vit B_{12}, 0.03 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; folic, 0.5 mg; biotin, 0.085 mg; ethoxyquin, 1,700 mg.

[z] Contained per mg per kg of diet: Fe, 35 mg; Zn, 60 mg; Mn, 85 mg; Cu, 70 mg; I, 1.6 mg; Se, 0.1 mg.

Sample analysis and measurements

The body weight gain (BWG) was calculated by measuring body weight for each individual on the 1st, 14th, and 28th day of the experiment. Feed intake (FI) was calculated by excluding the remaining amount from the feeding amount when measuring body weight, and the feed conversion ratio (FCR) was calculated by dividing the BWG by the FI. Water holding capacity (WHC) was analyzed according to the method of Laakkonen et al. (1970). Drip loss (DL) was measured as the weight ratio (%) of the initial sample by measuring the DL generated while shaping a 2 cm thick breast in a circular shape, putting it in a polypropylene bag, vacuum-packing it, and storing it in a refrigerator at 4℃ for 24 hours. Cooking loss (CL) was calculated by measuring the weight after shaping a 3 cm thick chicken breast into a circular shape, heating it to a core temperature of 70℃ in a hot water heater, allowing it to cool for 30 minutes. The meat color was measured with a spectro colormeter (Model JX-777, Color Techno. System Co., Tokyo, Japan) standardized with a white plate (lightness; L*, 94.04; redness; a*, 0.13; yellowness; b*,-0.51). At this time, a white fluorescent lamp (D65) was used as the light source. Shearing force (SF) was subjected to a shear force cutting test using a Rheometer (Compac-100, Sun Scientific co., Tokyo, Japan). The program used was R.D.S (Rheology Data System) Ver 2.01 (Sun Scientific Co., Tokyo, Japan). The pH was homogenized using a homogenizer (Bihon seiki, Ace, Osaka, Japan) and then measured with a pH meter (Mteeler Delta 340, Mettler-toledo, Ltd., Cambridge, UK). Thiobarbituric acid reactive substances (TBARS) was measured by the 2-thiobarbituric acid

(TBA) value according to the extraction method of Witte et al. (1970). The cordycepin content in the meat is degreased to extract the cordycepin contained in the chicken meat, then put 1 g of a sample into a glass container with a stopper, pour 100 mL of distilled water for high performance liquid chromatography (HPLC), and shake well to mix evenly. After that, the mixture was extracted with stirring at a speed of 90 rpm for 12 hours in a shaking water bath at 90℃. After cooling the extracted sample at room temperature, it was filtered using a filter paper, and 50 mL of the filtrate was put in an erlenmeyer flask, and 100 mL of acetone for HPLC was well mixed and shaken, and then cooled at 4℃ for 12 hours and filtered. The filtered sample was concentrated under reduced pressure to obtain a dried product, dissolved in 5 mL of HPLC water, filtered through a 0.45 μm membrane filter, and subjected to HPLC analysis.

Statistical analysis

All data were determined differences among means were Separated with repeated t-test using the least squares means procedure of SAS (SAS Institute, Cary, NC, USA). All statistical analysis differences were taken to be significant at $p < 0.05$, and $p < 0.01$ were considered near significant.

Results

Growth performance

Table 2 shows the effect of addition of CMP in broiler feed for a total of 28 days on the growth performance of broilers. At the end of the experiment, the CMP treatment showed significantly ($p < 0.05$) higher body weight than the control group. The CMP treatment showed significantly higher ($p < 0.05$) BWG than the control group on the entire period. The FI and FCR were significantly lower ($p < 0.05$) in the CMP treatment group than in the control group. The FCR tended to decrease ($p = 0.038$) with the addition of CMP over the entire period (1 - 28 d).

Meat quality characteristics

Table 3 shows the effect of the addition of CMP in feed on meat quality characteristics in broiler. At the end of the experiment, there was no significant difference ($p > 0.05$) on water content and DL in breast meat among treatment groups. In WHC, CMP treatment showed significantly higher results ($p < 0.05$). In CL, the CMP treatment showed significantly the lowest results ($p < 0.05$). In meat color, L* value, b* value did not show a significant difference between treatment groups ($p > 0.05$), but a* value was significantly lower in the CMP treatment group ($p < 0.05$) than the control group. The SF was significantly higher ($p < 0.05$) in the CMP treatment than the control group.

Meat quality storage characteristics

Table 4 shows the effect of the addition of CMP in feed on the storage properties of meat quality in broilers. The pH was significantly increased ($p < 0.05$) in CMP treatment compared to the control group. TBARS, which measures the degree of rancidity of fat, showed the lowest value in the CMP treatment compared to the control group ($p < 0.05$).

Table 2. Effect of supplementation CMP on growth performance in broiler.

Item	CON	CMP	SE	p-value
BW (g)				
Initial	42.2	42.2	0.3	0.406
14 day	390b	400a	3	0.042
Final	1,211	1,232	17	0.032
BWG (g)				
1 - 14 day	349b	358a	3	0.021
15 - 28 day	820	832	15	0.032
1 - 28 day	1,169	1,190	17	0.001
FI (g)				
1 - 14 day	388a	382b	1	< 0.0048
15 - 28 day	1,268	1,254	7	0.010
1 - 28 day	1,656	1,637	7	0.008
FCR ($g \cdot g^{-1}$)				
1 - 14 day	1.112a	1.069b	0.008	0.001
15 - 28 day	1.555	1.512	0.026	0.030
1 - 28 day	1.421	1.378	0.018	0.038

CON, basal diet; CMP, basal diet + 0.5% *Cordyceps militaris* with Probiotics supplement; SE, standard errors; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Table 3. Effect of CMP on meat quality characteristics of chicken breast from broiler.

Item	CON	CMP	SE	p-value
Water content (%)	75.58	75.40	0.22	0.791
Water holding capacity (%)	54.21b	62.81a	0.56	< 0.001
Drip loss (%)	3.05	3.04	0.13	0.885
Cooking loss (%)	15.28a	13.71b	0.40	< 0.001
Hunter color				
L*	50.01	49.64	0.81	0.612
a*	5.25a	4.90b	0.35	< 0.001
b*	9.48	9.32	0.44	0.700
Shearing force (g)	1,523b	2,101a	48	< 0.001

CON, basal diet; CMP, basal diet + 0.5% *Cordyceps militaris* with probiotics supplement; SE, standard errors; L*, lightness; a*, redness; b*, yellowness.

a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Table 4. Effect of CMP on storage characteristics of chicken breast from broiler.

Item	CON	CMP	SE	p-value
pH	5.82b	5.88a	0.01	0.04
TBARS (mg $MA \cdot kg^{-1}$)	0.27a	0.13b	0.01	< 0.002

CON, basal diet; CMP, basal diet + 0.5% *Cordyceps militaris* with probiotics supplement; SE, standard errors; TBARS, thiobarbituric acid reactive substances; MA, malondialdehyde.

a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Cordycepin content in meat

Table 5 shows the effect of the addition of CMP in feed on the content of cordycepin in meat for broilers. The cordycepin content was significantly increased ($p < 0.05$) in the CMP treatment compared to the control group.

Table 5. Effect of CMP on the composition of cordycepin in the breast from broiler.

Item	CON	CMP	SE	p-value
Cordycepin ($mg \cdot kg^{-1}$)	13.43b	14.22a	0.10	< 0.0001

CON, basal diet; CMP, basal diet + 0.5% *Cordyceps militaris* with probiotics supplement; SE, standard errors.
a, b: Means in the same row with different superscripts differ ($p < 0.05$).

Discussion

Growth performance

In this study, when CMP was added to the broiler feed, the FI decreased on the entire period, thereby improving FCR. These results were in agreement with those of Park (2011a) in which the addition of Cordyceps with fly pupa significantly increased BWG and FI in broilers (Park, 2011a). Similarly, Kang et al. (2003) reported that when 5% of feed was replaced by fermented *Cordyceps takaomontana*, the weight gain and feeding efficiency of starting broilers were significantly increased. The improved growth performance is attributed to cordycepin, ergosterol, and polysaccharides, which are physiologically active substances of Cordyceps that improve the broilers intestinal environment through improving the immunity, antioxidant and antimicrobial activities, and promote the growth of beneficial bacteria in the appendix of broilers (Lee, 1981; Furuya et al., 1983; Lin et al., 1999; Kwon, 2007; Park, 2011a). In addition, cordycepin in Cordyceps plays an important role in the early growth by enhancing the broilers' immunity (Kang et al., 2003). However, Kang et al. (2003) reported that replacing 20% of feed with Cordyceps fermented yarns significantly decreased the rate of weight gain in the finishing broilers, and the feed efficiency increased significantly in the group 10% replacement than those with 5% and 20% replacement (Kang et al., 2003). 20% of feed was replaced with fermented *Cordyceps militaris*, FI was significantly reduced, resulting in a decrease in BWG. Together, this study and previous studies' findings, demonstrate *Cordyceps militaris* to be more effective in the starting than in the finishing broiler period.

The meat WHC refers to the ability to retain moisture when an external physical force such as cutting or heat treatment is applied. It affects the softness, texture, color, and meat juiciness of the meat, and increases with protein structure change and ionic strength (Wu and Smith, 1987; Choi et al., 2009). In addition, meat color plays an important in judging the meat quality, while the lightness (L*) value is important in white muscle quality and is related to the DL and pH (Park, 2011b). Similar to a previous study, in this study, the water content and WHC were significantly higher after treatment with 0.5% of CMP, and the meat color showed significant changes, but all the values were within the normal range (Kralik et al., 2014). These findings were also in agreement with a report by Park (2011b), where 2.0% of Cordyceps with fly pupa was added in broiler feed, and as a result, the moisture content and WHC of broiler breast meat increased, but there was no significant effect on the meat color.

The storage loss refers to the submerged material generated by the formation of gaps in the muscle fibers over time after slaughter, and it is affected by pH changes; when the pH decreases, the storage loss tends to decrease (Froning et al., 1978; Barbut, 1993; Northcutt et al., 1994). In this test, since the pH value was within the normal range, it is estimated that there was no significant difference in the storage loss between treatment intervals.

Regardless of the heating method, when the meat is heated, the meat WHC decreases due to the contraction of the muscle fibers and shortening of the root excision, resulting in a low heating content (Cho et al., 2008). Also, as the storage period increases, the meat SF decreases as the softness increases, and the SF decreases increases with increase in the enzymes in the muscle, and enzymes from microorganisms; this is attributed to the increase of non-protein nitrogen compounds (Khan and Van den Berg, 1964). In the present study, the CL and SF were significantly improved with the addition of CMP, but there was no significant effect on DL. This improvement in meat quality is attributed to the enhanced immune, antioxidant, anti-inflammatory, and antibacterial effects of the cordycepin. However, more precise studies are needed because previous studies on storage loss, CL, and SF are insufficient.

Meat quality storage characteristics

The pH value is an important indicator of meat quality because it is closely related to the x meat quality aspects such as WHC and SF. According to Karaoglu et al. (2004), 1 $g \cdot kg^{-1}$ probiotic treatment in broilers showed the lowest pH value than a basal diet and 2 $g \cdot kg^{-1}$ probiotic. On the other hand, some studies reported increased pH values in broilers on a diet supplemented with probiotics (Aksu et al., 2005). In general, after slaughter, the pH in the muscle drops from pH 7.0 to 5.4 - 6.0 within 24 hours (Penny, 1977). On contrary, in a previous study, there was no significant difference in pH between the treatment groups when Cordyceps with fly pupa was added to broiler feed (Park, 2011b). Similarly, in the present study, all pH values are within the normal range. This indicates that the addition of the emulsifier did not have a significant effect on the pH.

Meat TBARS is an index indicating the degree of lipid oxidation, and it increases as lipids are decomposed by lipolytic enzymes and microbial metabolism, thereby lowering the quality of meat (Brewer et al., 1992; Raharjo and Sofos, 1993). In previous studies, the addition of Cordyceps with fly pupa in broiler feed significantly reduced the meat TBARS value meat quality, which was similar to the results of this study. The low TBARS value is believed to be a result of the antioxidative and antibacterial activity of the Cordyceps with fly pupa (Park, 2011b). Also, according to Aksu et al. (2005) report, lower TBARS values were reported in broilers on a diet supplemented with probiotics. However, it did not affect TBARS in pigs fed with probiotics. When the TBARS value of the meat is less than 0.2 mg malondialdehyde $(MA) \cdot kg^{-1}$, it is fresh while the value is above 4.0 mg $MA \cdot kg^{-1}$ is considered as rancidity (Brewer et al., 1992). In this study, all the values were within the normal range, although the addition of CMP had resulted in a significant difference in the TBARS values, and therefore the effect was thought to be insignificant.

Cordycepin content in meat

In this study, the addition of CMP to broiler feed significantly increased the cordycepin content of the breast meat. According to previous studies, the content of cordycepin in the breast and leg meat of broilers fed with *Cordyceps takaomontana* strain inoculation was twice as high as that of the controls (Kang et al., 2003). However, this study showed contrary results. This could be because, in the case of the previous study, a strain of Cordyceps takaomontana was inoculated into feed and fed to broilers, while in this study, a probiotic containing CMP was added to the feed; the difference in the CMP forms or amounts of the active ingredient could have resulted in different effects on the broilers. In addition, since *Cordyceps militaris* contain a high percentage of cordycepin, it is believed that the cordycepin is absorbed in the broilers body after ingesting Cordyceps and remains in the chicken meat. Similarly, according to Kang et al. (2003), an experiment which was conducted in an enclosed space, Paecilo-myces japonica was transmitted through airborne transmission in a CON feed experiment. This suggested that there is a high content of cordycepin in the CON. Since this study investigated the content of cordycepin in 0.5% of CMP only, more studies on the optimal amount of cordycepin feed additive are needed.

Conclusion

In this test, the addition of CMP in broiler feed improved the growth performance of broiler chickens and showed the effect of improving the WHC, CL and SF of chicken meat. Also, it improved the storage ability by reducing TBARS and showed the effect of increasing the content of cordycepin in the muscle of broiler chicken. Through this, the possibility of producing functional chicken meat containing cordycepin was confirmed, and the addition of CMP is thought to have a positive economic effect on broiler growth performance and meat quality.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

Authors Information

Jae Woo An, https://orcid.org/0000-0002-5602-5499
Ji Hwan Lee, https://orcid.org/0000-0001-8161-4853
Han Jin Oh, https://orcid.org/0000-0002-3396-483X
Yong Ju Kim, https://orcid.org/0000-0002-0960-0884
Se Yeon Chang, https://orcid.org/0000-0002-5238-2982
Young Bin Go, https://orcid.org/0000-0002-5351-6970
Dong Cheol Song, https://orcid.org/0000-0002-5704-603X
Hyun Ah Cho, https://orcid.org/0000-0003-3469-6715
Jin Ho Cho, https://orcid.org/0000-0001-7151-0778

References

Aksu MI, Karaoglu M, Esenbuga N, Kaya M, Macit M, Ockerman HW. 2005. Effect of a dietary probiotic on some quality characteristics of raw broiler drumsticks and breast meat. Journal of Muscle Foods 16:306-317.

Barbut S. 1993. Colour measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. Food Research International 26:39-43.

Boontiam W, Wachirapakorn C, Wattanachai S. 2020. Growth performance and hematological changes in growing pigs treated with *Cordyceps militaris* spent mushroom substrate. Veterinary World 13:768-773.

Brewer MS, Ikins WIG, Harbers CAA. 1992. TBA values, sensory characteristics, and volatiles in ground pork during long‐term frozen storage: Effects of packaging. Journal of Food Science 57:558-563.

Cheng YH, Wen CM, Dybus A, Proskura WS. 2016. Fermentation products of *Cordyceps militaris* enhance performance and modulate immune response of weaned piglets. South African Journal of Animal Science 46:121-128.

Choi YS, Choi JH, Han DJ, Kim HY, Lee MA, Kim HW, Jeong JY, Kim CJ. 2009. Characteristics of low-fat meat emulsion systems with pork fat replaced by vegetable oils and rice bran fiber. Meat Science 82:266-271.

Choi YY, Lee YY, Jung YY, Kwon TY, Chung YJ. 2004. Properties in formation of protein-binding polysaccharide. Korean Journal of Food and Nutrient 17:177-185. [in Korean]

Cho SH, Kim JH, Seong PN, Cho YM, Chung WT, Park BY, Chung MO, Kim DH, Lee JM, Ahn CN. 2008. Physico-chemical meat quality properties and nutritional composition of Hanwoo steer beef with 1++ quality grade. Food Science of Animal Resources 28:422-430. [in Korean]

Froning GW, Babji AS, Mather FB. 1978. The effect of preslaughter temperature, stress, struggle and anesthetization on color and textural characteristics of turkey muscle. Poultry Science 57:630-633.

Furuya T, Hirotani M, Matsuzawa M. 1983. N6-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelia of Cordyceps and Isaria species. Phytochemistry 22:2509-2512.

Han JC, Qu HX, Wang JG, Yan YF, Zhang JL, Yang L, Zhang M, Cheng YH. 2015. Effects of fermentation products of *Cordyceps militaris* on growth performance and bone mineralization of broiler chicks. Journal of Applied Animal Research 43:236-241.

Hashemi SR, Davoodi H. 2010. Phytogenics as new class of feed additive in poultry industry. Journal of Animal and Veterinary Advances 9:2295-2304.

Jeong JW, Choi YH. 2015. Anti-cancer properties and relevant mechanisms of Cordyceps, an active ingredient of the insect fungus Cordyceps spp. Journal of Life Science 25:607-614. [in Korean]

Joshi M, Sagar A, Kanwar SS, Singh S. 2019. Anticancer, antibacterial and antioxidant activities of *Cordyceps militaris*. Indian Journal of Experimental Biology 57:15-20.

Kang HS, Lee KW, Kim SK, Cho BW, Shin TS, Jeon HY, Son JH. 2003. Effects of inoculated diet with *Paecilomyces japonica* on broiler performance. Korean Journal of Poultry Science 30:49-54. [in Korean]

Karaoglu M, Aksu MI, Esenbuga N, Kaya M, Macit M, Durdag H. 2004. Effect of dietary probiotic on the pH and colour characteristics of carcasses, breast fillets and drumsticks of broilers. Animal Science 78:253-259.

Khan AW, Van den Berg L. 1964. Some protein changes during post‐mortem tenderization in poultry meat. Journal of Food Science 29:597-601.

Koo BS, Lee MS. 2004. Effects of *Cordyceps militaris* extract powder on plasma lipids and glucose in rats. Journal of the Korean Society of Food Culture 19:217-222. [in Korean]

Kralik G, Djurkin I, Kralik Z, Skrtic Z, Radisic Z. 2014. Quality indicators of broiler breast meat in relation to colour. Animal Science Papers and Reports 32:173-178.

Kuo YC, Tsai WJ, Shiao MS, Chen CF, Lin CY. 1996. *Cordyceps sinensis* as an immunomodulatory agent. The American Journal of Chinese Medicine 24:111-125.

Kwon HH, Shin S, Lee S, Lee S. 2007. Inununomodulatory effect of CMB, water extract of *Cordyceps Militaris* fruit bodies: Enhancement of cytokines and bioactive substances production through macrophage activation. Fall General Meeting and Academic Conference, The Pharmaceutical Society of Korea, Seoul, Korea.

Laakkonen E, Wellington GH, Sherbon JN. 1970. Low‐temperature, long‐time heating of bovine muscle 1. Changes in tenderness, water‐binding capacity, pH and amount of water‐soluble components. Journal of Food Science 35:175-177.

Lee H, Kim YJ, Kim HW, Lee DH, Sung MK, Park T. 2006. Induction of apoptosis by *Cordyceps militaris* through activation of caspase-3 in leukemia HL-60 cells. Biological and Pharmaceutical Bulletin 29:670-674.

Lee HH, Park C, Jeong JW, Kim MJ, Seo MJ, Kang BW, Park JU, Kim GY, Choi BT, Choi HY, Jeong YK. 2013a. Apoptosis induction of human prostate carcinoma cells by cordycepin through reactive oxygen species-mediated mitochondrial death pathway. International Journal of Oncology 42:1036-1044.

Lee SI. 1981. Bonchohak. Suseowon, Seoul, Korea. [in Korean]

Lee SY, Debnath T, Kim SK, Lim BO. 2013b. Anti-cancer effect and apoptosis induction of cordycepin through DR3 pathway in the human colonic cancer cell HT-29. Food and Chemical Toxicology 60:439-447.

Lin CY, Ku FM, Kuo YC, Chen CF, Chen WP, Chen A, Shiao MS. 1999. Inhibition of activated human mesangial cell proliferation by the natural product of *Cordyceps sinensis* (H1-A): An implication for treatment of IgA mesangial nephropathy. Journal of Laboratory and Clinical Medicine 133:55-63.

Northcutt JK, Foegeding EA, Edens FW. 1994. Water-holding properties of thermally preconditioned chicken breast and leg meat. Poultry Science 73:308-316.

NRC (National Research Council). 1994. Nutrient requirement of poultry, 9th rev. ed. NRC, Washington, D.C., USA.

Oh HJ, Song MH, Yun W, Lee JH, An JS, Kim YJ, Kim GM, Kim HB, Cho JH. 2020. Effects of replacing soybean meal with perilla seed meal on growth performance, and meat quality of broilers. Journal of Animal Science and Technology 62:495-503.

Ohmori T, Tamura K, Fukui K, Kawanishi G, Mitsuyama M, Nomoto K, Miyazaki T. 1989. Isolation of galactosaminoglycan moiety (CO-N) from protein-bound polysaccharide of *Cordyceps ophioglossoides* and its effects against murine tumors. Chemical and Pharmaceutical Bulletin 37:1019-1022.

Park BS. 2011a. Effect of feeding cordyceps with fly pupa on growth performance in broiler chickens. Journal of Life Science 21:1541-1548. [in Korean]

Park BS. 2011b. Effect of feeding diet containing cordyceps with fly pupa on blood lipid, fatty acid and TBARS in broiler chickens. Journal of the Korean Applied Science and Technology 28:273-283. [in Korean]

Park C, Hong SH, Lee JY, Kim GY, Choi BT, Lee YT, Park DI, Park YM, Jeong YK, Choi YH. 2005. Growth inhibition of U937 leukemia cells by aqueous extract of *Cordyceps militaris* through induction of apoptosis. Oncology Reports 13:1211-1216.

Penny IF. 1977. The effect of temperature on the drip, denaturation and extracellular space of pork longissimus dorsi muscle. Journal of the Science of Food and Agriculture 28:329-338.

Raharjo S, Sofos JN. 1993. Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: A review. Meat Science 35:145-169.

Shimizu DS. 1997. Illustrated vegetable wasps and plant worms in colour, 1-437. IeNoHikari Association, Tokyo, Japan.

Shim JY, Lee YS, Lim SS, Shin KH, Hyun JL, Kim SY, Lee EB. 2000. Pharmacological effects of Paecilomyces tenuipes. Korean Journal of Pharmacognosy 31:163-167. [in Korean]

Van Doan H, Hoseinifar SH, Dawood MA, Chitmanat C, Tayyamath K. 2017. Effects of *Cordyceps militaris* spent mushroom substrate and Lactobacillus plantarum on mucosal, serum immunology and growth performance of Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology 70:87-94.

Wang BJ, Won SJ, Yu ZR, Su CL. 2005. Free radical scavenging and apoptotic effects of *Cordyceps sinensis* fractionated by supercritical carbon dioxide. Food and Chemical Toxicology 43:543-552.

Witte VC, Krause GF, Bailey ME. 1970. A new extraction method for determining 2 ‐ thiobarbituric acid values of pork and beef during storage. Journal of food Science 35:582-585.

Won SY, Park EH. 2005. Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. Journal of Ethnopharmacology 96:555-561.

Wu FY, Smith SB. 1987. Ionic strength and myofibrillar protein solubilization. Journal of Animal Science 65:597-608.

Ying CC. 1987. Icons of medicinal fungi from China. Science Press, Beijing, China.

Yong-Lu L, Yin L, Jun-Wang Y, Chang-Xiao L. 1997. Studies on pharmacological activities of cultivated *Cordyceps sinensis*. Phytotherapy Research: An International Journal Devoted to Medical and Scientific Research on Plants and Plant Products 11:237-239.

Zhu JL, Liu C. 1992. Modulating effects of extractum semen Persicae and cultivated *Cordyceps hyphae* on immunodysfunction of inpatients with posthepatitic cirrhosis. Chinese Journal of Integrated Traditional and Western Medicine 12:207-209.