

Efficacy of Bactocell® and Toyocerin® as Probiotics on Growth Performance, Blood Parameters and Intestinal Morphometry of Turkey Poults

Research Article

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ABSTRACT

A feeding trial was conducted to investigate the efficacy of *Pediococcus acidilactici* (Bactocell®) or *Bacillus cereus* (Toyocerin®)-based probiotics on turkey's performance, carcass yield, blood parameters and intestinal morphology. A total of 240 1-day-old male Nicholas turkey poults were allocated into three dietary groups with four replicates. A corn-soybean-based diet was used as a basal diet. The dietary groups were 1) control (basal diet), 2) basal diet plus Bactocell® (1×10^9 cfu of *Pediococcus acidilactici*/g of the diet) 3) basal diet plus Toyocerin® (1×10^9 cfu of *Bacillus cereus*/g of the diet). Poults assigned in a normal condition from 1 to 84 days of age. Birds raised on floored pen and in an environmentally controlled house. Birds given *ad libitum* access to water and diet. Poults weighted at every week by pen basis to determine average body weight. Feed intake per pen recorded at the same age and feed conversion ratio calculated for whole period. Eight birds per treatment randomly selected to measure the serum cholesterol, triglyceride, heterophil, lymphocyte levels and histomorphological measurements of small intestine on day 84. The result of this study shown that there was not a significant association between added probiotics to feed and weight growth, feed conversion ratio, cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels ($P > 0.05$). There was a significant result of decrease epithelium thickness, increase villus height and increase cell area of Goblet ($P < 0.05$). Birds fed diet supplemented with probiotics also had lower ($P < 0.05$) Coliforms number of the ileum than that of the control. It could be concluded that under the condition of the current study, the probiotic affected small intestinal morphology of turkey poults significantly, but inclusion of these probiotics at the recommended dose (1 g/kg in diet) has no adverse effect on blood parameters and performance of turkey poults. The key points of using probiotics are in maintaining gastrointestinal health and resistance to pathogens and prevent digestive disorders that may express.

KEY WORDS Bactocell®, intestinal morphometry, performance, Toyocerin®, turkey.

INTRODUCTION

Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics of poultry industry. Therefore, it is highly essential to improve feed efficiency of poultry to produce meat economically and also food safety is more seriously considered than before. Over the

last decade, the importance of gastrointestinal tract health in broiler chicken has been increasingly recognized due to its contribution to their overall health and performance (Mountzouris *et al.* 2007). In recent years, concerns about antimicrobial resistance have grown, but the main concerns have been focused specifically on resistance within the food supply (Cui *et al.* 2005). Based on these statements, using

alternative growth promoters such as probiotics become important every day. Probiotic is a live microbial feed supplement, which beneficial to the host animal by improving its intestinal microbial population. It has been used for the alternative tools for helping chicks colonize normal microflora as conventionally hatched chicks do (Fuller, 1989). Due to their several negative effects, antibiotics have gradually been replaced by probiotics in controlling intestinal pathogenic bacteria (Fuller, 1992).

Probiotics represent a functional nutritional approach targeting gut function and health and provide a potential alternative to antimicrobial growth promoters in broiler nutrition (Zulkifli *et al.* 2000; Mountzouris *et al.* 2007; Applegate *et al.* 2010). On the other hand, to achieve high levels of economic efficiency, poultry are raised under intensive rearing systems in densely populated colonies of flocks. During the process of intensive production rearing, chickens are stressed by several factors such as transportation to the growing site, overcrowding, vaccination, chilling and / or overheating (Panda *et al.* 1999). These stressors can be alleviated by early establishment and maintenance of a favourable microbial population in the digestive tract, which in turn improve the assimilation of the food particle and performance (Panda *et al.* 2000). Evidence is presented showing that treatment with probiotics moderates growth retardation in chickens and the probiotic protection occurs whether the disease is induced by an environmental, microbial or nutritional stressor (Dunham *et al.* 1998).

One of the main roles of probiotics is control of serum cholesterol and triacylglycerol levels (Lin *et al.* 1989; Taranto *et al.* 1998). There are some evidences suggesting that *Lactobacillus* feed supplementation reduces the cholesterol and fatty acid composition of broiler serum (Kalavathy *et al.* 2006). Supplementation of probiotic in chicken has been shown to reduce the cholesterol concentration in egg yolk (Abdulrahim *et al.* 1996; Haddadin *et al.* 1996) and serum (Mohan *et al.* 1996; Jin *et al.* 1998). However, results under field conditions have generally been inconsistent (Stavric *et al.* 1992). Results from trials conducted with broiler fed various probiotics were inconsistent. Some researchers reported positive responses of weight gain and feed conversion ratio in chickens due to consumption of probiotics (Chiang and Hsieh, 1994; Mohan *et al.* 1996; Cavazzoni *et al.* 1998; Jin *et al.* 1998; Joy and Samuel, 1997; Kumprecht and Zobac, 1998; Fritts *et al.* 2000), while others reported no beneficial effects (Maiolino *et al.* 1992; Choudhury *et al.* 1998; Panda *et al.* 1999; Kahraman *et al.* 2000). Scarce information is available relating to probiotic feeding on the performance and blood parameters of turkey poults. To reveal the genetic potential of turkey for optimal production, they must be free from disease as well as consume diets that meet their requirements. Recently, the

use of probiotics in the poultry diet for control of pathogens and performance enhancement has gained attention due to the increasing restriction of antibiotics as growth promoting agents.

This study conducted us to investigate the efficacy of two kinds of probiotics (Bactocell® and Toyocerin®) respectively based on *Pediococcus acidilactic* and *Bacillus cereus* genera on growth performance, intestinal morphology and Coliforms number of the ileum of turkeys in a optimal condition from 1 to 84 days of age and also to study some blood parameters that have been usually used to examine the probiotic effects of feed supplements in turkey nutrition such as serum cholesterol, triglyceride, LDL, HDL, heterophil and lymphocyte levels.

MATERIALS AND METHODS

Birds and dietary treatments

Two hundred and forty 1-day-old Nicholas male turkey poults (initial weight 55 ± 2 g), randomly divided into 3 groups. Each treatment had 4 replicates and each replicate assigned to a pen with 20 turkey chicks. Birds raised on floored pen (2.8×2.5×1.5 meter) and in an environmentally controlled house. Birds had *ad libitum* access to water and diet.

The experimental treatments received a basal diet that supplemented as follows: "Control," with no additives; "Bactocell®," 1×10^9 cfu of *Pediococcus acidilactici*/g of the diet, "Toyocerin®," 1×10^9 cfu of *Bacillus cereus*/g of the diet. The bacterial flora in the Bactocell® probiotic was *Pediococcus acidilactici* in a concentration of 10^9 cfu/g.

Bactocell® was manufactured by Lallemand-France and was used at a dose of 1 kg/ton ration. Toyocerin® (Toyo Jozo Co., Japan) was a probiotic containing live spores of *Bacillus cereus var. toyoi* spores in a dry powder form (at least 10^9 *Bacillus toyoi* spores per kg of product), which was incorporated in the feed. Before formulation, each ingredient analyzed in triplicate for dry matter (DM), crude protein (CP), ether extracts (EE), crude fibre (CF) and ash, calcium and phosphorus following AOAC (1995). The basal diet was a corn-soybean meal diet in mash form that was formulated to meet Nicholas male nutrient requirements (Aviagen Turkeys-Commercial Feeding Program Calculator, 2009), (Table 1).

Growth performance traits and carcass yield percentages

Poults weighted each week by pen basis to determine average body weight. Feed intake per pen recorded weekly and feed conversion ratio calculated for whole period. At the end of the experiment, after weighing, 8 birds per treatment were randomly selected and killed by cervical dislocation.

Table 1 Ingredient and chemical composition of the basal diet

	1-2 weeks	3-5 weeks	6-9 weeks	10-12 weeks
Ingredients (%)				
Corn	47.50	55.50	58.70	62.50
Soybean meal	40.00	38.50	35.00	31.00
Wheat gluten	6.00	0.00	0.00	0.00
Vegetable oil	0.00	0.25	1.10	1.70
DL-Met	0.20	0.25	0.22	0.20
L-Lys	0.35	0.30	0.28	0.25
Di calcium phosphate	3.00	2.60	2.30	2.05
Limestone	1.40	1.10	1.10	1.00
Salt	0.25	0.25	0.25	0.25
Soda	0.20	0.15	0.15	0.15
Vitamin premix ¹	0.50	0.50	0.40	0.40
Mineral premix ²	0.50	0.50	0.40	0.40
Filler	0.10	0.10	0.10	0.10
Analysis (%)				
ME, kcal/kg	2800	2800	2900	3000
Crude Protein	25.60	22.20	21.00	19.20
Lysine	1.55	1.43	1.33	1.21
Methionine	0.59	0.57	0.52	0.48
Met + Cys	1.00	0.92	0.86	0.79
Threonine	0.96	0.86	0.80	0.75
Valine	1.19	1.03	0.96	0.89
Calcium	1.20	1.10	1.00	0.94
Ava. phosphorous	0.60	0.55	0.50	0.46
Potassium	0.96	0.93	0.87	0.84
Sodium	0.16	0.16	0.16	0.16
Chlorine	0.23	0.23	0.23	0.23
DCAB, mEq/kg	250.00	242.00	227.00	220.00

¹ The vitamin premix supplied the following per kilogram of complete feed: vitamin E (DL- α -tocopheryl acetate): 50 IU; Cholecalciferol: 3500 IU; vitamin A (retinyl acetate): 9000 IU; Menadione: 2 mg; vitamin B₁₂: 0.02 mg; Riboflavin: 5 mg; Thiamine: 2 mg; Pantothenic acid: 16 mg; Niacin: 65 mg; Choline: 500 mg; Folic acid: 2 mg; Biotin: 0.2 mg and Pyridoxine: 4 mg.

² The mineral premix supplied the following per kilogram of complete feed: Copper (CuSO₄·5H₂O): 12 mg; Manganese (MnSO₄·H₂O): 120 mg; Cobalt (CoCl₂): 0.4 mg; Selenium (Na₂SeO₃): 0.3 mg; Zinc (ZnO): 100 mg; Iron (FeSO₄·7H₂O): 80 mg and Iodine (Iodized NaCl): 2 mg.
ME: metabolizable energy and DCAB: dietary cation-anion balance.

The heart, gizzard, liver and fat pad were excised and weighed. The gastrointestinal tract was weighed after removal of the content. Then, the birds were defeathered and then head, neck, and feet were removed and carcasses were eviscerated. The carcass weight was then determined and the carcass yield percentage was calculated by dividing the carcass weight by the live body weight of birds multiplied by 100. Carcasses were weighed and cut up in commercial parts: breast, drumstick, thighs, feet, back, neck, and wings. Parts yield (drumstick, thighs, breast) was calculated as the ratio between the average part weight representative of each experimental unit and carcass weight.

Analysis of blood samples

Twenty four birds (eight birds per treatment) randomly selected to measure the serum cholesterol and triglyceride levels. The blood samples of these birds were taken on day 84. The blood was collected in a test tube to obtain serum. The concentration of total cholesterol in serum samples analyzed by duplicate, which using an automatic biochemical analyzer following the instructions of the supplier of the kit (Pars Azmoon, Cat. No. 1 500 010, Iran). The numbers and proportions (percentage) of heterophils and lympho-

cytes were determined using a Cell-Dyn Emerald Abbott Hematology Analyzer which uses electronic impedance and laser light scattering and has been standardized for analysis of turkey blood. Heterophil/lymphocyte ratios, an indicator of stress in birds, were calculated by dividing the number of heterophils by the number of lymphocytes in 1 mL of peripheral blood.

Intestinal morphology measurement

Twenty four birds (eight birds per treatment) on day 84 were randomly selected for intestinal morphology measurement.

A 1-cm segment of the midpoint of the duodenum, jejunum, and ileum removed, wash in physiological saline solution, and fix in 10% buffered formalin. Each segment then embedded in paraffin and a 2- μ m section of each sample placed on a glass slide and stain with hematoxylin and eosin for examination (Sakamoto *et al.* 2000). Histological sections examined with a phase contrast microscope. Villus height measured from the top of the villus to the top of the lamina propria. Fifteen measurements took per bird for this variable; for purposes of statistical analysis, the average of these values used.

Coliforms count

Twenty four birds (eight birds per treatment) on day 84 that were selected for intestinal morphology measurement were used for Coliforms counts. One gram of ileal digesta was sampled.

Samples were serially diluted and subsequently plated on duplicate Mac Conkey agar media (Himedia, India) for the enumeration of Coliforms. Plates incubated at 37 °C for 24 to 72 h, aerobically.

Statistical analysis

Data analyzed in a completely randomized design using the General Linear Model procedures of SAS 9.1 (SAS, 2003), and means compared by Duncan's multiple range test at 5% probability.

RESULTS AND DISCUSSION

Growth performance

The result of this study shows no significant association between added probiotics to feed intake and weight growth or feed conversion ratio changes ($P>0.05$; Table 2).

Carcass yield

There was a significant association decrease of gizzard weight in probiotic groups compared with control and an increase of breast meat yield in Bactocell® group ($P<0.05$; Table 3).

Table 2 Growth performance of turkey poults fed diets containing probiotics during 84 days rearing period

	1-2 weeks	3-5 weeks	6-9 weeks	10-12 weeks	Overall
Feed intake					
Control	0.36	1.90	6.20	10.69	19.15
Bactocell®	0.36	2.00	6.51	11.96	20.84
Toyocerin®	0.38	2.00	6.82	12.23	21.43
SEM	0.02	0.05	0.27	0.50	0.75
Weight gain					
Control	0.25	1.02	2.72	4.35	8.33
Bactocell®	0.26	1.04	2.93	4.18	8.41
Toyocerin®	0.27	1.05	2.98	4.45	8.75
SEM	0.01	0.03	0.07	0.12	0.13
Feed conversion ratio					
Control	1.43	1.87	2.29	2.46	2.30
Bactocell®	1.41	1.93	2.22	2.86	2.48
Toyocerin®	1.40	1.91	2.29	2.75	2.45
SEM	0.03	0.04	0.09	0.09	0.07

SEM: standard error of the means.

Table 3 Carcass characteristics of turkey poults fed diets containing probiotics at 84 days of age (percentage of the carcass weight)

	Pre-slaughter (kg)	Carcass (%)	Breast (%)	Thigh (%)	Drumstick (%)	Gizzard (%)	Liver (%)	Fat (%)	Heart (%)
Control	8.65	86.70	23.50 ^b	10.90	12.80	1.60 ^a	1.61	0.27	0.39
Bactocell®	9.20	87.90	24.50 ^a	11.10	12.20	1.48 ^b	1.79	0.31	0.31
Toyocerin®	9.20	87.70	23.30 ^b	11.00	12.60	1.43 ^b	1.85	0.38	0.31
SEM	0.30	0.50	0.30	0.10	0.10	0.03	0.08	0.05	0.04

The means within the same column with at least one common letter, do not have significant difference ($P>0.05$).

SEM: standard error of the means.

Blood parameters

The results of the blood test show that there was not a significant effect of the probiotics on cholesterol, triglyceride, HDL, LDL, heterophil and lymphocyte levels. In addition, there was a non-significant of an increased the ratio of HDL to LDL and ratio of heterophil to lymphocyte ($P>0.05$; Table 4).

Coliforms count

As the result of this study shows, the Coliforms number of the ileum decreased significantly ($P<0.05$) in Bactocell® and Toyocerin® groups when compared with control (Table 5).

Intestinal morphometry

Intestinal morphology measurements were improved by supplementation of Bactocell® and Toyocerin® in the turkey diet (Tables 6, 7 and 8).

Duodenum

The villus height, villus surface area, crypt depth and number of goblet cells were increased ($P<0.05$) by adding Bactocell® and Toyocerin® in turkey diet. Bactocell® supplementation decreased epithelial thickness significantly ($P<0.05$) and Toyocerin® decreased the epithelial thickness numerically compared with the control.

Jejunum

Toyocerin® supplementation increased villus height significantly ($P<0.05$) and Bactocell® increased the villus height numerically compared with the control. The villus surface area, crypt depth and number of goblet cells were significantly increased for Bactocell® and Toyocerin® supplementations compared with control ($P<0.05$).

Ileum

The villus height, villus surface area, crypt depth and number of goblet cells were significantly increased for Bactocell® and Toyocerin® supplementations compared with control ($P<0.05$). Moreover, Bactocell® and Toyocerin® supplementation decreased the epithelial thickness significantly ($P<0.05$) compared with control. In the current study, the addition of Bactocell® and Toyocerin® in turkey poult diets did not show any positive effect on performance. Similarly, Willis and Reid (2008) reported that the dietary inclusion of probiotics (minimum presence of 1.04×10^8 cfu/g) had no significant effect on broiler growth performance. Generally, results reported in the literature on the beneficial effects of probiotics on broiler growth performance are inconsistent. Factors such as bird characteristics (species, age, stage of production), nutrition, type and dosage of feed additives, management, and environmental conditions can affect broiler responses to probiotics (Yang *et*

al. 2009), thereby many of these factors accounted for the contrasting results.

Rearing condition is an important factor contributing to these variable results.

Table 4 Blood parameters of turkey poult fed diets containing probiotics at 84 days of age

	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	HDL/LDL	Heterophil	Lymphocyte	H/L
Control	123	43	53	39	1.40	24.30	72.30	0.34
Bactocell®	109	30	68	42	1.70	25.50	69.50	0.37
Toyocerin®	103	27	63	41	1.60	26.00	70.50	0.37
SEM	8.00	4.80	5.00	6.10	0.20	1.40	1.80	0.30

SEM: standard error of the means.

HDL: high-density lipoprotein and LDL: low-density lipoprotein.

Table 5 Effect of Bactocell® and Toyocerin® supplementations on total bacterial count and Coliforms number of ileum of turkeys

	Total bacteria count (cfu×10 ³ /g)	Coliform count (cfu×10 ³ /g)	Coliform:TBC (%)
Control	196 ^b	5.7 ^a	3.9 ^a
Bactocell®	775 ^a	2.2 ^b	0.3 ^b
Toyocerin®	83 ^b	0.6 ^b	1.1 ^b
SEM	38.7	0.7	0.9

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 6 Effect of Bactocell® and Toyocerin® supplementations on histomorphological parameters of the duodenum in turkeys

	Villus height (µm)	Crypt depth (µm)	Villus surface area (µm ²)	Epithelial thickness (µm)	Goblet cell number
Control	1490 ^c	179.8 ^c	134500 ^c	48.1 ^a	39.0 ^c
Bactocell®	2087 ^b	250.5 ^b	187625 ^b	40.5 ^b	100.5 ^b
Toyocerin®	2359 ^a	288.0 ^a	215875 ^a	46.4 ^a	128.8 ^a
SEM	64.1	6.2	4550.8	0.6	1.4

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 7 Effect of Bactocell® and Toyocerin® supplementations on histomorphological parameters of the jejunum in turkeys

	Villus height (µm)	Crypt depth (µm)	Villus surface area (µm ²)	Goblet cell number
Control	847.5 ^b	127.8 ^b	64525 ^c	9.5 ^c
Bactocell®	920.0 ^b	372.0 ^a	87000 ^b	20.8 ^b
Toyocerin®	1322.5 ^a	425.3 ^a	101200 ^a	26.5 ^a
SEM	119.2	25.6	3135.6	0.6

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 8 Effect of Bactocell® and Toyocerin® supplementations on histomorphological parameters of the ileum in turkeys

	Villus Height (µm)	Crypt Depth (µm)	Villus surface area (µm ²)	Epithelial thickness (µm)	Goblet cell number
Control	773.5 ^c	100.3 ^c	55900 ^c	37.4 ^a	10.3 ^c
Bactocell®	1080 ^b	169.0 ^b	77750 ^b	31.1 ^c	20.0 ^b
Toyocerin®	1205 ^a	188.0 ^a	87950 ^a	35.0 ^b	24.0 ^a
SEM	29.9	4.4	2081.3	0.4	1.1

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Angel *et al.* (2005) concluded that dietary supplementation with probiotics under favourable rearing conditions, without any disease or stress, had no beneficial effects on broiler growth performance. In another study, Timmerman *et al.* (2006) reported that the positive effects of probiotics in high performance broilers are lower than of low performing birds.

Nevertheless, no differences were seen in carcass yield due to the use of probiotics, and these results were similar to findings reported by Maiorka *et al.* (2001), Moreira *et al.* (2001) and Vargas *et al.* (2002). The results of the blood test showed slightly improvement in blood cholesterol and triglyceride levels in turkey poult fed the probiotic compared with control birds, but cholesterol and triglyceride

levels were not significantly different among treatment groups. Therefore, it was concluded that supplementation of the Bactocell® or Toyocerin® to turkey poults in the level of 1 g/kg diet has not the best performance.

The role of a diet is not only to provide nutrients to fulfill requirements of the body, but also to modulate various functions of the body. Improvement in growth performance and feed efficiency of broiler chickens fed probiotics (Kabir *et al.* 2004; Cavazzoni *et al.* 1998; Zulkifli *et al.* 2000; Mountzouris *et al.* 2007; Samli *et al.* 2007; Jin *et al.* 1998) is thought to be induced by the total effects of probiotic action including the maintenance of beneficial microbial population (Fuller, 1989), improving feed intake (Nahanshon *et al.* 1992; Nahanshon *et al.* 1993) and altering bacterial metabolism (Jin *et al.* 1997; Cole *et al.* 1987).

The decreased number of Coliforms of ileum was observed by addition of Bactocell® and Toyocerin® in the turkey diet. Probiotics exert antagonism against other microorganisms, including enteric pathogens, primarily through the production of lactic acid (Daeschel and Klaenhammer, 1985).

The increased villus height observed in the current study may be explained by the enhanced efficiency of digestion and absorption of the intestine due to a population of beneficial bacteria that supply nutrients and stimulate vascularization and enlargement of intestinal villus (Bedford, 2000; Gilmore and Ferretti, 2003). Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients (Caspary, 1992).

The intestinal epithelial cells originating in the crypt migrate along the villus surface upward to the villus tip and are extruded into the intestinal lumen within 48 to 96 h (Imondi and Bird, 1966; Potten, 1998). A shortening of the villi and deeper crypts may lead to poor nutrient absorption, increased secretion in the gastrointestinal tract and lower performance (Xu *et al.* 2003). In contrast, increases in the villus height and villus height: crypt depth ratio is directly correlated with increased epithelial cell turnover (Fan *et al.* 1997) and longer villi are associated with activated cell mitosis (Samanya and Yamauchi, 2002).

CONCLUSION

As the result of this study shows, there is a significant increase in cell area of goblet. The increased number of goblet cells, increased mucus secretion in the gastrointestinal mucosa, and as a result prevents it from binding to the mucosal pathogens. The histomorphological changes in the intestine of turkey poults reported in the present study provide new information regarding the potential for using probiotics in turkey feed.

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