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Effects of Dietary Supplementation of Enzyme complex on Growth Performance, Carcass Characteristics and Meat storability in Broiler Chickens

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사료 내 복합효소제의 첨가가 육계 생산성 및 도체특성 및 저장성에 미치는 영향

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Abstract This study was conducted to investigate the effects of dietary supplementation of enzyme complex (metalloprotease and xylanase) on growth performance, carcass characteristics and meat storability of broiler chickens. The experiment utilized a 2 (basal and low spec diets) × 3 (0, 0.5 or 1 g/kg of enzyme supplementation) factorial arrangement. A total of 360 male Ross broilers were randomly assigned into the following six groups: BD-T0 (basal diet + enzyme complex 0 g/kg), BD-T1 (basal diet + enzyme complex 0.5 g/kg), BD-T2 (basal diet + enzyme complex 1 g/kg), LD-T0 (low spec diet + enzyme complex 0 g/kg), LD-T1 (low spec diet + enzyme complex 0.5 g/kg), LD-T2 (low spec diet + enzyme complex 1 g/kg). Feed and water were provided ad libitum for 42 days, during which time feed intake and body weight were measured at one-week intervals. After the feeding trial, chickens were slaughtered by exsanguination and samples were collected. Feed intake was lower in the enzyme supplemented groups than the non-supplemented groups ($p<0.05$), as were body weight and gain ($p<0.05$). Thus, the feed conversion ratio improved in the enzyme supplemented groups relative to the non-supplemented groups ($p<0.05$). The carcass rate and production index were also significantly improved by supplementation with enzyme complex ($p<0.05$). Finally, the thiobarbituric acid reactive substances (TBARS) values were lower in the enzyme supplementation groups after 6 storage days ($p<0.05$). In conclusion, dietary supplementation with enzyme complex including metalloprotease and xylanase improved growth performance, carcass characteristics, and storability of broiler chicken meat.

요 약 본 연구는 육계사료 내 금속단백질분해효소와 xylanase를 포함하는 복합 효소제 첨가 급여가 육계 생산성, 도체특성 및 계육 저장성에 미치는 영향을 조사하기 위하여 실시하였다. 실험 디자인은 2 (기초사료, 저영양사료) x 3 (0, 0.5, 1 g/kg 효소첨가) 요인 배치로 설계하였다. 실험동물은 ross broilers 수컷 360수를 공시하여 다음의 6개의 시험 그룹에 완전임의 배치하였다. BD-T0(기초사료+복합효소제 0 g/kg), BD-T1(기초사료+복합효소제 0.5 g/kg), BD-T2(기초사료+복합효소제 1 g/kg), LD-T0(저영양사료+복합효소제 0 g/kg), LD-T1(저영양사료+복합효소제 0.5 g/kg), LD-T2(저영양사료+복합효소제 1 g/kg). 사료와 물은 42일 동안 자유채식을 실시하였으며, 사료섭취량과 체중은 1주 간격으로 측정하였다. 사양시험 종료 후에 방혈로 도살하고 분석에 필요한 시료를 채취하였다. 사료 섭취량은 효소첨가 그룹이 효소 비첨가 그룹에 비해서 유의하게 감소하였으며, 체중과 일당증체량은 효소첨가 그룹이 효소 비첨가 그룹에 비하여 유의하게 증가하였다. 따라서 사료요구율은 복합효소첨가에 의해 유의하게 개선되었다($p<0.05$). 복합효소첨가 급여에 의하여 도체율과 생산지수 또한 유의하게 개선되었다($p<0.05$). TBARS는 계육 저장 6일차에 효소첨가 그룹이 효소 비첨가 그룹보다 낮은 값을 나타내었다($p<0.05$). 본 연구의 결과를 종합해보면, 사료 내 복합효소제의 첨가 급여는 생산성과 도체성적 그리고 저장성을 유의하게 개선시켰다.

Keywords : Metalloprotease, Xylanase, Growth performance, Meat storability, Broiler chickens

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1. Introduction

Recently, alternative resources of fossil fuel have been widely developed due to an expansion of problems on the abnormal climate and reduction of carbon. Use of corn as an alternative material of fossil fuel is steadily increasing to produce bio-energy. Unfortunately, corn is one of the primary feed ingredients in livestock. Increased use of corn for bio-energy production makes more difficult to supply corn for animal feed. In addition, the increase in international oil price and an abrupt exchange rate fluctuation cause to the increase of the grain price and transportation costs. Higher grain price and transportation costs lead to an increase of production costs for livestock products. To solve these problem, in recent decades, many researchers has been conducting various studies to demonstrate the effects of using of probiotics, enzymes, and functional materials on productivity improvement in livestock [1-3]. These researches suggest that use of enzyme to increase the digestibility of nutrients has been considerably significant [4,5].

Some feed ingredients, which has the high insoluble nutrients, result in slow growth rate in livestock because digestion and absorption of nutrients is inefficient in digestive tract [6,7]. Various exogenous breakdown enzymes such as β-glucanase, xylanase, pectinase, protease, cellulase, phytase, and amylase have been used to elevate the usefulness of unavailable nutrients such as non-starch polysaccharide (NSP) in monogastric animal [8,9]. Dietary supplementation of these exogenous enzymes has been reported to have some positive effects such as decline of feed cost, enhancement of productivity, and the reduction of environmental burden through decreasing livestock execrations [9-14].

The digestive enzymes (metalloprotease) having exorcist spider (*Nephila clavata*), which mainly inhabits in South Korea, Japan except for Hokkaido, Taiwan, and China, are known that have high biological activity in a widely ranges of temperature and pH. The metalloprotease derived from bacteria, *Serratia proteamaculans*, isolated from this spider has high proteolytic activity, and the activity is increased by 3 or 4 times under the presence of metal ions. Metalloprotease also has an anti-inflammatory function decomposing the inflammation substance [15].

Xylan is a group of hemicelluloses that are found in plant cell walls. It is degraded by xylanase [16]. However, monogastric animals have no these digestive enzyme. Dietary supplementation of xylanase increases the availability of NSP in the digestive tract of broiler [17]. We hypothesized that dietary supplementation of metalloprotease may make it increased of feed efficiency, and the use of xylanase enables the improvement of NSP availability.

Thus, the aim of this study was to investigate the effects of dietary supplementation of metalloprotease and xylanase on growth performance, carcasses characteristics and meat quality in broiler chickens.

2. Materials and Methods

2.1 Experimental design and diets

All experimental animals were cared for according to the Guide for the Care and Use of Laboratory Animals (Animal Care Committee, Hankyong National University). A total of 360 of one-day-old male Ross broiler chicks were obtained from a local hatchery, and were randomly allotted to 18 floor pens. A floor space of 0.105 m^2 per bird was allowed in an open sided house. The treatments were divided into 2 by 3 factorial experimental design as follows; the experimental diets consist of 2 level energy diets (basal diet (BD) and low-spec diet (LD)) and 3 doses (0 (T0), 0.5 (T1) or 1 (T2) g/kg) of an enzyme complex. The BD was formulated to meet KRC (2007) requirements, and the LD was formulated with lower protein and energy levels compared to basal diet. Ingredients and chemical composition of experimental diets are shown

in Table 1. Used enzyme complex in this study was including metalloprotease 340,000 IU and xylanase 300,000 IU per kg. Feed and water were available *ad libitum* for 42 days.

Table 1. Ingredients and chemical composition of experimental diets

	Basal Diets (BD)		Low spec Diet (LD)	
	1-21 d	22-42 d	1-21 d	22-42 d
Ingredients, %				
Corn	40.56	41.08	44.36	46.52
Wheat	18	18	18	18
Soybean meal	31.9	31.3	30.3	28.3
Beef tallow	5.91	6.62	3.64	4.09
Limestone	0.8	0.3	0.9	0.4
Dicalcium phosphate	1.7	1.9	1.7	1.9
Salt	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.025	0.024	0.025	0.024
Lysine(powder)	0.17	-	0.18	-
DL-Methionine	0.26	0.18	0.22	0.17
L-Threonine	0.07	-	0.07	-
Vitamin mixture[1)]	0.1	0.1	0.1	0.1
Mineral mixture[2)]	0.1	0.1	0.1	0.1
Choline(liquid)	0.1	0.05	0.1	0.05
Clopidol	0.05	-	0.05	-
Salinomycin	-	0.1	-	0.1
Total	100	100	100	100
Chemical composition				
Moisture, %	12.3	12.3	12.7	12.7
Crude protein, %	21	19	20.5	18
Ether extracts, %	7.73	8.44	5.61	6.11
Crude fiber, %	3.78	3.75	3.78	3.71
Crude ash, %	5.96	5.59	5.96	5.49
Ca, %	0.89	0.74	0.92	0.77
P, %	0.6	0.63	0.6	0.63
ME, kcal/kg	3,100	3,150	3,000	3,050

[1)]Vitamin mixture(content per kg diet): vit A, 12,500 IU; Vit D3, 5,000 IU; Vit E, 70 mg; Vit K3, 4 mg; Vit B1, 4 mg; Vit B2, 8 mg; Vit B6, 3.644 mg; Vit B12, 30 μg; Calcium pantothenate, 19.6 mg; Niacin, 70 mg; Folic acid, 1.94 mg; Biotin, 0.2 mg; Oxytrap, 25 mg.
[2)]Mineral mixture(content per kg diet): Mn, 100 mg; Zn, 80 mg; Se, 0.3 mg; Fe, 80 mg; Cu, 40 mg; I, 1 mg,; Co, 0.2 mg.

2.2 Growth performance

The body weight and feed intake were recorded weekly, and body weight gain and feed conversion ratio (FCR) were calculated from those data. Production index was calculated using the following equation;

Production index = (livability × body weight) / (FCR × feeding day) x 100

2.3 Carcass characteristics

At the end of experiment (42days), three chicks corresponding to the average weight from each group were selected, and were slaughtered by exsanguination. The carcass weight was measured after the removal of head, feet, and internal organs. Then breast and thigh meat were harvested and weighed.

2.4 Storability test in meat

Changes in thiobarbituric acid reactive substances (TBARS) and volatile basic nitrogen (VBN) values of breast and thigh meat were measured during storage period (0 day, 3 day, and 6 day). The TBARS value was determined as described by Reitznerova et al. [18] and expressed as mg malonaldehyde equivalents per kg sample. Briefly, 5 g of meat sample was added to 45 mL of 20% trichloroacetic acid (in 2 M phosphate solution) and homogenized using Ultra-Turrex T25 tissue homogenizer (Janke and Kenkel, IKA, Labor Tecnik, Germany), and the solution was filtered through Whatman No. 1 filter paper. After mixing 5 mL of filtrate with 5 mL of 2-thiobarbituric acid (0.005 M in water) in test tube, the test tubes were kept at room temperature in the dark for 15 h, and measured the absorbance at 531 nm using spectrophotometer (X-ma 1000, Human Co., Korea). VBN was measured by modified micro-diffusion assay according to the Standards for Processing & Ingredient Specifications of Livestock Product method (SPISLP, 2011) [19], and was calculated by following;

$$VBN\ (mg\%) = [(a-b)\times(0.14\times f\times 100\times d)/S]$$

where, a is the sample titer, b is the blank titer, f is a reagent factor, d is the sample dilution, and S is sample weight (g).

2.6 Statistical analysis

The statistical analysis of data was performed using SPSS software program (ver. 17.0). Growth performance, carcass characteristics, and storability among treatment groups were analyzed by 2 × 3

Table 2. Effects of dietary supplementation of enzyme complex on the growth performance in broiler chickens

Items	Treatments[1]						SEM[2]	*P* value		
	BD-T0	BD-T1	BD-T2	LD-T0	LD-T1	LD-T2		Diet (D)	TRT (T)	D×T
Feed intake, g	3,852	3,605	3,684	3,832	3,601	3,603	42.4	ns[4]	0.066	ns
Body weight										
Initial, g	174	172	172	174	172	173	0.3	ns	ns	ns
Final, g	2,341	2,470	2,466	2,303	2,390	2,365	19	0.018	0.012	ns
BW gain, g	2,167	2,298	2,294	2,129	2,218	2,193	19	0.017	0.011	ns
FCR[3]	1.78	1.57	1.61	1.8	1.62	1.65	0.03	ns	0.014	ns
Production index	298	360	351	289	337	337	8.85	ns	0.017	ns

[1]BD-T0, basal diet + 0 g/kg enzyme complex; BD-T1, basal diet + 0.5 g/kg enzyme complex; BD-T2, basal diet + 1 g/kg enzyme complex; LD-T0, low spec diet + 0 g/kg enzyme complex; LD-T1, low spec diet + 0.5 g/kg enzyme complex; LD-T2, low spec diet + 1 g/kg enzyme complex
[2]SEM, Standard Error Mean
[3]FCR, Feed Conversion Ratio
[4]ns, non-significant (p>0.05)

factorial ANOVA. Significant differences on the change of meat storability as the time passed were analyzed by repeated-ANOVA. The significant differences among treatment groups were considered at P<0.05.

3. Results

3.1 Growth performance

The effects of dietary supplementation of enzyme complex on growth performance and production index are presented in Table 2. Although, the feed intake did not affected by the level of dietary energy and/or enzyme supplementation (p>0.05), it showed decreasing tendency by enzyme supplementation in both BD and LD groups (p=0.066). Final body weight and weight gain were significantly higher in BD group than LD group (p<0.05). The supplementation of enzymes significantly increased the final body weight and body weight gain compared to non-enzyme supplemented groups (p<0.05). However, there was no significant difference between the level of supplemented enzyme (p>0.05). Although, FCR was not affected by the dietary energy levels, it was significantly improved by enzyme supplementation (p<0.05), and production index had also similar results with FCR.

3.2 Carcass characteristics

The effects of dietary supplementation of enzyme complex on carcass rate and meat percent are presented in Table 3. There were no significant changes in carcass rate between BD and LD groups. However, the carcass rate was significantly improved by supplementation of enzyme complex (p<0.05). Meat

Table 3. Effects of dietary supplementation of enzyme complex on the carcass characteristics in broiler chickens

Items	Treatments[1]						SEM[2]	*P* value		
	BD-T0	BD-T1	BD-T2	LD-T0	LD-T1	LD-T2		Diet (D)	TRT (T)	D×T
Carcass ratio, %	73.1	76.1	75.7	74.0	75.2	75.0	0.25	ns[3]	<0.001	0.003
Meat percent, %										
Breast	20.6	22.9	23.5	23.1	22.0	22.5	0.47	ns	ns	ns
Tight	8.0	8.2	7.6	8.3	7.9	8.0	0.17	ns	ns	ns

[1]BD-T0, basal diet + 0 g/kg enzyme complex; BD-T1, basal diet + 0.5 g/kg enzyme complex; BD-T2, basal diet + 1 g/kg enzyme complex; LD-T0, low spec diet + 0 g/kg enzyme complex; LD-T1, low spec diet + 0.5 g/kg enzyme complex; LD-T2, low spec diet + 1 g/kg enzyme complex
[2]SEM, Standard Error Mean
[3]ns, non-significant (p>0.05)

percent was not affected by diet treatment and/or enzyme treatment.

3.3 Storability test in meat

The changes of TBARS and VBN of broiler breast and thigh meat during the storage periods were presented in Table 4 and 5. The values of TBARS and VBN were significantly increased in all groups as storage times passed (p<0.05). The TBARS of breast meat was significantly higher in BD groups compared to LD group at 0 and 6th day of storage (p<0.05). Although there was no significant difference between BD and LD groups, the TBARS in BD groups was observed to be higher than those of LD groups at 3rd day of storage. And, TBARS in thigh meat of BD groups also showed the higher value than LD groups at 6th day of storage (p<0.05). The dietary supplementation of enzyme complex did not affect to the changes in TBARS in thigh meat during overall storage periods. However, the value of TBARS in breast meat was significantly decreased in enzyme supplemented groups compared to non-supplemented enzyme groups at 6th day of storage (p<0.05).

Table 4. Changes in TBARS (mg malondialdehyde/kg sample)[1)] of breast and thigh meat in broiler chickens during storage periods

Items	Treatments[2)]						SEM[3)]	*P* value		
	BD-T0	BD-T1	BD-T2	LD-T0	LD-T1	LD-T2		Diet (D)	TRT (T)	D×T
Breast										
0 day	0.26x	0.33x	0.35x	0.23x	0.24x	0.19x	0.02	0.003	ns[4)]	ns
3 day	0.35x	0.38x	0.36x	0.26x	0.31x	0.23x	0.03	ns	ns	ns
6 day	0.97y	0.87y	0.72y	0.76y	0.70y	0.71y	0.03	0.003	0.013	ns
Thigh										
0 day	0.33x	0.38x	0.33x	0.30x	0.26x	0.25x	0.02	ns	ns	ns
3 day	0.70y	0.72y	0.61y	0.61y	0.61y	0.52x	0.02	0.038	ns	ns
6 day	1.07z	1.07z	0.95z	0.97z	0.89z	0.85y	0.03	0.014	ns	ns

[1)]TBARS, thiobarbituric acid reactive substances
[2)]BD-T0, basal diet + 0 g/kg enzyme complex; BD-T1, basal diet + 0.5 g/kg enzyme complex; BD-T2, basal diet + 1 g/kg enzyme complex; LD-T0, low spec diet + 0 g/kg enzyme complex; LD-T1, low spec diet + 0.5 g/kg enzyme complex; LD-T2, low spec diet + 1 g/kg enzyme complex
[3)]SEM, Standard Error Mean
[4)]ns, non-significant (p>0.05)
[x-z]Means within same column of each group without same superscripts are significantly different (p<0.05).

Table 5. Changes in VBN (mg %)[1)] of breast and thigh meat in broiler chickens during storage periods

Items	Treatments[2)]						SEM[3)]	*P* value		
	BD-T0	BD-T1	BD-T2	LD-T0	LD-T1	LD-T2		Diet (D)	TRT (T)	D×T
Breast										
0 day	24.9x	23.9x	27.3x	30.5x	26.6x	25.5x	0.83	ns[4)]	ns	ns
3 day	41.2y	40.8y	39.0x	41.0y	46.8y	41.9y	1.25	ns	ns	ns
6 day	55.4z	52.3z	56.0y	60.4z	58.6y	61.7z	1.14	0.016	ns	ns
Thigh										
0 day	30.7x	30.3x	26.6x	26.6x	28.4x	27.4x	1.33	ns	ns	ns
3 day	39.5x	39.5x	38.5x	41.9x	42.0xy	39.6x	1.04	ns	ns	ns
6 day	63.0y	68.0y	61.9y	64.4y	56.3y	57.4y	1.75	ns	ns	ns

[1)]VBN, volatile basic nitrogen
[2)]BD-T0, basal diet + 0 g/kg enzyme complex; BD-T1, basal diet + 0.5 g/kg enzyme complex; BD-T2, basal diet + 1 g/kg enzyme complex; LD-T0, low spec diet + 0 g/kg enzyme complex; LD-T1, low spec diet + 0.5 g/kg enzyme complex; LD-T2, low spec diet + 1 g/kg enzyme complex
[3)]SEM, Standard Error Mean
[4)]ns, non-significant (p>0.05)
[x-z]Means within same column of each group without same superscripts are significantly different (p<0.05).

4. Discussion

Mushtaq et al. reported that feed intake was decreased to 4.67% by addition of xylanase and glucanase in chicken diet [20], and Yu et al. also resulted that the supplementation of protease to broiler feed decreases the feed intake during growing stage (22 to 38 days old) [21]. The result of present study corresponded with those previous reports. In this study, there was no significant difference in feed intake between BD and LD groups. Normally, reducing the energy content of the diet results in an increase in the feed intake [22]. This might mean that the level of reduced dietary energy in this study was insufficient to affect the feed intake. Many previous studies had reported that the dietary supplementation of enzyme, such as xylanase, amylase and protease, improved weight gain and FCR in broiler [17,20,23,24]. Wang et al. reported the degradation effect of enzyme supplementation on dietary components [4]. In addition, Gracia et al. found that enzymes improved the retention of N and the digestibility of ether extract [25]. Meanwhile, Cowieson and Ravindran concluded that the addition of a combination of xylanase, amylase and protease into broiler diets was effective in improving productive performance despite the response of dietary component digestibility was very limited [9]. Although, the nutrient digestibility was not analyzed in this study, our results showed similar result with those previous studies on growth performance and FCR. More studies on nutrients digestibility of enzyme complex used in this study are necessary.

Wu and Ravindran found that there was no effect of the xylanase supplementation into wheat-based feed on breast muscle in chicken [23]. Kim also reported that the addition of enzymes in feeds with different energy and protein levels does not affect the meat percent in broiler chickens [26]. From the results mentioned above, we demonstrated that the dietary supplementation of metalloprotease and xylanase could improve the productivity and carcass rate without any negative influences such as reducing meat percent in broiler chickens.

Excessive reactive oxygen species (ROS) oxidize the lipid and fatty acid, and induce the oxidative stress [27], then these oxidative stress causes an inflammatory reaction [28]. In the previous study of Lee et al., they reported that metalloprotease has anti-inflammatory activity [15]. This effect of metalloprotease might be proceeded from inhibition of ROS formation. In present study, the decreased TBARS in breast meat of enzyme supplementation groups at 6th day of storage might be occurred by anti-oxidant effect of metalloprotease. The anti-oxidative mechanism of metalloprotease has to be studied further. The VBN values were higher in the LD groups than BD groups at 6th day of storage ($p<0.05$), however there was no significant difference in VBN of breast and thigh meat between supplemented group and non-supplemented group of enzyme complex during storage period ($p>0.05$). From above results, the supplementation of metalloprotease and xylanase might have positive effect for expanding the storage period for broiler meat.

5. Conclusion

The dietary supplementation of metalloprotease and xylanase improves the productivity without negative effect on decline of carcass characteristics. Furthermore, the enzyme supplementation inhibits the lipid oxidation of breast meat during storage periods. Based on those results, it can be suggested that the metalloprotease and xylanase is useful as a feed additives for broiler.

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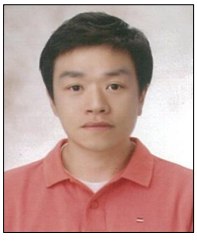

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