academicJournals

Vol. 10(26), pp. 985-993, 14 July, 2016
DOI: 10.5897/AJMR2016.8114
Article Number: BA9DB3959334
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Efficacy of *Rhodopseudomonas* containing multi-microbe probiotic on growth performance, mortality and cecal microflora in broilers

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Received 16 May 2016, Accepted 8 June, 2016.

Combinations of probiotic microorganisms are the study of interest due to beneficial impact through synergistic actions. Several researchers including our previous result found positive effects of various combinations of probiotic inclusion in broiler diet. In the current study, the efficacy of combinations of probiotic organisms by adding *Rhodopseudomonas* spp. was the primary interest on growth performance, mortality, immunity and cecal microflora in broilers. According to the completely randomized design, three hundred day-old Ross broiler chicks (initial BW, 45.08 ± 0.79 g) were randomly allocated to three dietary treatments with ten replications of 10 birds per replicate. Broilers were reared for 5 weeks, where experimental diets were provided for starter (0 to 3 weeks) and finisher (4 to 5 weeks) period. The dietary treatments were, CON = control (corn-soybean meal based basal diet); ABT = basal diet + chlortetracycline-HCl; RCMP = basal diet + multi-microbe probiotic, (*Bacillus* spp. + *Lactobacillus* spp. + *Saccharomyces* spp. + *Rhodopseudomonas* spp.). Present study revealed that, dietary supplementation of RCMP significantly improved overall average daily gain and feed conversion ratio compared to CON (P<0.05). Mortality rate was significantly reduced in RCMP and ABT supplemented group compared to CON (P<0.05). Serum immunoglobulin level was upgraded after RCMP and ABT supplementation relative to CON but remained nonsignificant (P>0.05). Moreover, cecal pathogenic *Escherichia coli* and *Salmonella* was significantly suppressed in RCMP and ABT supplemented group compared to CON (P<0.05); while number of beneficial microorganisms were higher in ABT and RCMP relative to CON but not significant (P>0.05). Thus, dietary supplementation of *Rhodopseudomonas*-based multi-microbe probiotic (RCMP) has the potentiality to be used as effective feed additives in broilers.

Key words: Broilers, multi-microbe probiotic combinations, immunity, mortality, performance.

INTRODUCTION

The use of antibiotics as prophylactic and growth promoting compounds has long been practiced in commercial poultry farming. However, increased antibiotic use has led to the development of antibiotic resistant microorganisms, production losses and increased risk of infection (Bager et al., 1998). In addition, tremendous use of antibiotic cause imbalance of the gut microflora causes health hazards, and antibiotic residues influence the

environment; and owing to these adverse effects, antibiotic growth promoters have been banned in many countries all over the world (Edens, 2003). Consequently, the entire poultry industry has been under pressure to identify viable alternatives to antibiotics. Probiotics, prebiotics and herbal feed additives and their different combinations became viable alternatives to antibiotic growth promoters in the poultry industry (Patterson and Burkholder, 2003; Kim et al., 2016).

Probiotics are commonly known as the mono or mixed cultures of living microorganisms that exerts beneficial effects on the host by balancing indigenous microbial population (Fuller, 1992; Havenaar et al., 1992). They have beneficial effects on growth performance (Kabir, 2009), health of the host (Guarner et al., 2003), increase nutrient digestibility (Mountzouris et al., 2010), modulate intestinal microflora (Teo and Tan, 2007), and facilitate development of humoral immunity (Waititu et al., 2014). There are variety of microrganisms widely used in the animal as well as poultry nutrition including *Bacillus, Lactobacillus, Lactococcus, Bifidobacterium, Enterococcus, Streptococcus, Pediococcus, Aspergillus, Candida* and *Saccharomyces* species, a variety of yeast species, and undefined mixed cultures (Patterson and Burkholder, 2003; Kabir, 2009). *Rhodopseudomonas* are non-sulphur phototrophic organisms under the family Athiorhodaceae, found in soils and many types of marine environments, currently, grew attention along with other probiotics in aquaculture, agriculture, biofuel production and waste management (Lee et al., 2008; Qi et al., 2009). Single strain use of probiotics are beneficial where Sanders and Veld (1999) proposed that multi-strain and multi-species probiotics are more effective; Timmerman et al. (2004) reported effectiveness of multi-microbe probiotic due to successful colonization. Xu et al. (2014) reported beneficial effects of single use of *Rhodopseudomonas* in broilers while Zhou et al. (2010) reported that *Rhodopseudomonas* with other multi-strain probiotic products have growth promoting efficacy in fish and plants. But there have been limited attempts to develop *Rhodopseudomonas* based multi-microbe probiotic products for livestock and poultry production. Beneficial effects of different combinations were found effective (*Bacillus*, *Lactobacillus*, *Saccharomyces, Enterococcus, Streptococcus* and *Clostridium* spp.) on broiler performance and immunity (Kim et al., 2012; Bostami et al., 2015). To the best of our knowledge, there were no previous reports on combination of different microorganisms along with *Rhodopseudomonas* species with other probiotic microorganisms, whether they exert positive or negative impact on broiler performance. It was hypothesized that, addition of purple non-sulphur microorganism *Rhodopseudomonas* with *Bacillus*, *Lactobacillus* and *Saccharomyces* probiotic bacteria would synergistically improve the growth performance, immunity; reduce the mortality and modulate the cecal microflora through microbial balance and nutrient utilization in broilers. Therefore, the present study was conducted to investigate the efficacy of *Rhodopseudomonas* containing multi-microbe combinations (RCMP) on growth performance, mortality, immunity and cecal microflora in broilers.

MATERIALS AND METHODS

The protocol for this experiment, use and care of broilers were carried out in accordance with the guidelines of the Animal Care and Use Committee of the Sunchon National University, Suncheon, Republic of Korea.

Birds, diet, experimental design and experimental care of birds

Broiler chicks for conducting the experiment were obtained from a local commercial hatchery (Yang Ji Company, Cheonan, Choongnam, South Korea). Three hundred day-old mixed sex Ross broiler chicks (initial BW, 45.08 ± 0.79 g) were randomly allocated to three dietary treatments. The dietary treatments were, CON = Control (Corn-soybean meal based basal diet); ABT = Basal diet + Chlortetracycline-HCl; and RCMP = Basal diet + Multi-microbe probiotic combinations (*Bacillus subtilis* + *Lactobacillus acidophilus* + *Saccharomyces cerevisiae* + *Rhodopseudomonas capsularis*); where each treatment had ten replications of 10 birds per replicate. Broilers were reared for a total of 5 weeks, where experimental diets were provided for two stages, namely the starter (0 to 3 weeks) and finisher (4 to 5 weeks).

Broilers were kept in a closed and well ventilated house with having well-arranged wire-floored cages of 100 cm long, 80 cm wide and 40 cm high, having a floor space of 800 cm^2/bird. The cages had a linear feeder in the front and a nipple drinker in the back to provide *ad libitum* feed and water throughout the entire experimental period. Temperature was maintained at 33°C from day 0 to 7, after which it was gradually reduced to 27°C at a rate of 3°C per week and then maintained at this temperature until the end of the experiment. The relative humidity (RH) was maintained at around 50% and continuous lighting was provided throughout the experimental period. The lighting regime for the study consisted of 23L: 1D for the entire experimental period.

A commercial corn soybean meal-based diet was used as the basal diet, which was formulated to meet the nutrient requirements of Ross broiler chickens following National Research Council guidelines (NRC, 1994).The starter diets were offered from 0 to 3 weeks and finisher from 4 to 5 weeks. The *Rhodopseudomonas* containing multi-microbe probiotic combinations (RCMP) and antibiotic were added at 0.1% (W/W) with basal diet in powdered form by replacing the equal amount of basal diet. Diets were supplied to the birds on weekly basis, where firstly total feed was

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Table 1. Feed ingredients and chemical compositions of the basal diets.

Items	Starter diet (0 to 3 weeks)	Finisher diet (4 to 5 weeks)
Ingredients (%, as fed basis)		
Corn grain	57.58	60.64
Soybean meal	26.80	24.90
Corn gluten	5.00	3.50
Soybean oil	2.20	2.20
Animal fats	4.50	5.00
Common salt	0.25	0.25
Dicalcium phosphate	2.14	2.00
Limestone	0.92	0.88
Vitamin-mineral premix [1]	0.30	0.30
Choline	0.08	0.07
L-lysine HCl (78%)	0.24	0.16
DL-Methionine	0.20	0.10
Calculated composition (% DM)		
ME (MJ/kg)	13.03	13.27
Moisture	12.07	13.08
Crude Protein	20.89	19.12
Ether extract	4.65	2.43
Crude Fiber	4.42	3.71
Crude Ash	5.63	5.61
Calcium	1.05	0.81
Available phosphorus	0.55	0.45
Lysine	1.42	1.10
Methionine	0.49	0.45

[1]Vitamin-mineral mixture provided the following nutrients per kilogram of diet: Vitamin A, 15,000 IU; vitamin D_3, 1,500 IU; vitamin E, 20.0 mg; vitamin K_3, 0.70 mg; vitamin B_{12}, 0.02 mg; niacin, 22.5 mg; thiamine, 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 (Bayer Korea Ltd., Dongjak-Ku, Seoul, Korea).

weighed for each treatment and then replace the basal diet at 0.1% to add similar amount of antibiotic and multi-microbe probiotic for the ABT and RCMP, respectively. Finally total feed was mixed properly and then supplied to the birds for each treatment and replications. *Rhodopseudomonas* containing multi-microbe probiotic combinations (RCMP) and feed sample was homogenized and analysed for microbial populations and chemical compositions. Dietary dry matter (DM), crude protein (CP), calcium, phosphorus and other chemical compositions were analysed and calculated according to the procedures described by Association of Official Analytical Chemists (AOAC, 2000). The ingredients and nutritional composition of the experimental basal diet are presented in Table 1.

The probiotics used in the present study were provided by the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea and Probion, Woogene B&G Co. Ltd., Seoul, South Korea. *Rhodopseudomonas capsularis* cells were grown under natural illuminations for 96 h in outdoor culture. In short, *R. capsularis* cells were collected by centrifugation (10,000×*g*) and then the residual cell mass mixed with the corn meal in the ratio of 1:5. After mixing properly, it was dried in a forced-air drying oven at a temperature of 60°C and finally stored in 4°C maintaining the temperature. A culture broth (CB) was prepared and then autoclaved before use. The culture broth medium was prepared with corn steep liquor (6%), molasses (4%), yeast extract (0.30%), KH_2PO_4 (0.50%) and K_2HPO_4 (0.25%). After autoclaving 2 L of culture broth and each microbial culture of 2 ml (*Lactobacillus*, *Bacillus*, *Saccharomyces*) was added and mixed properly and then subjected to fermentation for 48 h. The microorganisms grown on the culture broth were then sprayed after drying at the temperature of 40°C for 72 h, on the corn soybean meal (1:1). A two-step fermentation method was accomplished using a commercial fermenter (W-1000; Wonbalhyo Industry Co., Icheon, South Korea). Firstly, 0.5% *L. acidophilus* KCTC 3111 and 0.5% *B. subtilis* KCTC 3239 were added to the solid substrate media and fermented at 40°C by the repetition of 5 h of anaerobic and 3 h of aerobic conditions for about 48 h. Second fermentation was performed by adding 0.5% *S. cerevisiae* for 72 h at a temperature of 40°C under aerobic condition. Following completion of the fermentation process, the probiotic products were then dried to less than 15% of moisture at the temperature of 80°C for 48 h using a drying oven. Then the product was stored at 4°C for further use. The *Rodopseudomonas* spp., *Lactobacillus* spp. *Bacillus* spp. and *Saccharomyces* spp. were mixed properly and for the determination of the number of cells, 1 g was taken and diluted with sterilized distilled water (10 ml) at room temperature. After about 10 min, 1 ml of the dilution was serially diluted 10-fold in NaCl (8.5 g/kg) solution and then cultured in the agar media. The culture plate was then

Table 2. Microbial population and composition of *Rhodopseudomonas* containing multi-microbe probiotic combinations (RCMP).

Microflora and strain	Concentration (cfu/g)
L. acidophilus (KCTC 3111)	3.2×10^7
B. subtilis (KCTC 3239)	2.6×10^7
S. cerevisiae (KCTC 7915)	6.2×10^9
R. capsularis (KCTC 2583)	2.5×10^8
Chemical composition	**(%)**
Moisture	40.36
Crude protein	10.23
Crude fat	2.21
Crude fiber	11.33
Crude ash	10.13
Nitrogen free extract	22.52

KCTC, Korean Collection for Type Cultures.

incubated at the temperature of 37°C for 24 to 48 h and the number of colonies were counted. The chemical compositions of *Rhodopseudomonas* containing mixed microbial combinations (RCMP) were determined by the method of AOAC (2000). The microbial concentrations and the chemical composition of RCMP were shown in Table 2.

Measurement of broilers performance and mortality

Body weight and feed intake of all birds were measured on pen basis (replications of treatments) every week. Feed intake was measured according to residual feed. Based on the weekly data of body weight and feed intake, average daily gain (ADG) and average daily feed intake (ADFI), and feed conversion efficiency (FCR) was calculated for all treatments. ADG, ADFI and FCR were calculated for starter (0 to 3 weeks), finisher (4 to 5 weeks) and overall period (0 to 5 weeks). The mortality of birds was recorded on daily basis.

Blood analysis

At the end of the feeding trial (5^{th} week), three birds were randomly selected from each replication of all treatments in order to perform immunological analyses. Birds of the similar weight of the replications of treatments were selected for blood collections; where blood samples were collected from selected birds' brachial vein. After collection, blood samples were quickly transferred into centrifuge tubes that were centrifuged for 15 min at 1610× *g* in a cold chamber (4°C). Sera were then carefully removed to plastic vials and stored at -20°C for immunoglobulin (Ig) analysis. Concentrations of serum IgG, IgM and IgA were assayed using Chicken IgG (Cat. No. E30-104), IgM (Cat. No. E10-101) and IgA (Cat. No. E30-103) ELISA Quantitation Kits (Bethyl Laboratories Inc., Montgomery, TX, USA), respectively according to the manufacturer's instructions. Each sample was run in duplicate. The absorbance of each well was measured within 30 min by using a micro-plate auto-reader (Thermo Lab Systems, Helsinki, Finland) at 450 nm.

Cecal microbial analysis

Three birds were randomly selected from each pen in order to perform microbial analysis. Selected birds were slaughtered, and cecal contents were collected to measure the microfloral count. Feed was withdrawn 12 h before slaughtering. Approximately 1 g of cecal content was aseptically collected into a 2-mL safe-lock Eppendorf tube (Thermo Fisher Scientific Inc., Seoul, South Korea) and immediately preserved at -40°C for subsequent microbial analysis. After thawing, 1 g of the cecal sample was serially diluted with 9 mL of 0.9% sterile saline (1:10 dilution) and thoroughly mixed. Viable bacterial counts from the cecal samples were conducted by plating serial 10- fold dilutions in duplicate into different agar plates to isolate different bacteria. The culture media used for *E. coli* was MacConkey Sorbitol agar; for *Lactobacillus* spp. was Mann, Rogosa & Sharpe agar; and for *Bacillus* spp. was Mannitol-Egg Yolk-Polymyxin agar; and for *Salmonella* spp. was Salmonella Shigella Agar. Supernatant (100 µl) was smeared onto agar plate and incubated anaerobically at 37°C for 24 to 48 h. After incubation, microbial colonies were counted and expressed as log10 CFU/ml.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the General Linear Model Procedures (GLM) of SAS (2003) with diet as the main effect. The pen was used as the experimental unit to analyse growth performance, whereas individual chicks were used as the experimental unit for analysis of blood and cecal microflora. Statistically significant effects were further analysed and means were compared using Duncan's multiple range tests (DMRT) when necessary. Probability value $P<0.05$ was considered as statistically significant.

RESULTS

Growth performance and mortality of birds

Average daily gain (ADG) was significantly improved during starter (0 to 3 weeks), finisher (4 to 5 weeks) and overall period (0 to 5 weeks) in the RCMP and ABT supplemented group ($P<0.05$); but RCMP and ABT did not differ significantly ($P>0.05$) (Table 3). In addition,

Table 3. Effect of *Rhodopseudomonas* containing multi-microbe probiotic combinations (RCMP) on broiler growth performance.

Parameters	Period	Dietary treatments			SEM	P-value
		CON	ABT	RCMP		
IBW (g/bird)		44.75	44.91	44.84	0.389	0.962
FBW (g/bird)		2061.48[b]	2257.16[a]	2289.60[a]	32.174	0.001
ADG (g/bird)	0-3 weeks	44.58[b]	48.50[a]	49.47[a]	0.812	0.005
	4-5 weeks	77.18[b]	85.27[a]	86.14[a]	1.527	0.005
	0-5 weeks	57.62[b]	63.21[a]	64.14[a]	0.923	0.001
ADFI (g/bird)	0-3 weeks	59.66	61.61	62.15	0.827	0.143
	4-5 weeks	145.44	148.67	149.48	2.563	0.539
	0-5 weeks	92.33	94.18	94.05	0.842	0.281
FCR (Feed/Gain)	0-3 weeks	1.34	1.27	1.26	0.034	0.276
	4-5 weeks	1.89[a]	1.75[b]	1.74[b]	0.043	0.061
	0-5 weeks	1.60[a]	1.49[b]	1.47[b]	0.027	0.009

[a,b] Means with different superscripts within the same row are significantly different (P<0.05). SEM = Standard error of mean. CON = Control (Corn-soybean meal based basal diet); ABT = Basal diet + Chlortetracycline-HCl; RCMP = Basal diet + Multi-microbe probiotic combinations (*B. subtilis* + *L. acidophilus* + *S. cerevisiae* + *R. capsularis*).

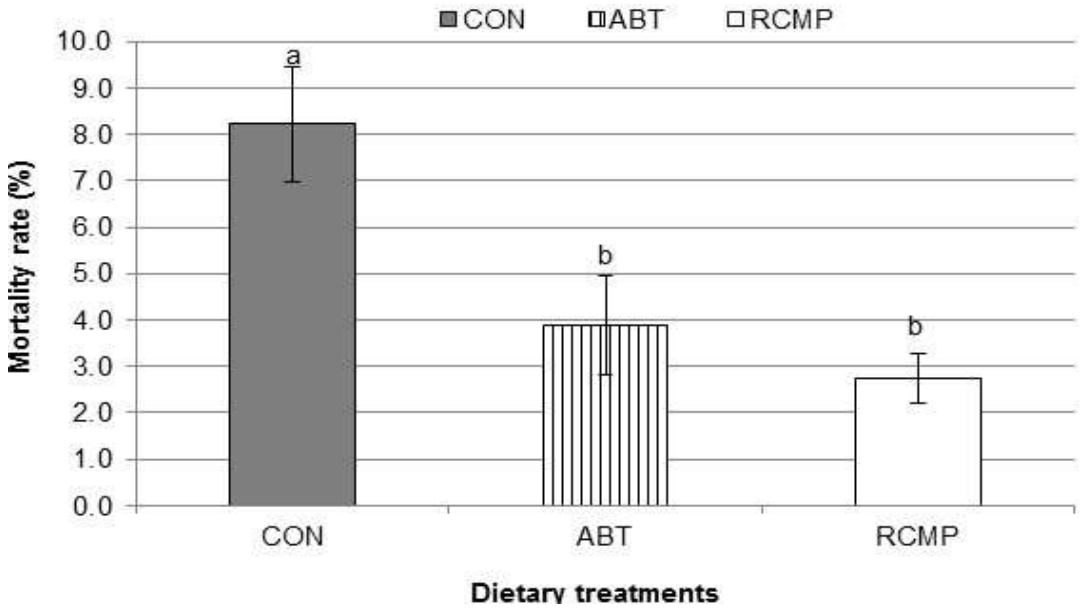

Figure 1. Effect of *Rhodopseudomonas* containing multi-microbe probiotic combinations (RCMP) on mortality of broilers. [a,b] Means with different superscripts within the similar bar are significantly different (P<0.05). Error bar indicated standard error. CON = Control (Corn-soybean meal based basal diet); ABT = Basal diet + Chlortetracycline-HCl; RCMP = Basal diet + Multi-microbe probiotic combinations (*Bacillus subtilis* + *Lactobacillus acidophilus* + *Saccharomyces cerevisiae* + *Rhodopseudomonas capsularis*).

average daily feed intake (ADFI) was statistically similar among the dietary treatments during starter, finisher and overall period (P>0.05). Furthermore, feed conversion ratio (FCR) was improved in RCMP and ABT during overall period compared to CON (P<0.05). Where during finisher period FCR tended to be improved in the RCMP and ABT relative to control (P<0.10).

The mortality of the broilers was lower in ABT (53%) and RCMP (67%) groups over the phases (0 to 5 weeks) (Figure 1) compared to CON (P<0.05); however, no difference was observed between RCMP and ABT during entire experimental period (P>0.05).

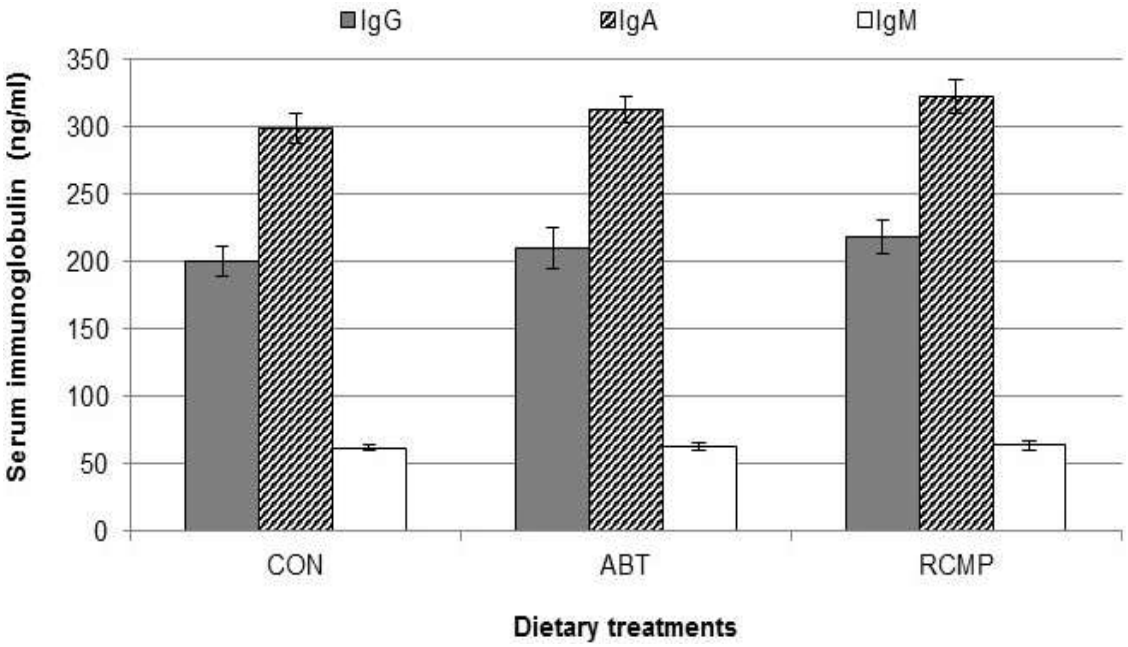

Figure 2. Effect of *Rhodopseudomonas* containing multi-microbe probiotic combinations (RCMP) on serum immunoglobulins of broilers. [a,b] Means with different superscripts within the similar bar are significantly different (P<0.05). Error bar indicated standard error. CON = Control (Corn-soybean meal based basal diet); ABT = Basal diet + Chlortetracycline-HCl; RCMP = Basal diet + Multi-microbe probiotic combinations (*B. subtilis* + *L. acidophilus* + *S. cerevisiae* + *R. capsularis*).

Serum immunoglobulins of broilers

It was observed from the present study that, there were higher value of the serum immunoglobulins, IgG, IgA and IgM of the birds (Figure 2) in RCMP and ABT supplemented groups in comparison to CON group, but there was no significant differences among the dietary treatments (P>0.05).

Cecal microbiology of broilers

As shown in the Table 4, the pathogenic *E. coli* and *Salmonella* was suppressed in the RCMP and ABT supplemented groups (P<0.05). There were no significant differences observed for the number of pathogenic *E. coli* and *Salmonella* between RCMP and ABT (P>0.05). However, the non-pathogenic bacteria (Lactobacillus and Bacillus) were numerically increased in RCMP and ABT supplemented group but did not differ significantly (P>0.05).

DISCUSSION

In the current study dietary supplementation of RCMP (*Bacillus*, *Lactobacillus*, *Saccharomyces,* and *Rhodopseudomonas*) improved the growth performance of broilers during 0 to 5 weeks of experimental period (Table 3). This is in agreement with Mountzouris et al. (2007) where growth promotion was reported with combination of multi-microbe probiotic (*Lactobacillus, Bifidobacterium, Enterococcus,* and *Pediococcus*). Photosynthetic bacteria, *Rhodopseudomonas* species stimulate the growth of fish (Zhang et al., 1988); and broiler (Xu et al., 2014). Zhou et al. (2010) reported that along with other beneficial microbes, *Rhodopseudomonas* species are able to improve the growth performance in fish through enhancement of immunity and health status. Improved body weight gain in the RCMP group of present study could be attributed to a better microbial environment in the gut, which in turn might have enhanced digestion, absorption and utilization of nutrients (Panda et al., 2000). Combination of probiotic microorganism exerts multifunctional effects such as enhancement of growth performance through synergistic (assisting the beneficial microbes) and antagonistic actions (inhibiting the pathogenic microbes) (Timmerman et al., 2004; Qi et al., 2009).

Generally, probiotic organisms help to maintain the intestinal microfloral balance by increasing digestive enzyme activity which consequently helps to improve feed intake and digestion (Jin et al., 1997). However, in the present study, feed intake did not differ significantly

Table 4. Effect of *Rhodopseudomonas* containing multi-microbe probiotic combinations (RCMP) on cecal microflora in broilers.

Microorganism (log_{10}CFU/g)	Dietary treatments			SEM	P-value
	CON	ABT	RCMP		
Bacillus	7.57	7.69	7.78	0.11	0.531
Lactobacillus	7.12	7.56	7.66	0.36	0.583
E. coli	7.47[a]	6.55[b]	6.34[b]	0.24	0.021
Salmonella	6.59[a]	6.21[b]	6.13[b]	0.06	0.001

[a,b] Means with different superscripts within the same row are significantly different (P<0.05). SEM = Standard error of mean. CON = Control (Corn-soybean meal based basal diet); ABT = Basal diet + Chlortetracycline-HCl; RCMP = Basal diet + Multi-microbe probiotic combinations (*B. subtilis* + *L. acidophilus* + *S. cerevisiae* + *R. capsularis*).

among the dietary treatments. Although the combinations of multi-microbe probiotic were different; Kim et al. (2012) observed significant improvements in feed conversion ratio (FCR) in response to *Bacillus, Lactobacillus,* and *Saccharomyces* supplemented diet, which supports the present findings of Ross broilers with combination of multi-microbe probiotic (*Bacillus* spp. + *Lactobacillus* spp. + *Saccharomyces* spp. + *Rhodopseudomonas* spp.). However, Salim et al. (2013) observed no effect on feed efficiency of broilers and this is consistent with starter period of the present study. It has been suggested that probiotic can promote feed efficiency by increasing the bioavailability of dietary micronutrients, modulating intestinal microflora, enhancing immuno-modulation and improving the health (Kabir, 2009; Yang et al., 2012). *Saccharomyces* can increase vitamin absorption, synthesis of enzymes, and protein metabolism (Crumplen et al., 1989); while *Rhodopseudomonas* can produce acetic, butyric and lactic acid, and extracellular polymeric substances and enzymes (Merugu et al., 2012). It has been postulated that the combination of *Rhodopseudomonas* spp. with other probiotic bacteria created probiotic niches, optimal pH, and successful colonization, and improved feed efficiency of broilers hence the observed better performances in the present study. The significant improvement of FCR in the RCMP supplementation in the current study additionally might be attributed to the purple non sulphur bacteria (*Rhodopseudomonas* spp.) as a source of single cell protein (for the poultry industry) (Salma et al., 2007); which have more digestible bacterial cell wall, and are rich in proteins, carotenoids, biological cofactors, and vitamins (Kobayashi and Kurata, 1978).

Mortality rate was reduced in the RCMP supplemented group (Figure 1) and this concurs with Khaksefidi and Rahimi (2005) who found reduced mortality rate in Ross broilers diet supplemented with multi-microbe probiotic. *Bacillus* can produce iturin and surfactin, and enzyme (Ferrari and Schmidt, 1993); *Lactobacillus* can produce lactic acid and bacteriocines (Yamato et al., 2003); have the antibacterial potential and produce fermentative enzymes. *Bacillus* and *Lactobacillus* inhibits the putrefactive bacterial enzymes and reduce mortality (Jin et al., 2000). The latter could be ascribed as the reason behind the reduced mortality in the current study. Zhou et al. (2010) reported that *Rhodopseudomonas* and *Bacillus* could enhance immunity and health status through secretion of immune substances. Combinations of probiotic strains can increase beneficial health effects owing to their synergistic and biotherapeutic effects (Musa et al., 2009). It could be inferred that the combination of the beneficial microorganisms exerted similar actions leading to reduced mortality in the RCMP groups in the present study.

Dietary supplementation of RCMP showed an increased trend of immunoglobulin status (IgG, IgA and IgM) in broilers in the present experiment (Figure 2). Several researchers have reported that probiotics in broilers enhanced humoral immune response (Koenen et al., 2004; Salim et al., 2013). Haghighi et al. (2005) reported that administration of a multispecies probiotic (*Lactobacillus, Bifidobacterium, Streptococcus*) enhanced the serum antibodies to several foreign antigens in case of chickens. Probiotics are involved in protection against a variety of pathogens in chickens (*Escherichia coli*, *Campylobacter* and *Salmonella*) and can reduce the mortality of birds (Cross, 2002; Gunal et al., 2006); which might be the ultimate fate of improvement of the immunoglobulin status. The gut and its resident microbiota play an essential role in shaping the immune system (Waititu et al., 2014); while probiotics promote such types of biological shaping of the immune system (Salminen et al., 1996).

The pathogenic *E. coli* and *Salmonella* were suppressed significantly, while the beneficial *Bacillus* and *Lactobacillus* were increased non-significantly in the RCMP supplemented group (Table 4). Consistent with present findings, Ceylan et al. (2003) and Ghadban et al. (1998) reported that multi-microbe probiotic could reduce pathogenic bacteria count (*Salmonella* and *E. coli)*. Supplementation of multi-microbe probiotic (*Lactobacillus*, *Bacillus* and *Clostridium*) could fortify beneficial micro-organisms, such as *Lactobacillus* and *Bifidobacterium* (Teo and Tan, 2007; Mountzouris et al., 2010). In addition,

probiotic helps in regulation of microbial homeostasis in the intestine which led to balancing the microorganisms in the gastrointestinal tract (Jin et al., 1997; Lee et al., 2010). This reduction could also be attributed to competitive exclusion, where the adhesion of beneficial bacteria to the intestinal mucosa prevents attachment by pathogenic bacteria (Kabir, 2009). Facilitation of antibody production (Ng et al., 2009), and secretion of biochemical substances by the probiotic organisms inhibit the growth and development of pathogenic bacteria (Patterson and Burkholder, 2003). *Rhodopseudomonas* provide the organic acids and energy resource for beneficial gut microflora and might exert proliferative effects on colonocytes; and antibacterial actions for pathogenic microorganisms (Guarner et al., 2003; Merugu et al., 2012). It could be inferred that the combination of the probiotic microbes exerted profound actions leading to a reduction in the pathogenic bacteria in the RCMP supplemented group in the current study.

Conclusion

The results of the current study suggested that supplementation with *Rhodopseudomonas*-based multi-microbe probiotic (RCMP) (*L. acidophilus* + *B. subtilis* + *S. cerevisiae* + *R. capsularis*) significantly improved the growth performance, reduced mortality; while significantly suppressed pathogenic *E. coli* and *Salmonella* in broilers. Thus, *Rhodopseudomonas*-based multi-microbe probiotic (RCMP) has the potential to be used as growth promoters in broiler nutrition.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

This work (Grants no. C0193546) was supported by Business for Academic-industrial Cooperative establishments funded Korea Small and Medium Business Administration in 2014.

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