Indian J. Anim. Res., 52 (7) 2018 : 1014-1017
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.ijaronline.in

Effects of dietary supplementation of endo-1,4-β-D-glucanase producing *Bacillus subtilis* sp.-fermented products on growth performance in broilers

Jeayoung Kim[†1,2], Byeongwoo Kim[†3], Seongkeun Cho[†3], Kyungjin Cho[2] and Jakyeom Seo[3]*

Division of management planning, Charmfre Inc,
Buan-gun, Republic of Korea, Korea.

Received: 25-01-2017 Accepted: 16-06-2017 DOI: 10.18805/ijar.v0iOF.9117

ABSTRACT

This study was conducted to investigate the effects of dietary supplementation of Endoglucanase producing *Bacillus subtilis* sp. fermented product (EBFP) on growth performance and meat characteristics in broilers. A total of 480, 1 day old ROSS male broiler chicks were obtained from a local hatchery and randomly allotted to 1 of 4 dietary treatments with 6 replicate pens consisting of 20 chicks. Three additional diets were prepared by mixing 0.1% of commercial cellulase, 0.1%, and 0.2% of EBFP with the control diet and all chicks were fed experimental diets and water ad libitum. Feed intake and body weight (BW) were recorded at 0, 3, and 5 week of the experiment. At the end of the experiment, birds fed a diet containing 0.1% of EBFP had the highest BW compared to birds in other groups ($P < 0.01$). During 0 to 5 week the experimental period, the dietary addition of 0.1% EBFP significantly increased ($P < 0.01$) BW gain that was higher than that of birds fed the diet in which only a commercial cellulase was added. No significant difference among treatments was observed in carcass weight, carcass yield, left breast, and thigh meat. These results suggest that dietary addition of cellulolytic probiotics may enhance growth performance compared with the single use of probiotics or enzymes.

Key words: Broilers, Feed enzymes, Growth performances, Probiotics.

INTRODUCTION

The use of feed additives including enzymes and probiotics has been widely spread in poultry industry. Among feed enzymes, non-starch polysaccharide (NSP) degrading enzyme comprise 50% in entire global feed enzyme market (Paloheimo *et al.*, 2010). Especially, the NSP degrading enzyme has an important role in poultry which do not possess the suitable enzymes to digest NSP within the digestive tract. Xylanase is a main NSP degrading enzyme used in poultry feeds to reduce viscosity of digesta caused by inclusion of high viscous grains (*e.g.* barely, wheat) in diets but also β-glucanase have been widely used because grain cell walls possess abundant carbohydrates linked by β-bond (Paloheimo *et al.*, 2010).

Probiotics are defined as "a live microbial feed supplement which beneficially affects the host animal" (Fuller, 1989), have been extensively applied to poultry industry since the first demonstration of the effect of orally administrated cecal extract from mature chickens on competitive pathogen exclusion in newly hatched chicks (Rantala and Nurmi, 1973). The rapid use of probiotics to establish a proper gut microflora in newly born chicks is critical because commercial birds are born in incubators which is controlled to sterilized condition as such, they do not develop an ideal gut flora found in healthy chicken (Lutful Kabir, 2009).

We hypothesized that there might be a synergistic effect on broiler's performance if the probiotics were developed to possess β-glucanase. In this study, thus, we tried to investigate the effects of dietary supplementation of probiotics which had endo-1,4-β-D-glucanase (endoglucanase) produced by the inoculant, cellulolytic *Bacillus subtilis* sp. (Park *et al.*, 2016) on growth performance and meat characteristics in broilers.

MATERIALS AND METHODS

Experimental diets, design and feeding management of experimental birds: A total of 480, 1 day old ROSS male broiler chicks were obtained from a local hatchery and housed in a floor pen in an environmentally controlled room. Feed and water were offered to all chicks ad libitum and the room temperature was gradually decreased from 34°C on day 0 to 25°C on day finished. All chicks were randomly allotted to 1 of 4 dietary treatments with 6 replicate pens consisting of 20 chicks. A two-phase feeding program with a starter diet from 0 to 3 week and a grower from 3 to 5 week was used in this study. The experimental diets were formulated to meet NRC (1994) requirements of broilers and used as a control diet (CON). Three additional diets were prepared by mixing 0.1% of commercial cellulase (Gwangdong Inc., China), 0.1%, and 0.2% of EBFP with

*Corresponding author's e-mail: jseo81@pusan.ac.kr
†These authors contributed equally to this study as the first authors.
[1]Division of Management Planning, Charmfre Inc., Buan-gun, Republic of Korea, [2] Genebiotech Inc., Seoul, Republic of Korea
[3]Life and Industry Convergence Research Institute, Department of Animal Science, Pusan National University, Miryang 50463, Republic of Korea

CON. Commercial cellulase was added to CON to prepare positive control (PC) diet.

To prepare endoglucanase producing *Bacillus subtilis* sp. fermented product (EBFP) in this experiment, endo-1,4-β-D-glucanase producing bacteria, *Bacillus subtilis* sp. were isolated from salted clams in previous study (Park *et al.*, 2016) and used as a inoculant. Selected bacteria was grown in 500 mL Erlenmeyer flasks containing 100 mL of liquid media (g/L of distilled water, 10 nutrient broth [Sigma and Aldrich, St. Louis, USA]) at 37°C for 12 h. To produce EBFP, the solid-state fermentation method was used. Briefly, the mixture of soybean meal and wheat bran in 6:4 ratio was prepared as a substrate. After the substrate was autoclaved at 121°C for 30 min, 1 kg of substrate, 10 mL of prepared inoculum, and 1 L of distilled water were thoroughly mixed in fermentation vessel. After mixing, the vessel was covered with 4 layer of cheese cloth then, incubated at 35°C for 72 h. At the end of the fermentation, EBFP was dried at 50°C for 2 days and stored at 4°C until used in this experiment.

Performance parameters: Feed intake and body weight (BW) recorded at 0, 3, and 5 week of the experiment. Feed conversion ratio was calculated as feed intake divided by body weight gain. Four birds with a BW close to the mean BW of each pen (24 birds per treatment), were killed at the end of the experiment then carcass weight, breast, and thigh meat were recorded.

Chemical analysis: The ingredients and chemical composition of experimental diets were listed in Table 1. Contents of dry matter ([DM], #930.15), crude protein ([CP], #990.03), ether extract ([EE], #920.39), crude fiber ([CF], #978.10), and ash (#942.05) were analyzed as described by AOAC (2005).

Statistical analysis: The data of growth performance and carcass characteristics were analyzed with PROC MIXED(SAS Institute Inc., Cary, NC). The fixed effect in this model was feed treatment and the random effect was animals within treatment. Statistical significance was declared at $P < 0.05$, and a trend was discussed at $0.05 \leq P < 0.1$.

RESULTS AND DISCUSSION

Effects of dietary supplementation of EBFP on growth performance and carcass characteristics were listed in Table 2. The initial BW of birds were not differed by treatments. At the end of the experiment, birds fed a diet containing 0.1% of EBFP had the highest BW compared to birds in other groups ($P < 0.01$). The significant difference of feed intake and feed conversion ratio between treatments was not observed. However, the addition of EBFP and commercial cellulase in the experimental diet significantly increased BW gain (BWG, $P < 0.01$) during 0 to 5 week of the experimental period. The BWG in birds fed a diet added 0.1% of EBFP were highest and the lowest was observed in birds fed CON. No significant difference among treatments was observed in carcass weight, carcass yield, left breast, and thigh meat.

The positive effects of probiotics (Patterson and Burkholder, 2003; Collins *et al.*, 2009; Lutful Kabir, 2009) or enzymes (Bedford, 2000; Khattak *et al.*, 2006; Bedford and Partridge, 2010) added in diets on broilers have been extensively reviewed. The use of both feed additives may bring a better animal performance than using each one separately, but the increase in feed costs should be also considered. Therefore, an alternative strategy which is the development of probiotics harboring NSP degrading enzyme, has been introduced (Cummins and Ho, 2005; Liu *et al.*, 2007). Liu *et al.* (2007) developed *Lactobacillus reuteri* sp. harboring rumen fungal xylanase and the liquid culture of transformed *Lactobacillus reuteri* sp. were used as a probiotics. The dietary addition of a liquid culture of transformed *Lactobacillus reuteri* sp. decreased viscosity of

Table 1: Ingredients and chemical composition of experimental diets (as-fed basis)

Item	Starter diet 0 to 3 wk	Grower diet 3 to 5 wk
Ingredients, g/kg		
Ground corn	422.3	448.0
Soybean meal	262.4	238.4
Wheat bran	150.0	150.0
Distiller's dried grains solubles	30.0	30.0
Whole soybean	50.0	50.0
Fish meal	15.0	15.0
Tallow	48.8	50.8
Lysine	0.4	0.6
DL-methionine	3.3	2.0
Choline chloride	1.3	1.5
Vitamin mixture1	2.0	1.5
Mineral mixture2	1.2	1.2
Dicalcium phosphate	8.8	7.2
Salt	2.5	2.5
Total	1000.0	1000.0
Chemical composition, % or as stated		
ME, kcal/kg	3,150	3,200
CP	21	20
Fat	8.15	8.39
Crude fiber	3.61	3.54
Ash	5.35	5.05
Ca	0.9	0.85
P	0.45	0.4
Lysine	1.25	1.15
Met + Cys	1	0.85

1 Vitamin mixture provided the following nutrients per kg: vitamin A, 26,000,000 IU; vitamin D3, 6,000,000 IU; vitamin E, 63,750 IU; vitamin K_3, 4,800mg; vitamin B_1, 2,400mg; vitamin B_2, 9,800mg; vitamin B_6, 5,400mg; vitamin B_{12}, 40,000 3; niacin, 55,000mg; pantothenic acid, 20,000mg; folic acid, 8,000mg.

2 Mineral mixture provided the following nutrients per kg: Fe, 57,630mg; Zn, 72,,000mg; Mn, 86,400mg; Co, 288mg; Cu, 6,000mg; I, 1,200mg; Se, 216mg.

Table 2: Dietary effects of supplementation of endoglucanase producing *Bacillus subtilis* sp. fermented product (EBFP) on feed intake, growth performances, and carcass characteristics in broilers.

Items	CON[1]	PC[1]	EBFP[1]		SEM	P - value
			0.1	0.2		
Body Weight, g/bird						
Initial	41.1	41.1	41.1	41.1	0.03	0.802
3 week	976	1000	1032	1004	16.85	0.218
5 week	2169[c]	2255[b]	2328[a]	2269[b]	9.58	< 0.01
Feed Intake, g/bird/day						
0 to 3 week	70.9	68.8	70.4	74.1	3.79	0.797
3 to 5 week	165	174	179	180	5.73	0.352
0 to 5 week	110	114	115	117	2.74	0.383
Body Weight Gain, g/bird/day						
0 to 3 week	46.7	48.0	50.0	48.1	0.84	0.217
3 to 5 week	85.3	89.6	92.6	90.4	2.34	0.262
0 to 5 week	62.6[c]	65.1[b]	67.3[a]	65.5[b]	0.50	< 0.01
Feed conversion ratio[2]						
0 to 3 week	1.52	1.44	1.42	1.54	0.08	0.703
3 to 5 week	1.95	1.94	1.94	1.99	0.04	0.825
0 to 5 week	1.76	1.75	1.70	1.79	0.03	0.307
Carcass characteristics						
Weight, g	1645	1624	1624	1587	18.27	0.224
Yield, %	70.6	71.0	70.5	69.6	0.61	0.522
Left Breast, % of carcass	14.3	13.8	14.8	14.1	0.33	0.174
Left thigh, % of carcass	13.5	13.3	13.4	13.1	0.18	0.484

[1]CON, basal diet; PC, basal diet + 0.1% commercial endoglucanase; EBFP 0.1, basal diet + 0.1% EBFP; EBFP 0.2, basal diet + 0.2% EBFP.[2] Feed conversion ratio = feed intake (g/bird) / body weight gain (g/bird)

intestinal digesta and increased BWG, ileal villus height and crypt depth during 0 to 21 d of age (Liu *et al.*, 2007). Genetically modified (GM) *Lactobacillus gasseri* and *Lactobacillus johnsonii* harboring endoglucanase gene from *Clostridium thermocellum* were developed by Cho *et al.* (2000) and they showed the endoglucanase activity of 0.722 and 0.759 Unit/mL respectively under liquid culture condition (Cho *et al.*, 2000). Nevertheless, with our knowledge, the use of GM probiotics in human nutrition has not been accepted because the safety of GM bacteria and ecological change by administration of GM probiotics in human gut was not fully demonstrated (Cummins and Ho, 2005). In this study, thus, we developed solid state fermented probiotics harboring endoglucanase produced by cellulolytic *Bacillus subtilis* sp. (Park *et al.*, 2016) which was not genetically modified.

During 0 to 5 week the experimental period, the dietary addition of 0.1% EBFP significantly increased ($P < 0.01$) BWG that was higher than that of birds fed the diet in which only a commercial cellulase was added, indicating that the use of probiotics harboring endoglucanase may exhibit better performance than the single administration of enzyme (Table 2). A similar improvement in BWG was also observed in the previous study in which probiotics with fibrolytic enzyme was used as a feed additive. Liu *et al.* (2007) reported that birds fed the diet containing liquid cultures of transformed *Lactobacillus reuteri*, which can produce xylanase, exhibited the increase in BWG suggesting that fibrolytic probiotics have a potential to provide additional benefits. However, the dietary addition of enzyme or EBFP did not improve carcass weight, yield, breast, and thigh meat contents although the live weight in birds fed a diet supplemented with 0.1% of EBFP was highest among treatments at the experiment end date. Horvatovic *et al.* (2015) also reported that the addition of exogenous enzyme had no effects on carcass traits in broilers indicating that the positive effect of fibrolytic enzyme supplementation on growth performances may not induce the improvement of carcass traits.

In conclusion, the dietary supplementation of EBFP increased BW, and BWG compared to CON and PC suggesting that probiotics having fibrolytic enzyme may enhance the positive effects on broiler's growth performance compared with the single use of enzymes. Further detailed studies would be guaranteed to investigate the mode of action of EBFP in gut metabolism.

ACKNOWLEDGEMENT

This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP; Ministry of Science, ICT & Future Planning) (No. NRF-2017R1C1B5017478).

REFERENCES

AOAC, (2005). Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists International, Gaithersburg, MD, USA.

Bedford, M.R. (2000). Exogenous enzymes in monogastric nutrition-their current value and future benefits. *Anim. Feed Sci. & Technol*., **86**: 1-13.

Bedford, M.R. and Partridge G.G. (2010). Enzymes in Farm Animal Nutrition. CABI.

Cho, J.S., Choi Y.J. and Chung D.K. (2000). Expression of *Clostridium thermocellum* endoglucanase gene in *Lactobacillus gasseri* and *Lactobacillus johnsonii* and characterization of the genetically modified probiotic *lactobacilli*. *Curr. Microbiol*., **40**: 257-263.

Collins, J., La Ragione, R.M., Woodward M.J., and Searle L.E.J. (2009). Application of prebiotics and probiotics in livestock, In: [Charalampopoulos, D., Rastall, R.A. (Eds.),] Prebiotics and probiotics science and technology, Springer, pp. 1123-1192.

Cummins, J. and Ho. M.-W. (2005). Genetically modified probiotics should be banned. Microb. *Ecol. Health Dis.*, **17:** 66-68.

Fuller, R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol*., **66:** 365-378.

Horvatovic, M.P., Glamocic, D., Zikic D., and. Hadnadjev T.D. (2015). Performance and some intestinal functions of broilers fed diets with different inclusion levels of sunflower meal and supplemented or not with enzymes. *Rev. Bras. Cienc. Avic*., **17:** 25-30.

Khattak, F., Pasha, T., Hayat Z. and Mahmud A. (2006). Enzymes in poultry nutrition. *J. Anim. Pl. Sci.*, **16:** 1-7.

Liu, J.R., Lai S.F. and Yu. B. (2007). Evaluation of an intestinal *Lactobacillus reuteri* strain expressing rumen fungal xylanase as a probiotic for broiler chickens fed on a wheat-based diet. *Br. Poult. Sci.*, **48**: 507-514.

Lutful Kabir, S.M. (2009). The Role of probiotics in the poultry industry. *Int. J. Mol. Sci.*, **10:** 3531-3546.

NRC, (1994). Nutrient Requirements of Poultry. 9th eds. The National Academies Press, Washington, DC.

Paloheimo, M., Piironen J. and Vehmaahperä J. (2010). Xylanases and cellulases as feed additives, In: Enzymes in Farm Animal Nutrition, [Bedford, M.R., Partridge, G.G. (Eds.)], CABI, pp. 12-53.

Park, E., Lee, S.S., Kim, B., Shin, T., Cho, B., Cho, S., Cho, K.J., Ha J.K. and Seo. J.(2016). Isolation of endo-1,4-β-D-glucanase producing Bacillus subtilis sp. from fermented foods and enhanced enzyme production by developing the mutant strain. Indian J. Anim. Res., accepted. DOI: 10.18805/ijar.v0iOF.7253

Patterson, J. and Burkholder K. (2003). Application of prebiotics and probiotics in poultry production. *Poult. Sci.*, **82:** 627-631.

Rantala, M. and Nurmi E. (1973). Prevention of the growth of *Salmonella infantis* in chicks by the flora of the alimentary tract of chickens. *Br. J. Poult. Sci.*, **14**: 627-630.

SAS Institute Inc, (2002). User' Guide: Statistics. SAS Institute, Inc, Cary, NC.