The effects of probiotic *Yeasture*® using at different rearing periods on performance, intestinal microbial population and carcass traits in broiler chickens

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Abstract

The present study was conducted to investigate the effects of using probiotic *Yeasture*® at different rearing periods on performance, intestinal microbial population and carcass traits in broiler chickens. A total of 378 one day old chickens (male and female) were randomly allotted to one of the 21 floor pens in a completely randomized design with seven treatments and three replicate groups and eighteen chickens in each group (9 male and 9 female). The Experiment treatments consisted of seven groups: A (control, without probiotic), B (prebiotics feeding between 1 to 10 days), C (prebiotics feeding between 1 to 24 days), D (prebiotics feeding between 1 to 42 days), E (prebiotics feeding between 11 to 24 days), F (prebiotics feeding between 11 to 42 days) and G (prebiotics feeding between 24 to 42 days). The chickens received the desired probiotic at different days of rearing period. The basal diets were corn and soybean meal, so 0.3 g/kg of basal diets in starter (1-10 days), 0.25 g/kg of basal diets in grower (11-24 d) and 0.2 g/kg of basal diets in finisher (24-42 d) period of probiotic was added to the basal diets for each other than treatment. The growth performance (evaluated through body weight gain, feed intake and feed conversion ratio), intestinal microbial population (the measurement of *Lactobacillus* and *Coliforms*) and carcass traits (relative weights of carcass, intestine, liver, gizzard and abdominal fat) were determined. Body weight gains and feed conversion ratio were significantly improved in broilers with added probiotic compared to the control group ($P<0.05$) whereas feed intake was not significantly altered. In addition, intestinal microbial population and carcass and gizzard weights markedly increased in the probiotic treated birds compared to control chickens ($P<0.05$). These results show that diet supplementation with probiotic *Yeasture*® at the early days of the rearing periods of broiler chickens especially, 1 to 24 and 1 to 42 days, had a positive effect on intestinal microbial population, leading to more nutrients being assimilated by the chickens and consequently greater performance in broiler chickens.

Introduction

Dietary antibiotic additions are demonstrated to have beneficial effects on birds' growth and feed conversion efficiency, and the inhibition of pathogen growth.[1,2] However, there is a great fear of using antibiotics as feed additives because of the public concern about antibiotic residues in poultry products and the potential evolving of antibiotic resistant bacteria; for this reason, the recent European Union ban on the prophylactic use of in feed antibiotics has escalated the search for alternatives for use within the poultry industry,[3] so antibiotics have been replaced by other products in controlling intestinal pathogenic bacteria.[4] Some probiotic microorganisms are an alternative to antibiotic to be used exclusively as a growth stimulant and for the improvement of feed conversion rate in farm animals.[4] Consequently, studies on probiotics such as growth promoters have recently gained a great attention.

Probiotic is considered as a live microbial feed supplementation that benefit from on avian by intestinal microbial balance improvement and are increasingly adopted as an alternative to antibiotic growth promoters in poultry diets.[5-7] Also, it plays an important role in the prevention of carcass contamination of intestinal pathogens during processing and growth stimulation rate and feed efficiency on growing chick.[8] Tortuero[9] stated that the probiotics include enzymes, yeast and live bacteria, which contribute to maintain the balance in intestinal micro flora. In the previous studies about the beneficial impact on poultry performance, it was shown that the diet supplemented with probiotic can have positive effects. For example, Kabir and colleagues[10] demonstrate that the addition of probiotic in diet has significantly increased the body weight gain and carcass yields in broilers throughout the whole experimental period (1 to 42 d). Lan and colleagues[11] reported that higher body weight gains in broilers were subjected to 2 probiotic species. In other studies, beneficial effects of probiotics on the performance of broiler,[12-14] modulation of intestinal microbial,[15,16] nutrient digestibility,[14,17] pathogen inhibition,[6,18] and immune-modulation and gut mucosal immunity[19-21] have been reported. Thus, the aims of this research were to investigate the effects of probiotic used at different rearing periods on performance, intestinal microbial population and carcass traits in broiler chickens.

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Materials and Methods

Birds, experimental design and management

A total of 378 one-day-old chickens (Ross 308) were used in the present study. The chickens were weighted and randomly allotted to cages such that each cage of chickens had a similar initial weight distribution. All the chicks were kept under similar management conditions according to Ross 308 strain catalogue. They were divided into seven equal groups (each group was constituted by three replicates of 18 birds per replicate) according to the probiotic contents incorporated in the diets (0.3 g/kg of basal diets in starter, 0.25 g/kg of basal diets in grower and 0.2 g/kg of basal diets in finisher period) at different rearing periods. Therefore, the experiment groups included, A (control, without probiotic), B (prebiotics feeding between 1 to 10 days), C (prebiotics feeding between 1 to 24 days), D (prebiotics feeding between 1 to 42 days), E (prebiotics feeding between 11 to 24 days), F (prebiotics feeding between 11 to 42 days) and G (prebiotics feeding between 24 to 42 days). The chickens received the desired probiotic at

different days of rearing period. The treatment diets were formulated to meet the requirements of broiler as recommended by the catalog of Ross 308 broilers (Table 1). The feed mixture of the experimental group was supplemented with the probiotic *Yeasture*®, made from *Saccharomyces cerevisiae, Lactobacillus casei*, *Lactobacillus acidophilus, Streptococcus faecium* and *Bacillus subtilis*. Birds had access to food and water *ad libitum*. All animal experimentation was conducted in accordance with the regulations of Islamic Azad University, Animal Ethics Committee.

In each pen, total chicken body weight, chicken numbers and the weight of unconsumed and added food were recorded on days 0, 10, 24 and 42. The mean body weight gain, food consumption and food conversion ratios were calculated for each cage (replicate) between 1 and 42 days. For each time period, the body weight gain was calculated and expressed as grams per bird. The food intake (g of food intake/bird) over the entire grow-out period was calculated by totaling food consumption in each time interval between each bird sampling. The food conversion ratio (g of food intake/g of body weight gain) was calculated by dividing the total food intake by the total weight gain in each cage.

Carcass characteristics

On day 42, six birds per experimental groups were randomly selected for organ weights. Birds were weighted and slaughtered by cervical dislocation and then the abdominal cavity was opened. The weight of carcass, gizzard, liver, intestine and abdominal fat were recorded and the corresponding percentages (% of live body weight) were calculated.

The measurement of intestinal microbial population

On days 10, 24 and 42, two birds per replicate were randomly selected for the measurement of intestinal microbial population. For this purpose, one gram of the composite gut sample from each chicken was diluted with 9 mL of 0.9% saline solution and mixed on a vortex. Viable counts of bacteria in the gut samples were then conducted by plating serial tenfold dilutions (in 1% peptone solution) into *Lactobacilli*de Man, Rogosa and Sharpe agar plates and MacConkey agar plates (to isolate the *Lactobacillus* and *Coliforms*). The *Lactobacilli* de Man, Rogosa and Sharpe agar plates were then incubated for 48 h at 37°C under anaerobic conditions. The *Lactobacillus* and *Coliforms* colonies were conducted immediately after removal from the incubator as described by Kang and colleagues.[22]

Statistical analysis

All the data were subjected to ANOVA procedures for completely randomized designs using the general linear model (GLM) procedure of the SAS program.[23] When data were in the form of percentages they were transformed by arc sin square root. Differences between treatments were compared by the Duncan's multiple range tests following ANOVA, and the values were considered statistically different at $P<0.05$.

Results

On the whole, there were no significant treatment effects on feed intake through the whole experimental period. The body weight gains ($P<0.05$) and feed conversion ratio ($P<0.05$) significantly improved in chickens treated by probiotic at different days comparison to the control group (Table 2). So that, using probiotic at different days of rearing period (1-24 d, 1-42 d and 11-42 d) increased body weight gain in comparison to the control group and treatment that had consumed probiotic only during 11-24 days. Also, adding probiotic into the diet at different days of rearing period (1-42 d) improved feed conversion ratio in comparison to the control group and treatment that had consumed probiotic only during 11-24 days. Furthermore, as shown in Table 3, the addition of probiotic in the diet of chickens had a significant effect on the microbial population. Besides, the microbial population significantly improved in chickens fed with probiotic in comparison to the control chicken. Chickens fed with probiotic had significantly higher *Lactobacillus* and lower *Coliforms* in comparison to the control chickens ($P<0.05$). In addition, the carcass weight and the relative gizzard weight significantly increased in 42-day-old birds supplemented with probiotic in comparison to control birds ($P<0.05$). So that, using probiotic at different days of rearing period, increased carcass yield in in comparison to the control group ($P<0.05$). In contrast, the liver, abdominal fat and intestinal weights were not markedly altered in the treated birds ($P>0.05$) (Table 4).

Discussion

Based on the results of the present study, the dietary supplementation of probiotic *Yeasture*® in chickens can be seen as an effective tool to improve the body weight gain and

Table 1. Composition of the basal diet (ingredient and nutrients) given to broiler chickens for 6 weeks.

Ingredient (%)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	56.64	57.05	61.16
Soybean meal (44% CP)	36.74	35.12	31.20
Sunflower oil	0.95	3.20	3.22
Di calcium phosphate	1.89	1.65	1.53
Oyster shell	1.35	1.12	1.08
Sodium bicarbonate	0.26	0.26	0.26
Salt	0.23	0.23	0.23
Vitamin premix*	0.25	0.25	0.25
Mineral premix°	0.25	0.25	0.25
DL-Methionine	0.42	0.26	0.25
L-Lysine mono-HCL	0.38	0.11	0.12
L-Threonine	0.64	0.50	0.45
Nutrients (calculated)			
ME, kcal/kg	2850	3000	3050
CP, %	22.14	20.95	19.54
Ca, %	1.05	0.90	0.85
Available P, %	0.50	0.45	0.42
Na, %	0.18	0.18	0.18
K, %	0.90	0.87	0.81
Cl, %	0.17	0.17	0.17
Met + Cys, %	1.07	0.90	0.86
Lys, %	1.43	1.18	1.09
Thr,%	0.94	0.29	0.74
Trp, %	0.94	0.80	0.26

*Vitamins mixture provide per 2.5 kilogram of diet: vitamin A, 12000000 IU; vitamin B_1, 4000 mg; vitamin B_2, 6000 mg; vitamin B_3, 18000 mg; vitamin B_6, 3000 mg; vitamin B_{12}, 15 mg; vitamin D3, 5000000 IU; vitamin E, 50000 IU; vitamin K_3, 3000 mg; vitamin B_9, 1500 mg; vitamin B_5, 70000 mg; vitamin H_2, 100 mg; choline chloride, 400000 mg.°Mineral mixture provide per 2.5 kilogram of diet: Mn, 120000 mg; Zn, 100000 mg; Fe, 40000 mg; Cu, 20000 mg; I, 1000 mg; Se, 300 mg.

feed conversion ratio, especially in chickens treated with probiotic throughout the whole experimental period (1 to 42 d) whereas, the difference in feed intake among all the diets were non-significant. These results are in line with the finding of Raceviciute and colleagues[24] who, demonstrate that, the body weight gain and food efficiency significantly improved in chickens fed with probiotic preparation *Yeasture*® in comparison to the control group. Karaoglu and Durdag[25] showed that prebiotic preparation consisted of *Saccharomyces cerevisiae* the weight of chickens at the middle of trial (14-28 days) increased from 3.62 to 7.57%, while in the latest trial periods (30-42 days) had no effect on the growth of chickens.

In the same trial, the feed efficiency at 1-7 days and 8-14 days were respectively by 8.5 and 16.67% lower than that in the control group. In parallel, Celik and colleagues[26] have found a positive effect of probiotic (*Saccharomyces cerevisiae)* at the end of conducted experiment (37 days) where the body weight of broilers was by 5.7% higher in comparison to the control group. On the other hand, Bai and colleagues[27] showed that, supplementing 0.1% probiotic product in diets, as an alternative to antimicrobial growth promoters, for better growth performance of broiler chickens during the starter phase. Likewise, Zulkifli and colleagues[28] and Yeo and Kim[29] reported that, the addition of 0.1 % probiotic *Lactobacillus* to the diet of broiler chickens improved weight gain and feed conversion ratio from 1 to 21 day of age, but not from 22 to 42 day of age. Li and colleagues[17] demonstrated that a commercial probiotic mixture of yeasts and other microbes improved growth performance in the starter phase. Therefore, the results of probiotic supplementation are consistent. In the previous studies, the broiler chickens fed diets containing a mixture of 12 *Lactobacillus* strains (1 strain of *Lactobacillus crispatus,* 2 strains of *Lactobacillus acidophilus*, 3 strains of *Lactobacillus fermentum*, and 6 strains of *Lactobacillus brevis*) had better body weight gain from 22 to 42 d of age,[30-32] and lower feed conversion ratio during the starter and grower periods.[30,33] Diets supplemented with

Table 2. Effects of probiotic *Yeasture*® using at different rearing periods on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broilers to 1-42 day of age.

Days of probiotic treated	BWG	FI	FCR
A (Control, without probiotic)	2051.16^{b}	4240.70	2.06^{a}
B (1-10 days)	2227.06^{ab}	7162.70	1.86^{abc}
C (1-24 days)	2280.02^{a}	4220.84	1.85^{bc}
D (1-42 days)	2376.78^{a}	4124.70	1.73^{c}
E (11-24 days)	2040.57^{b}	4065.70	1.99^{ab}
F (11-42 days)	2385.03^{a}	4322.20	1.81^{bc}
G (24-42 days)	2185.95^{ab}	4081.10	1.88^{abc}
SEM	44.33	90.99	0.04
P-Value	0.0002	0.4412	0.0020

$^{a-b}$Averages in a column with different superscript letters are significantly different (P<0.05).

Table 3. Effects of probiotic *Yeasture*® using at different rearing periods on intestinal microbial population (log 10 CFU/g) of broilers at days 10, 24 and 42.

Days of probiotic treated	10 day		24 day		42 day	
	*Lactobacillus** (log 10 CFU/g)	*Coliforms* (log 10 CFU/g)	*Lactobacillus**	*Coliforms*	*Lactobacillus**	*Coliforms*
A (Control, without probiotic)	3.96^{b}	6.79	6.50^{ab}	6.82^{a}	7.13	6.69^{ab}
B (1-10 days)	4.43^{ab}	6.40	5.89^{b}	6.09^{ab}	7.45	6.83^{ab}
C (1-24 days)	6.76^{ab}	3.87	7.36^{ab}	5.45^{ab}	7.41	7.43^{a}
D (1-42 days)	8.26^{a}	4.22	8.16^{a}	6.18^{ab}	8.46	5.14^{b}
E (11-24 days)	4.69^{ab}	5.85	6.83^{ab}	5.47^{ab}	6.79	6.59^{ab}
F (11-42 days)	5.42^{ab}	5.16	7.78^{ab}	4.07^{b}	8.15	5.62^{ab}
G (24-42 days)	3.82^{b}	5.05	6.02^{b}	6.09^{ab}	8.58	5.92^{ab}
SEM	0.93	0.70	0.47	0.50	0.50	0.48
P-value	0.0102	0.0512	0.0063	0.0110	0.1010	0.0159

$^{a-c}$Averages in a column with different superscript letters are significantly different (P<0.05). *Lactobacillus population detected could come from both native gut microbiome and added prebiotics.

Table 4. Effects of probiotic *Yeasture*® using at different rearing periods on carcass, intestine, liver, gizzard and abdominal fat of from broiler chickens when they were 42 days old.

Days of probiotic treated	Carcass (%)*	Intestine (%)	Liver (%)	Gizzard (%)	Abdominal fat (%)
A (Control, without probiotic)	73.65^{b}	3.09	2.50	2.31^{c}	1.67
B (1-10 days)	78.30^{ab}	3.95	2.41	2.82^{abc}	1.50
C (1-24 days)	77.29^{ab}	3.81	3.32	2.36^{bc}	1.47
D (1-42 days)	82.55^{a}	3.75	2.91	2.97^{a}	1.66
E (11-24 days)	79.58^{ab}	3.69	2.86	2.73^{abc}	1.50
F (11-42 days)	78.96^{ab}	3.69	2.76	2.86^{ab}	1.59
G (24-42 days)	81.00^{a}	3.75	3.11	2.61^{abc}	1.67
SEM	3.04	0.19	0.20	0.24	0.08
P-Value	0.0317	0.1019	0.0534	0.0036	0.4593

$^{a-c}$Averages in a column with different superscript letters are significantly different (P<0.05). *% of live body weight.

Saccharomyces cerevisiae products (from 0.25 to 0.75%) had no significant effect on the performance of broiler chickens on 1 to 21 d, while, improved performance[34-36] and decreased feed efficiency[25,37] in broilers after the 21st day of age. Thus, the discrepancy between our results and those of earlier studies may be due to the differences in probiotic concentrations, microbial species or strains of microorganisms used, or yeast product formulations.

The reason for the improvements in body weight gain and feed conversion ratio of broilers fed with probiotic supplement were probably not only the increased feed intake, but also the improved nutrient digestibility. Several studies stated that supplemental yeast culture improved Ca and P digestibility.[34,38] In addition, diets supplemented with a mixture of yeasts and other microbes improved the digestibility of dry matter, energy, crud protein Ca, P, and some amino acids in broilers.[17] The improved nutrient digestibility may cause better growth performance of broilers. On the whole, the positive effects of probiotics on growth performance have been reported by Cmiljanic and colleagues;[39] Anjum and colleagues;[40] Aftahi and colleagues.[41]

Furthermore, chickens fed probiotic *Yeasture*® had significantly higher *Lactobacillus* and lower *Coliforms* counts compared with control chickens. In agreement, Sherief and colleagues[42] reported that, the concentrations of bacteria belonging to *Lactobacillus* in the duodenum and jejunum at d 42 were significantly higher in prebiotic supplemented broilers in comparison to the control group whereas, *Coliforms* colony count were not significantly affected by any of the dietary probiotic treatments.

In this experiment, the addition of probiotic *Yeasture*® to the diet of chickens at different rearing periods provided and improved nutrient assimilation by reducing the growth of harmful bacteria such as *Coliforms,* and the stimulation of the growth of useful bacteria such as *Lactobacillus* in the intestinal tracts of chickens. This condition improves the growth performance of broiler chickens.

In the present study, the carcass and the relative gizzard weights also significantly improved in chickens supplemented with probiotic *Yeasture*®, whereas the relative weights of liver, intestine and abdominal fat were not significantly altered. These results were in contrast with those reported by Raceviciute and colleagues[24] showing no significant effects of probiotic preparation *Yeasture*®-*w* in broiler chickens on carcass yield weight on day 56 of age. Baidya and colleagues[43] and Al-Barwary and colleagues[44] demonstrate that probiotics feeding did not have any influence on the carcass weight. Likewise, Islam and colleagues[45] stated that, there were no significant treatment effects on thigh, wing and liver of broiler chickens on day 35 of age.

Conclusions

In conclusion, this study showed that diet supplementation with probiotic *Yeasture*® at the early days of the rearing periods of broiler chickens especially, 1 to 24 and 1 to 42 days, had a positive effect on intestinal microbial population, leading to more nutrients being assimilated by the chickens and consequently to a greater performance in broiler chickens.

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