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Usage of Green Fluorescent Protein for Tracing Probiotic Bacteria in Alimentary Tract and Efficacy Evaluation of Different Probiotic Administration Methods in Broilers

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ABSTRACT

Lactobacilli, like the other gut commensal bacteria, are well known for their use in poultry nutrition and for their probiotic properties. However, little is known about their interaction with the gastro-intestinal tract when administered *in vivo*. To specifically monitor the passage of lactobacilli after administered in hatchery, *Lactobacillus plantarum* was transformed with the recombinant vector pLEM415::gfp. A total of 200 one-day old chicks (ROSS 308) were assigned to five experimental groups including the control and four in-hatchery probiotic administration method groups comprised of *in ovo* injection, oral gavage, spray, and vent lip application. At 0, 4, 12, 24, 48, and 72 hours post-probiotic administration, 6 chicks were sacrificed from each group. Adhered bacteria were sampled from intestinal sections. Polymerize chain reaction (PCR) was used to trace the transformed *L. plantarum* in the alimentary tract of the birds. The GFP transformed bacteria were detected in intestinal samples of oral gavage, spray, and *in ovo* injection groups; while in vent lip method no GFP transformed bacteria were detectable. Oral gavage method of probiotic administration was the most effective route, which seemed to be the result of direct delivery of the full dose of probiotic microorganisms into the target sites. Based on the results of this trial, administration of probiotic in hatchery had a positive effect on the morphology of the intestine and *in ovo* injection route, and oral gavage method seemed to be more effective. In this experiment, the utility of transformed probiotic bacteria with GFP was shown to monitor the fate of the probiotics when administered via various routes to poultry.

Keywords: Broiler, Labeling, Poultry, Polymerize Chain Reaction, Probiotic.

INTRODUCTION

The lactic acid bacteria constitute a family of gram-positive bacteria that are widely found as commensals of the animal and human gut (Marteau and Rambaud, 1993). Among them, lactobacilli play important roles. Lactobacilli possess many physiological functions, such as controlling gut infections, stabilizing the cholesterol level of serum, and anti-carcinogenic activity (Bloksma *et al*., 1979). Lactobacilli are also able to immunomodulate the cytokine response of intestinal mucosal cells in response to luminal antigens, and they stimulate local cell immunity of intestinal and humoral immunity (Qing-Hua *et al*., 2007). In many field experiments which were performed through application of probiotics in poultry nutrition, the results were inconsistent. The efficiency of probiotics depended on strain and dosage of probiotic, age of the bird, and also on the route of administration. Different methods of probiotic administration in hatchery were described as *in ovo* injection (Cox *et al*., 1992; Edens *et al*., 1997), spray administration (Wolfenden *et al*., 2007), oral

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gavage (Sterzo *et al*., 2005; Higgins *et al*., 2007), and vent lip (Filho *et al*., 2007; Higgins *et al*., 2008).

However, considering the route of administration, distribution and movement of lactobacilli in the gastro-intestinal tract, as well as their colonization, are not clear. The detailed mechanisms by which probiotic bacteria mediate their effects are just beginning to be elucidated, but still they are not well understood. The mechanisms seem complex and varied among different probiotic preparations. A good method to answer these questions is to mark the lactobacilli and trace them. Green fluorescent protein (GFP) from jellyfish *Acquoria victoria* is a highly useful fluorescent tag for studying the localization, structure, and dynamics of living cells (Margolin, 2000). The GFP has been transformed already in many bacteria. Moreover, the expression of GFP did not alter bacterial interactions with the host cells and bacteria producing GFP could be visualized within live mammalian cells (Kohler *et al*., 1999; Chen *et al*., 2012).

This study aimed at describing, for the first time, tracing GFP transformed *L. plantarum* in the gastrointestinal tract of broilers after using them in hatchery with various methods of probiotic administration.

MATERIALS AND METHODS

In this experiment, lactobacilli were originally purchased from Razi Vaccine and Serum Research Institute (Karaj, Iran) and transformed with a recombinant expression vector pLEM415::gfp encoding a stable *GFPuv* gene (Gory *et al*., 2001). The *GFPuv* gene was under the control of the constitutive L-lactate dehydrogenase promoter (pldhL), allowing detection of lactobacilli expressing GFP through PCR in chicken gut.

L. plantarum was grown in MRS medium (Merck, Germany) at 37°C. Transformed *L. plantarum* was cultured at 37°C in MRS medium containing ampicillin at a final concentration of 1 µg mL^{-1}. *E. coli* strain DH5α harboring pLEM415 was grown at 37°C with shaking in LB medium containing ampicillin at a final concentration of 50 µg mL^{-1}.

Plasmid and DNA Manipulations

The plasmid pLEM415, donated by Qing-Hua Yu (Qing-Hua Yu, Key Lab of Animal Physiology and Biochemistry, Ministry of Agriculture, Nanjing Agricultural University, Nanjing, China), is an *E. coli/L. reuteri* shuttle vector (Serror *et al*., 2002). It contains a multi-cloning site and genes for ampicillin resistance (Amp^r). Isolation of plasmid from *E. coli* and *L. plantarum* was done by the alkaline lysis method (Sambrook *et al*., 1989). Agarose gel electrophoresis of isolated DNA was performed on 1% (w/v) agarose gel in TAE buffer at 60 V. The DNA was visualized and photographed on an UV transilluminator after staining with ethidium bromide solution (0.5 µg mL^{-1}) for 15 minutes.

Basic Protocol of Glass Bead Transformation

To obtain lysozyme-treated cells of *L. plantarum*, a culture of *L. plantarum* was grown at 37°C in 30 mL of MRS medium to mid log phase. Cells were harvested by centrifugation at 5,000×g for 5 minutes, washed in distilled water, and suspended in 5.0 mL of 0.5M sorbitol in 0.01M Tris-HCl (pH 7.0). The cell suspension was mixed with lysozyme (Merck, Germany) (at a final concentration of 1 mg mL^{-1}) and then incubated at 37°C for 30 minutes. Lysozyme–treated cells were pelleted at 5,000×g for 5 minutes, washed in 10 mL of transformation buffer (0.5M sorbitol, 0.02M maleic acid, 0.02M $MgCl_2$, pH 6.5), and suspended in 1 mL of transformation buffer.

Transformation of *L. plantarum* using glass beads was conducted as follows. Aliquots of 0.5 mL of lysozyme-treated cell

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suspension were placed in 15 mL conical disposable polypropylene centrifuge tubes. To lysozyme-treated cells, we added 1 µg of plasmid DNA, and immediately after, 500 µL of 30% polyethylene glycol 6,000 (PEG 6000). Then, 0.3 g acid washed glass beads (212–300 µm in diameter, Sigma) sterilized by baking at 250°C for 3 hours were added. Lysozyme-treated cells were agitated at the highest speed on a vortex mixer for 15 seconds and then diluted by the addition of 10 mL of transformation buffer. After the beads were allowed to settle, the agitated lysozyme-treated cell suspension was transferred to a new 15 mL conical tube. The cells were pelleted by centrifugation at 5,000×g for 5 minutes and suspended in 1 mL of MRS medium supplemented with 0.5M sorbitol. To allow phenotypic expression of the plasmid encoded ampicillin resistance, the lysozyme-treated cell culture was incubated at 37°C for 1 hour. Transformants were recovered by plating a 0.1 mL aliquot of the lysozyme-treated cell culture onto MRS agar containing 0.5M sorbitol and ampicillin at a final concentration of 1 µg mL^{-1} and incubated at 37°C for 1 day. Transformation frequency was expressed as ampicillin resistant colonies per 1 µg of pLEM415 (Pongsak and Parichat, 2009).

Plasmid Stability Testing

The stability of gfp-containing plasmids in GFP-labeled *L. plantarum* was determined under nonselective conditions. *L. plantarum* GFP-labeled isolates were tested to assess stability of plasmid. Overnight cultures of GFP-labeled strains grown in the presence of antibiotics were used to inoculate (1:1,000 dilution) MRS broth without antibiotics. Bacteria were grown in broth without the presence of antibiotics and were transferred daily (24-hour intervals) at a 1:1,000 dilution into fresh medium for 5 consecutive days. At 2 and 5 days, culture samples were diluted and spread onto agar plates without antibiotics (MRS agar). The plates were incubated until colonies appeared, and the numbers of total and green fluorescent colonies were determined. Under these growth conditions, cultures reached approximately 10^8–10^9 CFU mL^{-1}, which corresponded to approximately 10 generations per transfer using the following formula:

Number of generations= [(Log cells at the end of incubation)-(Log cells at the beginning of inoculation)]/0.301

The cell number at the beginning of inoculation was 10^5–10^6 CFU mL^{-1} (ave. 5.5 log), whereas at the end of incubation, it was 10^8–10^9 CFU mL^{-1} (ave. 8.5 log). Plasmid loss was calculated as the ratio between the plate counts of green fluorescent colonies and total counts on nonselective plates.

Intestinal Distribution and Kinetics of GFP Transformed *L. plantarum* in Chickens

Two hundred one-day old broilers (ROSS 308) were assigned to one of each four groups of different methods of probiotic administration and also to a control group. Each group, comprising of 40 chicks, was kept in a cage battery (90×60×40 cm^3) and fed *ad libitum* during the 4 days of the experiment. The four administration method groups were: (1) *in ovo* injection group: after 18 days of incubation, 50 fertile eggs were injected (into their air cells) with 0.1 mL of probiotic containing 7×10^{10} CFU mL^{-1} per egg in sterile PBS; (2) oral gavage group: 0.1 mL of probiotic suspension containing 7×10^{10} CFU was administrated through gavage into the crop; (3) spray administration: conducted by confining chicks in their shipping box and being directly sprayed with 0.25 mL of probiotic suspension containing 7×10^{10} CFU mL^{-1} per chick. The chicks were held in their shipping box for 30 minutes before being placed in the cage; (4) vent lip group: each chick received 25 µL of probiotic suspension containing 2.8×10^{11} CFU mL^{-1} deposited on the vent lip. The drop was sucked inside the

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cloaca within a few seconds; (5) Control group: Chicks did not receive any probiotic treatment.

Transformed *L. plantarum* Recovery

At 0, 4, 12, 24, 48, and 72 hour post-administration of probiotic, 6 animals were sacrificed at each time point. Samples from luminal contents in the proventriculus, Duodenum, ileum, and cecum were weighed, homogenized, and transferred into 3 mL PBS buffer.

DNA Extraction

Cells were harvested from 3 mL of PBS buffer by centrifugation at 8,000×g for 5 minutes. DNA extraction was carried out according to Hai-Rong and Ning (2006).

PCR

Universal primer, Unibac f (5'-CGTGCCAGCCGCGGTATACG-3') and Unibac r (5'-GGGTTGCGCCGTTGCGGACTTAACCCAACAT-3') (611 bp) identify all known bacteria using the invariant region in the 16S rRNA gene of the bacteria (Amit-Romatch and Uni, 2004). The universal primer set was used for determining the total bacterial population. The following primers were used for tracing transformed *Lactobacillus plantarum*; ldhL-GFP1 (5'-TTAGGGCCCACTGAGAAGTTGCTCTC-3') and ldhL-GFP2 (5'-TTAATCGATTTATTTGTAGAGCTCATCC-3') (1,001 bp).

For PCR amplification, 5 µL of DNA extract was added to 45 µL of the PCR mixture containing 30.875 µL of nuclease free water, 2 µL of each primer (10 µM), 2 µL dNTP mix (10 mM), 5 µL PCR buffer, and 0.0625 µL Taq polymerase. PCR components were provided by Cinagen, Iran. The PCR was conducted in a programmable thermal controller (BioRad, USA). The amplification conditions for the universal primers were: 1 cycle of 94°C for 4 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 1 minute and 68°C for 1.5 minutes, and finally 1 cycle of 68°C for 1.5 minutes (Amit-Romach and Uni, 2004) and for ldhL-GFP were: 1 cycle of 94°C for 4 minutes, 30 cycles of 94°C for 1 minute, 67°C for 1 minute and 72°C for 1 minute, and finally 1 cycle of 72°C for 4 minutes (Qing-Hua *et al*., 2007). PCR products were visualized by agarose gel (1%) electrophoresis containing ethidium bromide (Serva, Germany). Densitometric evaluation of different bands was carried out using Photo Capt software version 12.4 (Vilber Lourmat, France). The densitometry results were reported as relative density of GFP bands to universal bands.

Morphology of Intestine

For the histomorphological examination of the intestine, tissue samples from duodenum and ileum were collected from 3 euthanized birds of each group at each sampling time (4, 8, 12, 24, 48 and 72 hours after post-hatch) and fixed in 10% buffered formalin-saline.

Tissues were dehydrated by immersing through a series of alcohol with increasing concentrations (from 70% to absolute ethanol), infiltrated with xylene, and embedded in paraffin. A rotary type microtome was used for cutting the paraffin sections. The blocks were properly trimmed and sections of 5 mm thickness were cut. The villi height was measured. The mean from 10 villi per sample was used as the average value for further analysis.

Statistical Analysis

The morphology data were analyzed in a completely randomized design model, the comparison of means being carried out through LSD Test. All statistical analyses

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were done using SAS program (SAS Institute, 1990).

RESULTS

One of the principal concerns raised regarding the use of a marker gene is the stability of the marker (plasmid). Plasmid stability tests i.e., determining the proportion of plasmid-bearing cells remaining after a specified period of time, were conducted by culturing the GFP-labeled strains in the absence of antibiotic selection for 20 generations. After 20 generation that plasmid lose of plasmid in *L. plantarum* was 15%.

The results of the test are shown in Table 1. No transformed bacteria were detected in the control and vent lip groups. In the *in ovo* injection group, in the first step of sampling, transformed bacteria were detected in duodenum and, in the second step, in cecum. They were not detectable anymore in the rest of sampling. In the oral gavage group at all stages of sampling in proventriculus and in duodenum at 0, 4, 12 hours after administration, the transformed bacteria were detectable (Figure 1). In the spray group, the transformed *Lactobacillus plantarum* were detectable. These transformed bacteria were detected in proventriculus at 12, 24, and 48 hours and in duodenum at 72 hours after administration.

Universal primers were used in these experiments to determine the relative contribution of transformed bacteria to the total microflora.

In the *in ovo* injection group at the first sampling in duodenum, the relative contribution of *L. plantarum* transformed with GFP was 95% and in cecum in the next stage it was 83.27 %.

Table 1. Tracing of *L. plantarum* transformed with Green Fluorescent Protein (GFP) in GI tract after administration with different methods in hatchery.

Treatment	GI [a]	Time (h)					
		0	4	12	24	48	72
Control	Proventriculus	- [b]	-	-	-	-	-
	Duodenum	-	-	-	-	-	-
	Ileum	-	-	-	-	-	-
	Cecum	-	-	-	-	-	-
In ovo injection	Proventriculus	-	-	-	-	-	-
	Duodenum	+ [c]	-	-	-	-	-
	Ileum	-	-	-	-	-	-
	Cecum	-	+	-	-	-	-
Oral gavage	Proventriculus	+	+	+	+	+	+
	Duodenum	+	+	+	-	-	
	Ileum	-	+	-	-	-	-
	Cecum	-	-	-	-	-	-
Spray	Proventriculus	-	-	+	+	+	
	Duodenum	-	-	-	-	-	+
	Ileum	-	-	-	-	-	-
	Cecum	-	-	-	-	-	-
Vent lip	Proventriculus	-	-	-	-	-	-
	Duodenum	-	-	-	-	-	-
	Ileum	-	-	-	-	-	-
	Cecum	-	-	-	-	-	-

[a] Gasteronial intestinal, [b] No detection and [c] Detection of *L. plantarum* transformed with GFP.

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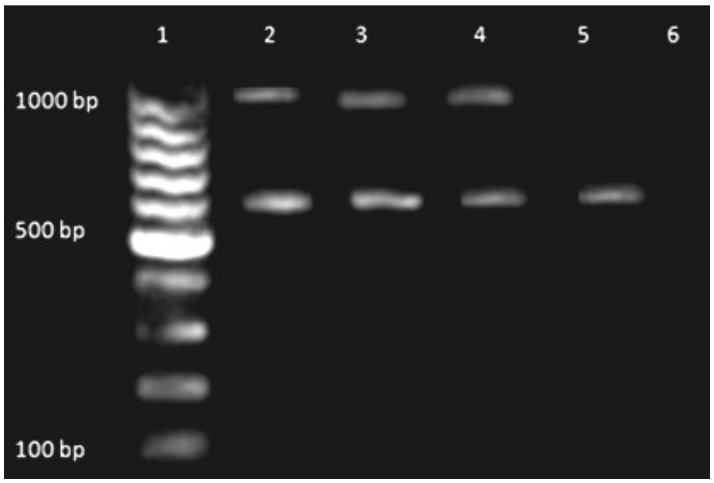

Figure 1. Agarose gel electrophoresis of PCR products from intestine contents of oral gavage treatment 4 hours after post-hatch chicks [Lane (1) 100 bp DNA size marker; (2) Proventriculus; (3) Duodenum; (4) Jejunum; (5) Cecum, and (6) Negative control]. GFP band (1,001 bp) and universal band (611bp).

In the oral gavage group, the relative contribution of transformed *L. plantarum* in proventriculus at the first, second, third, fourth, fifth, and the last sampling was, respectively, 96.5, 93.2, 85, 72.1, 68.9, and 65.21%, while in duodenum, in the first sampling it was 93.2% and at 12 hours after hatch 91.4%. In ileum, 4 hours after hatch, the relative contribution was 94.3 percent.

In the spray group, the relative contribution in proventriculus at 12 hours after hatch was (88%), 24 hours (85%), and at 48 hours after administration, probiotic was 60%, while in duodenum at 72 hours, it was 81.25%.

The impact of different treatments on the length of villi in duodenum is presented in Table 2. Different methods of administration of probiotic in hatchery had a significant effect on villi height of duodenum at different times of sampling, except in the first sampling (4 hours after hatch). In the first sampling, no significant difference was observed between different treatments (P> 0.01). Eight hours after administration of probiotic, the highest length of villi was observed in the *in ovo* injection group, which had no difference with oral gavage group (P< 0.01). At the next three samplings, the highest and lowest length of villi were observed in the *in ovo* injection and the control group, respectively (P< 0.01). In the final sampling, the highest villi length was observed in the *in ovo* injection

Table 2. Height of villi in duodenum at different sampling times (4, 8, 12, 24, 48, 72 hours post- hatch).

Treatment	H_4 [ns]	H_8 [a]	H_{12} [a]	H_{24} [a]	H_{48} [a]	H_{72} [a]
Control	0.19	0.19[b]	0.22[c]	0.27[cd]	0.27[b]	0.29[d]
In ovo	0.19	0.33[a]	0.37[a]	0.39[a]	0.43[a]	0.44[a]
Oral gavage	0.23	0.24[ab]	0.30[b]	0.30[bc]	0.31[b]	0.39[ab]
Spray	0.21	0.21[b]	0.24[c]	0.24[d]	0.28[b]	0.36[bc]
Vent lip	0.19	0.19b	0.25c	0.32b	0.30b	0.30dc
SEM [b]	0.01	0.013	0.017	0.014	0.016	0.018

[a] Significant difference (P< 0.01), [b] Standard Error of mean; [ns] No significant difference.

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group, which showed no significant difference with the oral gavage method.

The effects of different treatments on the villi height in ileum are presented in Table 3. Various methods of administration of probiotic in hatchery impacted on villi height in ileum at different times of sampling. Four hours after administration of probiotic in hatchery, *in ovo* injection group had the highest villus and had no significant difference with other groups, except the control group (P< 0.01). In the second sampling, the highest length of villi was observed in the *in ovo* injection group, which showed significant difference with the other groups (P< 0.01). In the third sampling, the highest and lowest height of villi were observed in the *in ovo* injection group and the vent lip group, respectively. Twenty four hours after post-hatch, the highest villi length was seen in the *in ovo* injection group, which had no statistically significant difference with other groups, except with the vent lip group. In the last two samplings, the highest villi length was observed with the *in ovo* injection and oral gavage treatments.

The difference between treatment groups was not statistically significant with the exception of group 3.

DISCUSSION

Lactobacilli, commensal bacteria found in animal intestine, are defined as viable nutritional agents conferring benefits to the health of the host animal. The alimentary tract of chicken is host to a variety of species of lactobacilli (Qing-Hua *et al*., 2007).

Different methods are used for administration of probiotics in poultry nutrition. However, little is known about the distribution and the movement of lactobacilli in the gastro-intestinal tract. It remains a challenge to study the fate of these microorganisms when administered in a complex microbial environment *in vivo*.

As a potential marker for tracking lactobacilli in animal intestine, the *GFP* gene was placed downstream of the constitutive ldhL promoter in plasmid pLEM415::gfp. The gene transfer systems for *Lactobacillus* strains using conjugation and protoplast transformation had a low efficiency and lack of reproducibility (De and Simons, 1994).

However, Pongsak and Parichat (2009) showed that lactobacilli were transformed reproducibly by the glass bead method with a high frequency and efficiency. This method was used in this experiment for transformation of *L. plantarum* with pLEM415::gfp.

As shown in this study, transformed bacteria were traced in the gastrointestinal tract after their administration in hatchery with different methods including oral gavage, spray and *in ovo* injection, but these bacteria were not traced in the vent lip group in neonatal broilers in any of the samplings.

Gory *et al*. (2001) expressed GFP in *Lactobacillus sakei* and demonstrated that expression of GFP did not alter bacteria growth. Qing-Hua *et al.* (2007) used GFP for transformation of *Lactobacillus delbrueckii* and used them for tracing bacteria in the gastrointestinal tract of

Table 3. Height of villi in Ileum at different sampling times (4, 8, 12, 24, 48, 72 hours post- hatch).

Treatment	H_4 [a]	H_8 [a]	H_{12} [a]	H_{24} [a]	H_{48} [a]	H_{72} [a]
Control	0.12[b]	0.16[b]	0.16[c]	0.19[ab]	0.20[ab]	0.23[b]
In ovo	0.16[a]	0.20[a]	0.23[a]	0.23[a]	0.23[a]	0.30[a]
Oral gavage	0.15[ab]	0.15[b]	0.16d[c]	0.19[ab]	0.20[a]	0.28[a]
Spray	0.13[ab]	0.15[b]	0.18[b]	0.19[ab]	0.19[b]	0.20[b]
Vent lip	0.14[ab]	0.14[b]	0.15[d]	0.18[b]	0.19[b]	0.20[b]
SEM	0.003	0.006	0.007	0.01	0.008	0.011

[a] Significant difference (P< 0.01).

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broiler chickens after oral gavage administration. Geoffroy *et al*. (2000) orally administered GFP-labelled *L. plantarum* to mice and found that fluorescent lactobacilli were mostly embedded in the intestinal mucus or were excited in the lumen, even though some bacteria seemed to be closely associated with epithelial cells. Schultz *et al.* (2005) used EcN-GFP (*E. coli* strain Nissle 1917 expressing GFP) and traced the labeled bacteria in the gastrointestinal tract of mice, and Satho *et al.* (2009) used GFP to trace the fate of exogenous micro-organisms inside the alimentary tract of mosquitoes. Ling *et al.* (2000) used them for transformation of *Edwardsiella Tarda* and traced them in fish.

In our study, following *in ovo* injection, spray, and oral gavage methods of administration, GFP-labeled *L. plantarum* were observed in gastronial tract of chicks, but in vent lip group no GFP-labeled *L. plantarum* was detected in GI.

In this experiment, the administration route which delivers the whole dose of the probiotic directly into the alimentary canal (oral gavage) was the most effective administration method.

The *in ovo* injection procedure produced only a primary displacement, however, durability was not observed. In several study in the case of probiotic administration method in hatchery to reduction *Salmonella* in gastrointestinal tract in broiler chicks more efficient of oral gavage method than *in ovo* injection and spray method were reported (Hosseini-Mansoub *et al*., 2011; Hashemzadeh *et al*., 2010). Meijerhof and Hulet (1997) showed higher efficiency of oral gavage than *in ovo* injection on performance in broiler chicks. Moghaddam *et al.* (2010) showed that the oral gavage method had much better impact on performance in broiler than other methods of administrating probiotic in hatchery.

As Table 4 shows, with aging of broiler chicks, the relative contribution of *L. plantarum* transformed with GFP to total microflora population decreased, which is normal. Although the alimentary tract of the newly hatched chick is usually sterile, organisms rapidly gain access from the surrounding environment such as feed, water, and hatchery floor. Previous studies have documented the changes of the microflora during the post hatch period (Mead and Adams, 1975; Cengiz *et al*., 2012).

Initial increases of height of villi occurred in the 1- to 4-day-old post-hatch chicks. Uni *et al*. (1996) stated that rapid

Table 4: Relative contribution of transformed *L. plantarum* to total microflora in GI after administration with different methods in hatchery.

Treatment	GI	Time (h)					
		0	4	12	24	48	72
In ovo injection	proventriculus	-	-	-	-	-	-
	Duodenum	95 [a]	-	-	-	-	-
	Ileum	- [b]	83.27	-	-	-	-
	cecum	-	-	-	-	-	-
Oral gavage	proventriculus	96.5	93.2	85	72.1	68.9	65.2
	Duodenum	93.2	92.5	91.4	-	-	
	Ileum	-	94.3	-	-	-	-
	cecum	-	-	-	-	-	-
Spray	proventriculus	-	-	88	85	60	
	Duodenum	-	-	-	-	-	81.2
	Ileum	-	-	-	-	-	-
	cecum	-	-	-	-	-	-

[a] Percentage of transformed *L. plantarum* to total microflora in GI tract, [b] No detection of *L. plantarum* transformed with Green Fluorescent Protein (GFP).

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increases in villi size occurred in the 1- to 2-day-old post-hatch chicks, and the rate of growth then declined, reaching a plateau 5 to 10 d after hatch.

A significant effect was observed on the length of villi in both segments of the small intestine (proximal and distal) among different methods applied for probiotic administration. In the case of villi height in the proximal segment of the intestine, duodenum, no significant difference was observed between *in ovo* injection and oral gavage groups at the final sampling. According to the length of villi in the distal segment of the small intestine, ileum, in the three last samplings, no significant difference was observed between the two groups. This result shows that elementary replacement of probiotic bacteria in intestine in *in ovo* injection and oral gavage groups had enhanced the length of villi.

Intestinal morphology characteristics are affected by administration of probiotic in hatchery. In this study, the results showed that the use of probiotics increased the length of villi in duodenum and ileum which can improve nutrient absorption and increase feed efficiency. In a study conducted by Chichlowisk *et al*. (2007), it has been stated that a probiotic containing lactobacilli, *Bifidobacterium thermophilum* and *Enterococcus faecium* increased the jejunum villi compared to the control group. In another study, a positive effect of the *in ovo* injection administration method was observed on villi height at duodenum and jejunum in broiler chicks (Hashemzadeh *et al*., 2010). These results are most probably due to enhanced SCFA (short chain fatty acid) formation induced by probiotics. Butyrate, propionate, and acetate, the most important SCFA, have a direct trophic action on the colonic mucosa. There is evidence, which proved Gastrointestinal absorption enhancement with increasing length of villi observed enhancement intestinal absorptions (Roediger and Era, 1982).

CONCLUSIONS

In this study, it has been demonstrated that GFP can be used as a useful marker in *Lactobacillus* to monitor the fate of the bacteria when administered to chickens. Transformation of lactobacilli with GFP allowed us, for the first time, to study the intestinal passage of administered lactobacilli in chicken with different methods in hatchery. This opens an array of experimental possibilities such as the study of microbe–host interactions, the influence of the resident microflora, and concurrent anti-microbial treatment on colonization. This will enable us to better understand the behavior of microorganisms and their influence on host.

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استفاده از پروتئین فلوئورسانت سبز برای ردیابی باکتری های پروبیوتیکی در دستگاه گوارش و ارزیابی روش های مختلف تجویز پروبیوتیک در مرغ گوشتی

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چکیده

لاکتوباسیلوس ها همانند دیگر باکتری های همزیست دستگاه گوارش به خاطر استفاده در تغذیه طیور و دارا بودن خصوصیات پروبیوتیکی شناخته شده می باشند. به هر حال اطلاعات کمی در مورد اثر متقابل آنها با دستگاه گوارش در بدن حیوانات در شرایط in ovo مزرعه ای وجود دارد. برای مشخص کردن مسیر لاکتوباسیل ها بعد از تجویز در هچری، لاکتوباسیلوس پلانتاروم با پلاسمید نوترکیب pLEM415::gfp ترانسفورم شد. تعداد ۲۰۰ جوجه گوشتی (راس ۳۰۸) یک روزه به پنج گروه آزمایشی شامل: یک گروه شاهد و چهار گروه تجویز پروبیوتیک در هچری که عبارتند از تزریق به تخم مرغ، گاواژ دهانی، افشانه و تلقیح به کلوآک تقسیم شدند. در مقاطع زمانی صفر، چهار، دوازده، بیست و چهار، چهل و هشت و هفتاد و دو ساعت پس از تجویز پروبیوتیک، شش پرنده از هر گروه کشتار شدند. از باکتری های چسبیده به قطعات مختلف دستگاه گوارش پس از هموژنیزه کردن و شستشو با بافر نمونه گیری انجام شد. از واکنش های زنجیره ای پلیمراز برای ردیابی لاکتوباسیلوس

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پلانتاروم ترانسفورم شده با GFP در دستگاه گوارش استفاده شد. باکتری های ترانسفورم شده در نمونه های گرفته شده از دستگاه گوارش در گروه های گاواژ دهانی، افشانه، تزریق به تخم مرغ ردیابی شدند؛ در گروه تلقیح به کلوآک هیچ باکتری ترانسفورم شده ای با GFP مشاهده نشد. به نظر می رسد که روش گاواژ دهانی موثرترین روش بود که نشان دهنده برتری تحویل دز کامل باکتری به سایت های هدف می باشد. بر اساس نتایج این آزمایش نشان داده شد که تجویز زودهنگام پروبیوتیک در هچری تاثیر مثبت بر مورفولوژی دستگاه گوارش دارد و گروه تزریق به تخم مرغ جنین دار و گاواژ دهانی تاثیر بیشتری داشتند. در این آزمایش کارایی باکتری های ترانسفورم شده با GFP برای بررسی ردیابی آنها پس از استفاده با شیوه های مختلف تجویز در طیور نشان داده شد.

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