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Original article

Comparison between the effects of two multi-strain probiotics and antibiotic on growth performance, carcass characteristics, gastrointestinal microbial population and serum biochemical values of broiler chickens

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ABSTRACT

This experiment was conducted to evaluate the effect of two types of probiotic and antibiotic virginiamycin on performance, carcass characteristics, gastrointestinal microbial population and Serum biochemical values of broiler chickens. Based on a completely randomized design, three hundred day old Ross 308 broilers were allotted into 4 treatments with 5 replicate pens per treatment and 15 broilers in each pen for 42 days. Dietary treatments were (1) an antibiotic-free corn-soybean meal mash basal diet (control), (2) control + 200 g/ton of virginiamycin, (3) control + 1500 g/ton of fermented probiotic produced and isolated from fermented poultry slaughterhouse wast, and (4) control + 1000 g/ton of commercial probiotic (BioPlus-2B®). The results indicated that the use of antibiotic improved body weight gain (BWG) (7.2 and 8.6 % respectively) and feed conversion ratio (FCR) (3.24 %) as compared to control and commercial probiotic treatments ($P<0.05$). Also, the birds under virginiamycin treatment had higher feed intake (FI) than those treated with commercial probiotic. The use of fermented probiotic relatively improved BWG (3.28 %) and FCR (1.0 %) as compared to control. Efficiency of dressed carcase, breast, thigh and internal organs including liver, heart, bursa of fabricius and spleen were not affected by these experimental treatments (P>0.05). Adding commercial probiotic to the basal diet increased the relative weight of gizzard than fermented probiotic and reduced the relative weight of abdominal fat pad than virginiamycin treatment (P<0.05). Both of these probiotics increased total bacterial population in crop when compared with virginiamycin (P<0.05). The use of fermented probiotic significantly increased the lactic acid bacteria population in crop than antibiotic treatment. Fermented probiotic supplementation significantly lowered the pH of ileum than those control and antibiotic treatments. Antibiotic virginiamycin and fermented probiotic were effective to reduce ileum coliforms population when compared to control and commercial probiotic treatments (P<0.05). The use of commercial probiotic reduced the blood concentration of cholesterol when compared to control birds (P<0.05). The serum concentration of triglyceride and very low density lipoprotein (VLDL) in birds under fermented probiotic significantly was lower compared to antibiotic treatment. None of these fed additives had significant effect on other Serum biochemical parameters including glucose, high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, albumin and globulin. According to the present study, fermented probiotic has been found to have a positive effect especially on body weight of broilers. The use of fermented probiotic is therefore recommended to effectively replace conventional antibiotics.

1. Introduction

Microflora of poultry gastrointestinal tract plays an important role in the nutrients digestion and absorption and control of pathogens. Proliferations of pathogenic bacteria in the gut often leads to intestinal inflammation and subsequently decreases the production rates and increase the mortality and contamination risk of poultry products (Baurhoo et al., 2009). Antibiotic growth promoters (AGP) improve the growth performance of birds by increase the beneficial microbial population and prevent the establishment of pathogenic bacteria in the gut. However, the use of such antibiotics was banned because of their adverse effects on human health (e.g. the risk of development of antibiotic-resistant bacteria and antibiotic residues in treated animal's food products) (Chauvin et al., 2005). Today, the combination of solutions such as genetic selection of resistant birds, strict sanitation in and around the poultry house, removal of pathogenic bacteria from food and water, vaccination and using of the appropriate feed additives in food and water are employed to achieve the high performance and elimination of AGP in poultry industry (Doyle and Erickson, 2006). Probiotics have been introduced as an effective alternative to antibiotics in animal feed (Patterson and Burkholder, 2003). In 2001, the term probiotic was defined as "A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host" (Schrezenmeir and De Vrese, 2001). AGP often reduce the population of Gram-positive bacteria such as Lactobacilli and bifidobacteria in the gastrointestinal tract (Baurhoo et al., 2007). In contrast, probiotics increase the population of these beneficial bacteria in the digestive tract (Sharifi et al., 2012). It has been reported that probiotics are effective to improvement of poultry performance, nutrient digestibility and modulation of intestinal microflora, biosynthesis of various vitamins and release of bacteriocins. The efficacy of probiotics depend on factors including microbial species composition (e.g., single or multiple strains) and viability, application method and frequency, administration level, diet composition, bird age, farm hygiene, and environmental stress factors (Mountzouris et al., 2010). A variety of microbial species including Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Streptococcus, and numerous yeast cultures have been used as probiotics, but more recently, the research on Lactobacillus in animal feed is increasing (Mikulski et al., 2012). Among the genus of Lactobacillus, the species of Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus rhamnosus and Lactobacillus fermentum were showed the high antimicrobial activity against pathogens (Koll et al., 2008). Furthermore, the probiotic properties of lactic acid bacteria such as Lactobacillus plantarum (Heres et al., 2003; Murry et al., 2006), Lactobacillus rhamnosus (Bouzaine et al., 2005) and Lactobacillus fermentum (Bouzaine et al., 2005) has been identified for broilers. In Iran, only the imported probiotics are available for use in poultry industry. Therefore, the access to domestic desirable probiotic products seems very important and needed. Since the microbial fermentation of segments of gastrointestinal tract (e.g., ceacum) could be a suitable method for isolation of probiotic microorganisms (Durant et al., 2000), and also according to the fermentability of poultry waste (Urlings et al., 1996), this experiment was conducted to compare the effects of fermented probiotic (derived from fermented poultry waste) with antibiotic virginiamycin and commercial probiotic (Bioplus-2B®) on performance, carcass characteristics, gastrointestinal microbial population and Serum Biochemical values of broiler chickens.

2. Materials and methods

2.1. Experimental design

In this study, 300 broiler chickens of the commercial Ross 308 strain were used in a completely randomized design with 4 treatment and 5 replicates in each treatment and 15 birds/replicates and reared on the floor pens for 42 days. Before beginning this study, the dry matter, crude protein, ether extract, crude fiber, and ash contents of corn and soybean meal were determined in the Laboratory to make sure of the presence of sufficient amounts of protein and crude fiber content of the ration (AOAC, 1984). A basal diet was formulated as control according to recommendation from Ross broiler nutrition specification for starter (0 to 10 days), grower (11-28 days) and finisher (29-42 days) periods (Table 1). The required amount of growth stimulating additives under study was added to the basal diet so that, In addition to the basal diet (control), antibiotic virginiamycin (200 g/ton) was added to the control treatment, while fermented probiotic (1500 g/ton), and commercial probiotic Bioplus-2B® (1000 g/ton) were supplemented into the other treatments respectively. All of these feed additives in powder form were mixed thoroughly in aforesaid quantities to a small amount of feed in a premixer. The resultant mixture was then mixed with the rest of the feed in a mechanical blender until a thorough and consistent mixture was obtained. Fermented probiotic provided by fermentation of poultry slaughterhouse wastes by starter culture of Lactobacillus plantarum (ATCC 1058) for 6 days under anaerobic condition at 30 ± 1 oC (Urlings et al., 1996) in fermentation chambers in Gorgan University of Agricultural Sciences and Natural Resources and then lactic acid bacteria were isolated and identified in the dried product according to the Durant et al (2000). This probiotic contained (about 65×108 CFU/g) species of Lactobacillus plantarum, Lactobacillus fermentum and Lactobacillus rhamnosus. Bioplus-2B (BioPlus 2B, CHR Hansen BioSystems, Denmark, RazakCo. Iran) is a kind of commercial probiotic that contains at least 3.2×109 CFU/g Bacillus licheniformis and Bacillus subtilis. All birds received feed and water ad libitum. Birds and feed were weighed on 1st and 42th days of the experiment on a pen basis and body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined accordingly.

2.2. Sample collection and analysis

2.2.1. Microbiology

On day 14, one bird from every pen (5 birds per replicate) was slaughtered via the cervical dislocation. After disinfection of the abdominal surface of the carcass and areas around it, the internal organs were removed. Then about 5 cm from the length of the ileum middle part (from the Meckel's diverticulum to cacal junction) and the crop and their content and mucosa were sampled. To determine the microbial population, one gram of crop and ileum contents was used to make serial 10 fold dilutions using buffered peptone water and then 0.1 ml of the appropriate crop and ileum dilutions were spread respectively on modified de Man, Rogosa, Sharpe agar (to detect lactic acid bacteria) and violet red bile agar (to detect coliforms) (Izat et al., 1990). The culture of lactic acid and coliform bacteria was incubated anaerobically at 37.5 oC for 24-48 hours. The plate count agar was used to count the total aerobic bacterial population of the ileum and the crop (Engberg et al., 2000). The plates were incubated aerobically at 37.5 oC for 48 hours. After counting the number of colonies in each plate, the number so obtained was multiplied by the inverse of the dilution and the result was stated as the number of colony forming unit (CFU) in 1 gram of the sample (Downes and Lto, 2001). All the processes were carried out in the faculty of animal sciences microbiology laboratory at the Gorgan University of Agricultural Sciences and Natural Resources.

Table 1
The basal diet composition and calculated analysis (g /kg) in different rearing periods.

Ingredients (As percent)	Starter (0-10d)	Grower (11-28d)	Finisher (28-42d)
Corn	558.9	589.6	626.7
Soybean meal	371.7	340.6	297.3
Soybean oil	22.1	29.5	37.5
Dicalcium phosphate	17.9	15.5	14.8
Oyster shell	13.0	10.6	10.5
Vitamin premix*	2.50	2.50	2.50
Mineral premix**	2.50	2.50	2.50
Salt	5.00	5.00	4.50
L-lysine	2.90	1.40	1.20
DL-Methionine	3.50	2.60	2.30
Nutrient content			
ME (Kcal /Kg)	2900	3000	3100
Crude protein	211.0	200.0	184.1
Calcium	10.00	8.60	8.20
Available phosphore	4.80	4.30	4.10
Sodium	2.10	2.10	1.90
Lysine	13.70	11.80	10.50
Methionine	6.80	5.70	5.20
Methionine + Cystine	10.20	9.00	8.30

*Supplied the following per kg of diet, vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg;
**Supplied the following per kg of diet, Choline chloraid, 200000 mg, Manganeze, 40000 mg, Iron, 20000 mg; Zinc, 40000 mg, coper, 4000mg; Iodine, 400 mg; Selenium, 80 mg.

2.2.2. pH measurement

For measuring the pH, about 1 gram of the crop and ileum content of each chicken was collected and transferred into 2 ml distilled water, then the pH levels were measured using a pH meter (Izat et al., 1990).

2.3. Blood sampling

On 42th day of experimental feeding 5 ml of blood was collected from wing vein from 5 birds in each treatment. Blood samples were centrifuged (at 2000 × g for 10 min) and serum was separated and then stored at -20°C until assayed for measuring blood parameters (glucose, total protein, albumin, cholesterol, tyiglycerid and high density lipoprotein (HDL)) using appropriate laboratory kits (Pars Azmoon®). The serum globulin was calculated by subtracting serum albumin from serum total protein levels. Very low density lipoprotein (VLDL) cholesterol was calculated from triglycerides by dividing the factor 5. The low density lipoprotein (LDL) cholesterol was calculated by using the formula, LDL cholesterol=Total cholesterol-HDL cholesterol -VLDL cholesterol. Then the birds were slaughtered for evaluation of carcasses and determination of relative weight of internal organs (Perreault and Leeson, 1992).

2.4. Statistical analysis

All data were analyzed using the GLM procedure of SAS® (SAS institute, 2003) for analysis of variance. Significant differences among treatments were identified at 5% level by Duncan's multiple range tests.

3. Results

3.1. Performance

The effect of experimental treatments on the performance of broiler chickens is given in Table 2. The growth performance of birds under fermented probiotic treatment has no significant different compared to other treatment (P<0.05). However, the use of fermented probiotic improves the BWG and FCR to 3.2 and 1.08 percent compared to the control treatment. Adding commercial probiotic to the basal diet had not significant effect on growth performance, but supplementation of antibiotic virginiamycin significantly improved the BWG and FCR compared to control treatments (P<0.05). Both of the antibiotic and fermented probiotic show no significant different on growth performance of the birds (P>0.05). The birds in the treatment supplemented with antibiotics only shows significantly high FI compared to the treatment supplemented with commercial probiotics (P<0.05). Numerous studies were conducted on the effects of probiotics on broilers feeding programs. In some of these studies, the use of probiotics in the diet of broiler birds has been found to improved the growth performance of broiler birds (Houshmand et al., 2011; Mountzouris et al., 2010; Awad et al., 2009). In contrast, other studies have reported non- significant effects of probiotics on performance of broilers (Knap et al., 2011; Mutus et al., 2006). These inconsistencies may be due to factors such as nutrition (Chen et al., 2013), management (Eckert et al., 2010) and rearing conditions (Angel et al., 2005). In the present study, fermented probiotic was more effective to improve the BWG and FCR when compared to commercial probiotic. These differences could be due to the bacterial composition of the probiotics (L. plantarum, L. fermentum and L. rhamnosus in fermented probiotic and B. licheniformis and B. subtilis in commercial probiotic), because the type of bacterial species is effective on performance of broiler chickens (Pelicano et al., 2003). It is reported that commercial probiotics are more effective on broilers performance in unfavorable environmental conditions such as high temperature (Rahimi and Khaksefidi, 2006) and high stocking density (Joaquin-Torres et al., 2013). In agreement to previous studies (Baurhoo et al., 2009; Belay and Teeter, 1994), antibiotic virginiamycin is found to improve the BWG and FCR of broiler chickens. Antibiotic virginiamycin decreases the presence of pathogens and their toxic products in the gut which cause the enteric inflammation and lower the absorption potential of gastrointestinal tract epithelium of broilers (Gunal et al., 2006). In the present study, antibiotic virginiamycin was more effective on growth performance of boiler chickens than both of the probiotics. The reason of this may be attributed to the fact that antibiotics even decrease the counts of beneficial indigenous- gram positive microflora such as lactobacilli and bifidobacteria which could compete for nutrients and energy with the birds. So, in these conditions more nutrients and energy will be available for the growth of birds (LaVorgna et al., 2013).

Table 2
Comparison effects of different probiotics and virginiamycin on growth performance of broiler chickens on 42 days of age.

Treatments	Initial weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion ratio (g/g)
Control	49	2834.3 ^{b}	5253.8 ^{ab}	1.85 ^{a}
Virginiamycin	49	3040.4 ^{a}	5459.1 ^{a}	1.79 ^{b}
F-probiotic*	49	2927.4 ^{ab}	5357.5 ^{ab}	1.83 ^{ab}
C-probiotic**	49	2798.7 ^{b}	5200.3 ^{b}	1.85 ^{a}
SEM	0.28	49.34	71.59	0.01

* Fermented probiotic, ** Commercial probiotic
a,b Means in each column with different superscripts have significantly different (p<0.05).

3.2. Carcass characteristics

The effect of the additives used on carcass efficiency (as percent of live body weight) are reported in Table 3. The relative weight of gizzard in birds supplemented with commercial probiotic was higher than that of the birds supplemented with fermented probiotic (P<0.05). The abdominal fat percentage in birds fed commercial probiotic was lower compared to the treatment fed virginiamycin (P<0.05). All the three feed additives used has no significant effect on the relative weight of all the other internal organs (P>0.05). It is reported that probiotic microorganisms could decrease the carcass fat content (Jin et al., 1998) and increase the gizzard weight (Radecki et al., 1992). Several studies (Baurhoo et al., 2009; Maiorka et al., 2001) indicated that use of virginiamycin and probiotic could not increase broilers carcass efficiency. Ahmadi (2011) reported that the supplementation of broiler chickens diet with virginiamycin had no significant effect on carcass efficiency and internal organs weight. In the study of Midilli et al., (2008), addition of a mixture of Bacillus licheniformis and Bacillus subtilis (4.6 × 108 cfu/g) to broiler chickens diet was found to have no effect on carcass efficiency (P>0.05). Although, in the present study, the difference of breast and thigh efficiency was not significant among treatments, but all of these fed additives improved their value as related to the findings of the previous studies (Piray et al., 2007; Plicano et al., 2003).

Table 3
The effect of different probiotics and virginiamycinon carcass characteristics (as percent of live body weight) of broiler chickens on 42 days of age.

	Treatments				
	Control	**Virginiamycin**	**F-probiotic***	**C-probiotic****	**SEM**
Dressed Carcass	70.44	71.13	68.25	70.60	1.58
Breast	23.56	24.87	24.45	23.78	0.69
Thigh	18.75	19.01	19	18.78	0.31
Abdominal fat	1.87^{ab}	1.90^{a}	1.79^{ab}	1.75^{b}	0.04
Gizzard	1.45^{ab}	1.37^{ab}	1.25^{b}	1.57^{a}	0.07
Liver	2.30	2.06	2.17	2.13	0.09
Heart	0.51	0.51	0.48	0.57	0.03
Bursa of fabricius	0.11	0.06	0.06	0.08	0.02
Spleen	0.16	0.14	0.14	0.13	0.01

* Fermented probiotic, ** Commercial probiotic
a,b means in each column with different superscripts are significantly different (p<0.05).

3.3. Microbial population

The effects of the additives used on gut microbial population are presented in Table 4. The use of virginiamycin in broiler chickens diet significantly decreased the total bacterial population in crop these birds compared to both of the probiotic treatments (P>0.05). Lactic acid bacteria population in crop of birds fed diet containing fermented probiotic was significantly higher than that fed virginiamycin (P<0.05). Supplementing of diet with fermented probiotic significantly decreased the ileum pH compared to the antibiotic and control treatments (P<0.05). Also, the use of antibiotic virginiamycin and fermented probiotic significantly lowered the population of coliforms in ileum than commercial probiotic and control treatments (P<0.05). None of these fed additives was effective on crop pH and total bacterial population of ileum (P>0.05). Unlike probiotics, antibiotic virginiamycin decreased the population of gram positive bacteria. Also, some gram negative bacteria are sensitive to the antibiotic virginiamycin (Nagaraja and Taylor, 1987). Therefore, this antibiotic is predominantly effective on improvement of broiler performance by decrease the total bacterial population in the gastrointestinal tract. The same results was observes by other researchers (Yakhkeshi et al., 2012). Probiotic bacteria are effective on improvement of broilers performance by producing of short-chain fatty acids. So, the reduction of pH in the ileum of birds received probiotic could be due to the more production of short-chain fatty acids in the contents of this section. The non-ionised form of these fatty acids can penetrate the cell wall and disrupt the normal physiology of the gram negative bacteria (e.g., coliforms) which eventually will lead to their death (Dhawale, 2005). However, different factors such as microorganisms composition and supplementation level are effective on the probiotic response (Midilli et al., 2008; Rahimi and Khaksefidi, 2006).

Table 4
The effect of the feed additives on gut microbial population (Log10 cfu/g) and pH of broiler chickens on 14 days of age.

	Treatments				
	Control	**Virginiamycin**	**F-probiotic***	**C-probiotic****	**SEM**
In crop					
pH	4.72	4.78	4.64	4.76	0.21
Total bacterial population	5.35 [ab]	4.75 [b]	5.98 [a]	5.96 [a]	0.40
Lactic acid bacteria	7.82 [ab]	7.30 [b]	8.30 [a]	7.72 [ab]	0.23
In ileum					
pH	6.68 [a]	6.66 [a]	6.31 [b]	6.52 [ab]	0.10
Total bacterial population	6.74	5.69	5.30	6.49	0.53
Coliforms	6.07 [a]	4.70 [b]	4.72 [b]	6.32 [a]	0.31

* Fermented probiotic, ** Commercial probiotic,
a,b means in each column with different superscripts are significantly different (p<0.05).

3.4. Serum biochemical values

The effect of these feed additives on blood parameters are presented in Table 5. Commercial probiotic significantly reduced serum cholesterol concentration when compared to the other treatments (P<0.05). Also, fermented probiotic significantly decreased the serum concentration of triglyceride and VLDL compared to virginiamycin treatment (P<0.05). In accordance with our findings, it is reported by (Rahimi and Khaksefidi, 2006) that the use of commercial probiotic in the diet of birds under heat stress, significantly decreased the serum cholesterol concentration. In contrast, several studies reported the use of virginiamycin (Ahmadi, 2011) and probiotic (Chawla et al., 2013; Yakhkeshi et al., 2012) had no significant effect on serum cholesterol, triglyceride, VLDL and LDL in broiler chickens. Probiotics lower the serum lipids profile through several mechanisms including enzymatic deconjugation of bile acids by bile salt hydrolase and thus excretion of them in the feces, using cholesterol in cell membrane and binding cholesterol to cell wall of probiotics in the intestine, conversion of cholesterol into coprostanol, inhabitation of hepatic cholesterol synthesis by short chain fatty acids such as propionate produced by probiotic bacteria and/or redistribution of cholesterol from plasma to the liver (Homayouni et al., 2012). Furthermore, probiotic microorgamisms decrease the serum cholesterol concentration through inhibiting the hydroxyl methylglutaryl coenzyme A which contributes to the cholesterol synthesis (Fukushima and Nakano, 1996). Decrease in serum triglyceride might be ceased due to the increase of lactic acid bacteria population in the gut (Homayouni et al., 2012). It is reported that the dietary supplementation of Bacillus subtilis ,in addition to reducing the amount of abdominal fat in carcass, could decrease the triglyceride concentration in serum, liver and carcasses of broiler chickens and effectively lower the activity of acetyl coenzyme A carboxylase (Santose et al., 1995).

Table 5
The effect of the feed additives on Serum biochemical values of broiler chickens fed different probiotics and virginiamycin

	Treatment				
	Control	**Virginiamycin**	**F-probiotic***	**C-probiotic****	**SEM**
Glucose (g/dl)	259	260	249.60	247.40	7.36
Cholesterol (mg/dl)	131 [a]	128.40 [ab]	120.20 [ab]	115.20 [b]	4.43
Triglyceride (mg/dl)	89.60 [ab]	95.80 [a]	69.40 [b]	85.80 [b]	7.10
HDL (mg/dl)	73.60	79.20	80.80	71.60	5.81
LDL (mg/dl)	39.48	30.04	25.52	26.44	4.38
VLDL (mg/dl)	17.92 [ab]	19.16 [a]	13.88 [b]	17.16 [b]	1.42
Total protein (g/dl)	3.24	3.36	3.64	3.32	0.17
Albumin (g/dl)	1.36	1.64	1.78	1.40	0.14
Globulin (g/dl)	1.88	1.72	1.86	1.92	0.09

* Fermented probiotic, ** Commercial probiotic
a,b means in each column with different superscripts are significantly different (p<0.05).

4. Conclusion

The results of this experiment indicated that the fermented probiotic, in addition to increase of beneficial bacteria and decrease of pathogenic bacteria population in the gastrointestinal tract, could improve the growth performance of broiler chickens. Therefore, it is recommended that fermented probiotic at a specify rate could be used as an alternatives to conventional antibiotics such as virginiamycin in feed of broiler chickens.

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