

Growth Performance, Blood Characteristics, and Meat Quality Attributes of Broiler Chickens Fed Direct-Fed Microbial (DFM) as an Alternative to Antibiotics

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Abstract

This study was conducted to investigate the supplementation of direct-fed microbials (DFM) as an alternative to antibiotic on growth performance, blood parameters, amino acid content, and quality of breast meat of broiler chicken. In total, 800 1-d-old male broiler chicks (Ross×Ross) were randomly distributed into four dietary treatments with four replicate pens per treatment (50 birds/replicate pen). The four dietary treatments fed for 35 d were: a corn-soybean meal basal diet without antibiotic as negative control (NC); NC plus 0.1% virginiamycin as positive control (PC); NC plus 0.1% direct-fed microbials (DFM 1); and NC plus 0.1% mixed direct-fed microbials (DFM 2). Growth performance, blood parameters, and amino acid content, chemical composition, quality attributes and sensory analysis of breast meat of broiler chickens were evaluated. No significant differences were found among the treatments for over all growth performance of broiler chickens at 35 day of age, but the body weight gain was numerically increased when birds were fed PC and DFM supplemented diets. The levels of triglycerides, glucose, total protein and Ca content in blood were not affected by the dietary treatments; however, the total blood cholesterol level was significantly decreased ($P < 0.05$) in PC and DFM supplemented groups compared with the NC group. In addition, the enzyme aspartate aminotransferase (AST) was significantly lower ($P < 0.05$) in PC and DFM supplemented groups compared with the NC group, and the enzymes alanine aminotransferase (ALT) was lower in DFM 2 compared with that of other treatments. Dietary supplementation of DFM was significantly increased ($P < 0.05$) the cystine, valine, isoleucine and proline contents of breast meat of broiler chickens; however, other meat amino acid contents were not affected by the dietary treatments. The shear force values of breast meat of broiler chickens were not significantly affected by the dietary treatments; however, the cooking loss was significantly decreased ($P < 0.05$) and the water holding capacity was significantly increased in PC and DFM supplemented groups compared with the NC group. The moisture content of breast meat was significantly lower in DFM 2 compared with NC and the lipid content was also significantly lower in PC and DFM supplemented group compared with the NC group. The protein content of meat was not affected by the dietary treatments; however, the ash content of meat was significantly increased in DFM compared with the NC. In addition, the DFM supplementation did not affect the tenderness and flavor of breast meat, but the juiciness was significantly increased in DFM 2 compared with the PC. It is concluded that dietary supplementation has a positive effect on growth performance of broiler chickens to some extent, but it decreases the cholesterol, AST and ALT levels in blood, and increases the meat quality attributes of broiler chickens.

Keywords: Direct-Fed Microbials; Blood Characteristics; Meat Quality; Sensory Analysis; Broiler Chickens

Introduction

The application of antibiotics in food animals for growth promotion and disease prevention may induce antibiotic resistance in human and animals [1], making them resistant to antibiotics when needed [2]. However, the emergence of antibiotic resistance is closely related to the amount of antibiotic residues in the environment [3]. The antibiotic resistance can spread directly by contact, and indirectly through the food chain, air, water, and soil. As a consequence, several countries has restricted the use of antibiotic in the livestock feeds to avoid harmful impact on public health. Very recently, the US Food and Drug Administration has issued an order to prohibit the certain uses of antimicrobial drugs in food animals which would be effective from April 5, 2012. In Asia, Korea is the first country to ban the use of antibiotic growth promoters (AGP) in animal feed completely from July 2011 [4]. In addition, the consumers are very conscious regarding this issue and, thus, it is a growing concern for the nutritionist, academics and for the livestock feed industry people to look for suitable alternatives to AGP to ensure the safety of animal products [5,6].

Several studies have demonstrated that the potential alternative feed additives to AGP includes: direct-fed microbials (DFM), different herbs or spices and essential oils, acidifiers and organic acids, prebiotics and different dietary enzymes [7]. Among them, DFM, a source of live beneficial microorganisms, has been practiced as an effective alternative to antibiotics in animal feed industry over the last few decades due to its diverse function on animal health and productivity. In general, *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Saccharomyces cerevisiae* are frequently used as DFM in the poultry feed industry. These microorganisms can influence on the intestinal microbiota as well as host health and welfare in different ways, such as; competitive exclusion of pathogenic bacteria [8], lowering the pH through acid fermentation, competing for mucosal attachment and nutrients, producing bacteriocins, stimulating the gut associated immune system [9], increasing the production of short-chain fatty acids [10], increasing epithelial integrity, reducing epithelial cell apoptosis, and stimulating the intraepithelial lymphocytes [11,12].

Currently, a greater array of DFM have been used by the broiler industry to promote the balance and quality of intestinal microflora for the host, but the functions of these products varies according to their production procedure and practical application. Several researchers have reported that feeding DFM improves the growth performance of broiler chickens [13,14] and egg production of laying hens [15]. By contrast, other researchers failed to observe a positive effect of feeding DFM on BW gain in broiler chickens [16] and in pigs [17]. The inconsistent results like this have been explained due to limited species of microorganism added as DFM. It is speculated that the potential benefit of DFM depends upon the microbial species, strain, concentration, production techniques, and storage condition. Evidence so far showed that better performance had been achieved by the use of mixtures of microorganism with different species rather than the single use of microbial species or strains [18]. Therefore, a feeding trial was conducted to investigate the supplementation of various DFMs as an alternative to antibiotics on growth performance, blood characteristics, and meat quality attributes of broiler chickens.

Materials and Methods

Experimental protocol

A total of 800 1-d-old male broiler chicks (Ross×Ross) was randomly allotted to four dietary treatments with four replicate pens (50 birds/replicate pen) for 35 d. The four dietary treatments are: a corn-soybean meal basal diet (Control); 0.1% virginiamycin, a streptogramin class of antibiotic (AGP); 0.1% direct feed microbials that contained *Lactobacillus reuteri* (DFM 1); and 0.1% direct feed microbials that contained a mixture of *Lactobacillus reuteri*, *Bacillus subtilis* and *Saccharomyces cerevisiae* (DFM 2). DFM 1 was provided by a commercial company (Daesung Microbiology Co., Ltd, Seoul, Korea) and DFM 2 was provided by a Youngju farmers' cooperative (Youngju-si, Gyeongsangbuk-do, Korea). Both of the DFM products are approved for feeding to animals in Korea and the actual concentration of DFM is presented in table 1. The basal diet was formulated to meet the National Research Council requirements [19] and was fed during the experiment in 2 phases, 0 to 21d and 22 to 35d (Table 2). All birds were raised on a rice hull-littered floor pens (0.093 m²/bird) in an environmentally controlled room. Continuous lighting was provided throughout the experimental period. The initial room temperature

was 32°C, and reduced by 3°C each wk until 35 d of age. Birds were allowed free access to feed and water throughout the whole feeding period.

Item	Concentration	
	DFM 1	DFM 2
<i>Lactobacillus reuteri</i>	1×10 ⁹ cfu ² /g	1×10 ⁹ cfu/g
<i>Bacillus subtilis</i>	-	1×10 ⁷ cfu/g
<i>Saccharomyces cerevisiae</i>	-	1×10 ⁸ cfu/g

Table 1: The concentration of direct-fed microbials (DFM)¹.

¹DFM 1 was provided by a commercial company (Daesung Microbiology Co., Ltd, Seoul, Korea) and DFM 2 was provided by a Youngju farmers' cooperative (Youngju-si, Gyeongsangbuk-do, Korea); Both of the DFM products were supplied as powder form. ²colony forming unit.

Name of ingredients	Starter diet (0-21d)	Finisher diet (22-35d)
Corn	53.44	61.64
Soybean Meal	33.65	27.88
Corn Gluten Meal	4.16	4.00
Soybean oil	4.68	3.06
Limestone	1.02	0.08
Tricalcium phosphate	2.01	0.05
Salt (NaCl)	0.25	0.23
DL-Methionine	0.27	1.31
Lysine-HCl	0.02	0.25
Vitamin-mineral mixture ¹	0.50	0.50
Total	100.0	100.0
Calculated composition (%)		
ME, kcal/kg	3,100	3,100
Crude protein	22.0	20.0
Lysine	1.10	1.00
Methionine	0.50	0.38
Methionine + cystine	0.87	0.72
Ca	1.00	0.90
Available P	0.50	0.35

Table 2: Composition of the basal diets (as-fed basis, %).

¹Vitamin-mineral mixture provided following nutrients per kg of diet: vitamin A, 15,000 IU; vitamin D₃, 1,500 IU; vitamin E, 20.0 mg; vitamin K₃, 0.70 mg; vitamin B₁₂, 0.02 mg; niacin, 22.5 mg; thiamin, 5.0 mg; folic acid, 0.70 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10.0 mg; Fe, 72 mg; Co, 2.5 mg.

Growth performance

The body weight (BW) and feed intake were measured weekly by pen. Feed conversion was calculated as the feed to gain ratio. The BW gain, feed intake, and feed conversion were corrected for dead birds.

Sample collection

At the termination of the feeding trial, two birds from each pen, close to the mean BW, were selected and killed by cervical dislocation. Immediately after cervical dislocation, blood samples (5 mL each) were collected by heart puncture using EDTA vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) and stored on ice and provided for immediate hematology analysis. Meat were collected and stored in plastic bags at 4°C for the shear force measurement of muscles. The rest of the samples were then stored at -20°C until the chemical analysis of meat, water holding capacity (WHC) and sensory evaluation were performed.

Analyses of blood samples

Blood samples were centrifuged at 2,000 × g at 4°C for 20 minutes to separate the plasma and were stored at -15°C until the plasma composition was measured. The various blood parameters were analyzed using Multi-species Hematology System (HEMAVET 950 FS, Drew Scientific Inc., Oxford, CT, USA). The plasma composition was measured using an automatic blood analyzer (Hitachi 747, Tokyo, Japan).

Measurement of shear force values

The shear force values of breast meat of broiler chicken were determined as described by [20] using an Instron Universal Testing Machine (Instron, Canton, MA) with a Warner-Bratzler shear attachment. Within 24 hours after slaughter a 36360:5 cm and a 46462 cm samples were collected from the back skin and breast muscle, respectively. Shear values were obtained using the Instron machine and a 500-kg load cell with a full scale load of 1, a preset crosshead speed of 250mm/min, and a proportional chart speed ratio of 2:1 (mm/min).

Measurement of WHC

The centrifugation method was performed for the determination of WHC of breast and thigh meat as described by [21] with some modifications. One gram of ground meat was placed on a round filter paper (No.4, Whatman Ltd. UK). The filter paper with meat was put into centrifuge tubes (Mobicols from MoBiTec, Göttingen) and centrifuged (CR 20B2, Hitachi Koki Co., Ltd., Japan) at 6,710g for 10 minutes. The released water content was measured and calculated as percentage of the initial moisture content of meat.

Sensory evaluation of meat

Sensory evaluation of meat were carried out for the cooked breast meat without skin. Ten panelists were selected from the meat science laboratory of our department and all had experience in poultry meat sensory analysis. Criteria for selection were: age between 20 to 40 years, not allergic to chicken, consumption of chicken at least once a wk, and willingness to evaluate meat from experimental chickens. Chicken breast meat contained in the air tied vinyl bags were thawed by heating for 20 minutes at 35°C in a water bath. The bags were then opened and pieces of meat (30g each) were placed in screw-capped flasks. These were heated at 75°C for 20 minutes in a 177°C electric oven and served to the panelists. Samples from all dietary treatments were randomly presented to each panelist in one session. They were asked to rank the meat samples using a 9-point category hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) [22]. Sensory analysis included three characteristics such as tenderness, juiciness and flavor. Water and unsalted crackers were provided to clean their plates between samples.

Statistical analyses

Data were subjected to one-way ANOVA using the General Linear Models procedure of the Statistical Analysis System [23]. Pen means were used as the experimental units for all variables evaluated. The mean differences were compared using Duncan’s multiple range tests. Significance was declared when the probability was less than 5% ($P < 0.05$).

Results and Discussion

Growth performance, blood parameters, and amino acid content, chemical composition, quality attributes and sensory analysis of breast meat of broiler chickens were evaluated in this experiment. No significant differences were found among the treatments for over all growth performance of broiler chickens at 35 day of age, but the body weight gain was numerically increased when birds were fed PC and DFM supplemented diets (Table 3). The levels of triglycerides, glucose, total protein and Ca content in blood were not affected by the dietary treatments; however, the total blood cholesterol level was significantly decreased ($P < 0.05$) in PC and DFM supplemented groups compared with the NC group. In addition, the enzyme aspartate aminotransferase (AST) was significantly lower ($P < 0.05$) in PC and DFM supplemented groups compared with the NC group, and the enzymes alanine aminotransferase (ALT) was lower in DFM 2 compared with that of other treatments (Table 4).

Performance	Treatment ¹			
	NC	PC	DFM 1	DFM 2
BW gain (g/bird)	2051.04 ± 10 ²	2065.97 ± 22	2074.55 ± 21	2078.93 ± 27
Feed intake (g/bird)	3071.15 ± 71	3079.33 ± 3.1	3160.67 ± 120	3075.62 ± 46
Feed efficiency (Feed/gain)	1.50 ± 0.01	1.49 ± 0.11	1.52 ± 0.09	1.48 ± 0.05

Table 3: Effect of direct fed microbials (DFM) supplementation on growth performance of broiler chickens (35 d).

¹NC: Negative Control (basal diet); PC: Positive Control (0.1% virginiamycin); CC: Commercial Control (0.1% probiotic made by the commercial company); T1, (0.1% probiotic made by Eongju). ^{a,b}: Means with different superscripts within a row differ significantly ($P < 0.05$).

²Mean ± SD.

Item	Treatment ¹			
	NC	PC	DFM 1	DFM 2
TCHO	107.60 ± 8.21a	88.20 ± 5.57b	89.83 ± 3.47b	85.88 ± 7.97b
TG	84.44 ± 10.22	71.88 ± 15.25	80.92 ± 13.69	78.02 ± 17.07
GLU	208.52 ± 8.57	206.00 ± 8.73	204.85 ± 8.57	204.30 ± 11.05
TP	2.68 ± 0.17	2.38 ± 0.19	2.70 ± 0.32	2.44 ± 0.26
Ca	5.97 ± 0.80	6.10 ± 0.60	6.23 ± 0.80	6.38 ± 0.75
AST	287.90 ± 26.62a	230.06 ± 21.82b	239.48 ± 28.89b	189.72 ± 25.04c
ALT	24.03 ± 1.26a	21.70 ± 2.49a	23.33 ± 1.77a	14.76 ± 1.23b

Table 4: Effect of direct-fed-microbial supplementation on blood parameters of broiler chickens.

¹NC: Negative Control (basal diet); PC: Positive Control (0.1% virginiamycin); CC: Commercial Control (0.1% direct feed microbial made by the commercial company); T1: (0.1% direct feed microbial made by Eongju); ^{a,b,c}: Means with different superscripts within a row differ significantly ($P < 0.05$).

Dietary supplementation of DFM was significantly increased ($P < 0.05$) the cystine, valine, isoleucine and proline contents of breast meat of broiler chickens; however, other meat amino acid contents were not affected by the dietary treatments (Table 5). The Shear force values of breast meat of broiler chickens were not significantly affected by the dietary treatments; however, the cooking loss was significantly decreased ($P < 0.05$) and the water holding capacity was significantly increased in PC and DFM supplemented groups compared with the NC group. The moisture content of breast meat was significantly lower in DFM 2 compared with NC and the lipid content was also significantly lower in PC and DFM supplemented group compared with the NC group. The protein content of meat was not affected by the dietary treatments; however, the ash content of meat was significantly increased in DFM compared with the NC. In addition, the DFM supplementation did not affect the tenderness and flavor of breast meat, but the juiciness was significantly increased in DFM 2 compared with the PC (Table 6).

Amino acids	Treatment ¹			
	NC	PC	DFM 1	DFM 2
Cystine (Cys)	0.24 ± 0.01bc	0.23 ± 0.00c	0.25 ± 0.00a	0.25 ± 0.01a
Methionine (Met)	0.52 ± 0.03	0.53 ± 0.02	0.53 ± 0.02	0.54 ± 0.02
Aspartic acid (Asp)	2.03 ± 0.06	2.10 ± 0.04	2.08 ± 0.06	2.11 ± 0.08
Threonine (Thr)	0.99 ± 0.03	1.02 ± 0.02	1.00 ± 0.04	1.01 ± 0.04
Serine (Ser)	0.84 ± 0.06	0.89 ± 0.02	0.88 ± 0.03	0.89 ± 0.03
Glutamic acid (Glu)	3.33 ± 0.09	3.43 ± 0.05	3.34 ± 0.15	3.37 ± 0.09
Glycine (Gly)	0.91 ± 0.07	0.94 ± 0.02	0.93 ± 0.02	0.96 ± 0.04
Alanine (Ala)	1.42 ± 0.03	1.44 ± 0.04	1.41 ± 0.04	1.42 ± 0.05
Valine (Val)	0.89 ± 0.08b	0.96 ± 0.02ab	0.95 ± 0.03ab	0.98 ± 0.04a
Isoleucine (Ile)	0.85 ± 0.08b	0.92 ± 0.02ab	0.91 ± 0.02ab	0.94 ± 0.04a
Leucine (Leu)	1.78 ± 0.03	1.84 ± 0.04	1.80 ± 0.06	1.83 ± 0.07
Tyrosine (Tyr)	0.68 ± 0.04	0.70 ± 0.01	0.69 ± 0.02	0.70 ± 0.03
Phenylalanine (Phe)	1.09 ± 0.10	1.11 ± 0.05	1.08 ± 0.06	1.09 ± 0.08
Lysine (Lys)	1.89 ± 0.07	1.92 ± 0.04	1.87 ± 0.04	1.91 ± 0.08
Histidine (His)	0.93 ± 0.08	1.00 ± 0.06	0.96 ± 0.05	0.95 ± 0.02
Arginine (Arg)	1.24 ± 0.09	1.33 ± 0.03	1.31 ± 0.06	1.30 ± 0.05
Proline (Pro)	0.79 ± 0.07b	0.86 ± 0.02a	0.86 ± 0.03a	0.90 ± 0.04a

Table 5: Effect of direct-fed-microbial supplementation on amino acid pattern in meats of broiler chickens.

¹NC: Negative Control (basal diet); PC: Positive Control (0.1% virginiamycin); CC: Commercial Control (0.1% direct feed microbial made by the commercial company); T1: (0.1% direct feed microbial made by Eongju); Eongju).

^{a,b,c}: Means with different superscripts within a row differ significantly ($P < 0.05$).

Meat quality attributes	Treatment ¹			
	NC	PC	DFM 1	DFM 2
Shear values	4.08 ± 0.17	3.29 ± 0.23	3.85 ± 0.09	3.23 ± 0.26
Cooking loss (%)	22.58 ± 2.33a	18.01 ± 1.71b	19.33 ± 0.81b	18.11 ± 1.44b
WHC (%)	54.56 ± 1.34b	57.94 ± 1.07a	58.33 ± 1.95a	58.01 ± 2.67a
Moisture (%)	76.28 ± 1.50a	75.07 ± 0.35ab	75.03 ± 0.47ab	74.63 ± 1.07b
Lipid (%)	1.22 ± 0.43a	0.60 ± 0.16b	0.64 ± 0.25b	0.76 ± 0.17b
Protein (%)	22.06 ± 0.42	22.63 ± 0.40	22.64 ± 0.60	22.48 ± 0.60
Ash (%)	0.78 ± 0.07b	0.86 ± 0.06ab	0.86 ± 0.04ab	0.92 ± 0.07a
Sensory analysis				
Tenderness	4.28 ± 0.54	3.88 ± 0.70	4.38 ± 0.47	4.58 ± 0.53
Juiciness	4.15 ± 0.42ab	3.65 ± 0.24b	3.80 ± 0.24ab	4.28 ± 0.38a
Flavor	4.05 ± 0.19	4.00 ± 0.23	3.78 ± 0.46	4.20 ± 0.35

Table 6: Effect of direct-fed-microbial supplementation on meat quality attributes of broiler chickens.

¹NC: Negative Control (basal diet); PC: Positive Control (0.1% virginiamycin); CC: Commercial Control (0.1% direct feed microbial made by the commercial company); T1, (0.1% direct feed microbial made by Eongju). ^{a,b}: Means with different superscripts within a row differ significantly ($P < 0.05$).

Conclusion

It is concluded that dietary supplementation of DFM may have a benefit to promote growth performance of broiler chickens, but it decreases the cholesterol, AST and ALT levels in blood, and increases the meat quality attributes of broiler chickens.

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