Original Research

Effect of *Lactobacillus reuteri* PIA16 Isolated from the Gastrointestinal Tract of Assam Indigenous Chicken on the Productive Performance of Broilers

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Abstract

Two Lactobacillus spp. harvested separately from caecum and jejunum of Assam indigenous chickens, India were characterized both as Lactobacillus reuteri and registered under GenBank, NCBI, India as Lactobacillus reuteri PIA16. Treating as individual entity, their effect on the productive performance of broiler chickens were evaluated when supplemented singly and with prebiotic, mannan oligosaccharide to the broiler diet and noted for variations between the strains. Daily broiler ration (20%) fermented daily with 20% of L. reuteri PIA16 culture at a dose of 10^8cfu/ml when fed to the birds, significantly ($P<0.05$) improved body weight gain, feed consumption and FCR were observed. The effect was further improved with addition of prebiotic (MOS). Despite the higher production cost per broiler in all the L. reuteri PIA16 fed groups, the gross profit was high when compared to control group and higher still in prebiotic added groups. It can, thus, be considered as potential probiotic agent.

Key words: Broilers, *Lactobacillus reuteri* PIA16, MOS, Prebiotic, Probiotic

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Introduction

In recent years, use of probiotics, prebiotics and synbiotics those that enrich certain bacterial population in the digestive system are considered as alternatives to antibiotic growth promoters in poultry nutrition (Patterson and Burkholder, 2009) pertaining to their potential to reduce chances of infection in poultry and subsequent contamination to poultry products. Fuller (1989) defined probiotics as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". It consists of live microbial cultures that are isolated from the gastro intestinal tract (GIT) of a healthy adult animal of the same species to which probiotics will be given, as it is usually species specific (O'Dea *et al*.,

2006). These beneficial microflora are known to increase the host's protective barrier against enteric bacteria, humoral immune reaction against pathogens and cell immunity response (Taheri *et al*., 2014). *Lactobacilli*, also considered as beneficial bacteria, were found to be the most prominent bacteria in the crop of birds after the first week of age (Frei *et al.*, 2001). The predominant species of *Lactobacillus* are *L. reuteri, L. salivarius* and *L. animalis* (Vineetha, 2014). Prebiotics are selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health (Gibson *et al.*, 2004). Mainly prebiotics are small fragments of carbohydrates and commercially available as oligosaccharides of galactose, fructose or mannose (Ganguly, 2015). Currently, combined administration of probiotics and prebiotics referred to as synbiotic, is gaining momentum in poultry production for its beneficial effects.

Assam local chickens reared under natural conditions without much intervention of modern day management and feeding practices adapt well to local climate and possesses disease resistance capacity (Kalita *et al.*, 2009). These special attributes of the local chickens may have been influenced by beneficial microflora like the *Lactobacilli* present in the gut boosting its immune system. Thus, these microbes when isolated and characterized from the gut of local chicken may prove useful as probiotic culture for commercial exploitation. Subsequently, *Lactobacillus reuteri* PIA16 was isolated from the gut of Assam local chicken and characterized at Institute of Microbial Technology (IMTECH), Chandigarh. To evaluate if *Lactobacillus reuteri* PIA16 was a putative probiotic, its effect on the productive performance of broilers and any synergic effect when supplemented with prebiotic were studied.

Materials and Methods

Two strains of *Lactobacillus* isolated from different gut region *i.e*., ACE5 (caecum) and AJ3 (jejunum) both showed 99.72 % genetic identity with *Lactobacillus reuteri* through the 16S rRNA gene sequence and BLAST search analysis conducted under the Institute of Microbial Technology (IMTECH), Chandigarh, India. The strain is registered as *Lactobacillus reuteri* PIA16 (accession No. KX260961) under GenBank, National Centre for Biotechnology Information (NCBI), India. These two strains were used for *in-vivo* growth bioassay as two separate entities so as to also examine any variation between the strains pertaining to the different gut regions they were harvested from. As premix, 20% of daily ration required for broilers was autoclaved (Patra, 2013; Vineetha, 2014) and daily inoculated with 20% of ACE5 and AJ3 broth culture separately and fermented at 37°C for 48 hrs. The fermented feed was added daily to the required daily ration and mixed well. The initial average count of the isolates ACE5 and AJ3 were 1.04×10^8 cfu/ml and 1.05×10^8 cfu/ml, respectively but after fermentation the count of *Lactobacillus* increased to 1.85×10^8 cfu/gm and 1.89×10^8 cfu/gm of fermented feed for ACE5 and AJ3, respectively. The commercially available prebiotic, mannan oligosaccharide (MOS) @ 0.25% (Kim *et al*., 2011) was used for the experiment.

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A total of 240 day-old chick broilers (Cobb) were randomly assigned into five groups of 48 birds containing six numbers in each of eight replicates of mixed sex per group. They were provided with different dietary treatments *i.e.* a control -basal diet (T_1), T_1 + ACE5 at $1.85x10^8$cfu fermented feed (T_2), T_1 + AJ3 at $1.89x10^8$cfu fermented feed (T_3), T_2 +ACE5-MOS (T_4) and T_3 +AJ3-MOS (T_5) at 0.25% of the total feed. The birds were housed in battery cages and reared under standard managemental conditions for duration of 5 weeks. The growth parameters were recorded weekly. Daily mortality, if any, was recorded for estimation of livability % during 0-3 and 4-5 weeks. Broiler Performance Efficiency Index (BPEI) and the cost of production per kg live broiler were calculated according to the formula described by Narahari and Kumararaj (2008) to evaluate the economy of feeding *Lactobacillus reuteri* PIA16 and MOS in broilers. Data obtained were statistically analyzed with one way Analysis of Variance and Duncan's multiple range tests using the software SAS System (Local, X64_7PRO).

Results and Discussion

Growth Performance

Difference in the body weight change among the groups were pronounced by 3rd week where all the *Lactobacillus reuteri* PIA16 fed groups showed significant ($P<0.05$) improvement in body weight compared to control (Table 1). The MOS supplemented groups showed significantly better result than T_2 and T_3 and most superior was the T_5 group. The body weight change in the remaining weeks also followed the same trend. This growth promoting effect of dietary *Lactobacillus* supplementation and prebiotic in broiler diet were also noted in several reports (Murry *et al*., 2006; Zhu *et al.*, 2009; Sherief and Sherief, 2011; Saiyed *et al*., 2015). The improvement observed in the study maybe due to the improvement of the intestinal environment with the presence of probiotic bacteria, thus, increasing the efficiency of digestion and nutrient absorption processes (Alkhalf *et al*., 2010). Moreover, prebiotic are known to play a role in augmenting the activity and/or the composition of the probiotic bacteria, thereby, improving the performance of the broilers (Gibson *et al.*, 2004).

All the treated groups showed significantly ($P<0.05$) better weight gain than control group. A noticeable increase ($P<0.05$) in body weight gain in groups supplemented with MOS compared to their respective counterparts were observed in the last two weeks. An increase in daily weight gain to the extent of 14 per cent by 4-day application of lactic acid bacteria culture was observed (Strompfova *et al.*, 2005). Similar reports also revealed significantly higher weight gain in the broilers fed with probiotic cultures containing *Lactobacillus* (Swamy and Upendra, 2013; Song *et al*., 2014). Better overall performance of the broilers fed with *Lactobacillus* and prebiotic in terms of feed efficiency and growth in the present study might be due to the improvement of the viability of the probiotic bacteria through the usage of the prebiotic as a substrate for fermentation by the probiotic bacteria which subsequently produced lactic acid, reducing

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further the pH of the GI tract and have suppressive effect on unwanted organisms and thus, controls the population of pathogenic micro-organisms, promoting the health status of the broilers (Bansal *et al*., 2011; Falaki *et al*., 2011).

Table 1: Growth performance of broiler chickens fed with *Lactobacillus reuteri* PIA16

Parameters	Experimental groups (48 nos. of birds/group)				
	Control (T1)	T_2	T_3	T_4	T_5
Body Weight Change (g/bird)					
Day old	$44.62^{a}\pm0.7$	$44.62^{a}\pm0.7$	$44.61^{a}\pm0.6$	$44.61^{a}\pm0.1$	$44.61^{a}\pm0.1$
1st week	$108.37^{a}\pm1.2$	$113.83^{bc}\pm1.6$	$116.81^{cd}\pm1.4$	$110.42^{ab}\pm1.1$	$117.73^{d}\pm0.9$
2nd week	$266.19^{a}\pm2.1$	$286.09^{b}\pm3.8$	$300.08^{cd}\pm2.3$	$295.67^{c}\pm3.3$	$307.92^{d}\pm2.2$
3rd week	$527.88^{a}\pm4.9$	$571.02^{b}\pm7.5$	$592.00^{c}\pm6.2$	$605.27^{c}\pm5.3$	$626.46^{d}\pm7.3$
4th week	$904.48^{a}\pm9.0$	$981.02^{b}\pm10.5$	$1008.00^{b}\pm12.1$	$1049.21^{c}\pm11.2$	$1080.79^{c}\pm15.3$
5th week	$1403.08^{a}\pm8.8$	$1521.81^{b}\pm12.3$	$1558.81^{b}\pm15.5$	$1634.69^{c}\pm13.9$	$1676.79^{d}\pm17.1$
Body Weight Gain (g/bird)					
1st week	$63.75^{a}\pm1.3$	$69.22^{bc}\pm1.7$	$72.21^{c}\pm1.6$	$65.81^{ab}\pm1.16$	$73.13^{c}\pm0.94$
2nd week	$157.82^{a}\pm2.6$	$172.25^{b}\pm3.0$	$183.27^{c}\pm1.8$	$185.25^{c}\pm3.2$	$190.19^{c}\pm2.4$
3rd week	$261.69^{a}\pm5.3$	$284.94^{b}\pm8.4$	$291.92^{bc}\pm6.0$	$309.60^{cd}\pm5.4$	$318.54^{d}\pm8.0$
4th week	$376.60^{a}\pm9.2$	$410.00^{b}\pm5.7$	$416.00^{b}\pm9.2$	$443.94^{c}\pm8.78$	$454.33^{c}\pm10.2$
5th week	$498.60^{a}\pm11.9$	$540.79^{b}\pm6.9$	$550.81^{b}\pm9.6$	$585.48^{c}\pm8.3$	$596.00^{c}\pm7.8$
Feed Consumption (g/bird)					
1st week	94.37	100.36	104.71	93.46	103.1
2nd week	249.36	265.28	280.39	274.16	249.57
3rd week	437.01	462.1	469.99	479.9	490.58
4th week	662.83	701.1	703.04	719.15	731.46
5th week	922.41	968.03	974.92	989.5	1001.28
Overall	2365.98	2496.87	2533.05	2556.17	2575.99
Feed Conversion Ratio					
1st week	1.48	1.45	1.45	1.42	1.41
2nd week	1.58	1.54	1.53	1.48	1.47
3rd week	1.67	1.63	1.61	1.55	1.54
4th week	1.76	1.71	1.69	1.62	1.61
5th week	1.85	1.79	1.77	1.69	1.68
Overall	1.69	1.64	1.62	1.56	1.55

^{abc}*Means bearing same superscripts in a row do not differ significantly (P≤0.05)*

The feed consumption of the broilers which consumed *L. reuteri* PIA16 in their ration were comparatively more than control birds by 3rd week but little difference was observed among the treated groups (Table 1). Similar finding was also reported by Falaki *et al*. (2011) on using synbiotic in broiler chickens. Total feed consumption per bird was recorded highest in group T_5 (2575.99 g) followed by group T_4 (2556.17 g), though there were no pronounced difference with the remaining groups. The improvement in feed intake may be accounted for the improved growth performance of the broilers fed with dietary supplementation of *Lactobacillus* and prebiotic resulting from the stimulation of favourable microbial balance in the gut and consequently improve the efficiency of digestion and nutrient absorption process of the host. In a more

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recent study, an overall increase in feed intake in chicken fed with multistrain probiotics than the control group was reported (Zhang and Kim, 2014).

A better FCR in T_4 and T_5 groups was observed than T_2 and T_3 groups while the least was recorded in T_1 group in the 1st week (Table 1). Same trend was observed in the following weeks and the groups supplemented with probiotic and prebiotic showed an augmented effect on FCR when compared with groups fed only with probiotic. This may be attributed to the synergism between probiotic and prebiotic followed by better absorption of nutrients by the GI system which is responsible for better feed conversion efficiency. Overall, T_5 group showed the best FCR. Broilers supplemented with *L. plantarum* and *L. reuteri* Pg4 were reported with improved FCR (Vandeplas *et al*., 2009; Yu *et al.,* 2007). Using probiotic culture containing *Lactobacillus* indicated lowered FCR in broiler chickens (Alkhalf *et al.,* 2010; Murry *et al*., 2006).

Economics of Production

No mortality was recorded during 0-5 weeks showing 100% livability in all the groups. The Broiler Performance Efficiency Index (BPEI) indicated T_5 (109.09) group to be most efficient followed by T_4 (105.16), T_3 (96.89), T_2 (96.30) and T_1 (83.83). The cost of production per bird (Rs.) was recorded highest in T_5 (136.86) group and lowest in T_1 (127.52) group with the remaining groups falling in between the range. The higher production cost in T_5 and T_4 might be due to higher feed consumption which resulted in higher body weight. Despite higher production cost in the *Lactobacillus* fed groups, the gross profit per broiler (Rs.) was recorded highest in T_5 (31.14) followed by T_4 (28.53), T_3 (22.17) and T_2 (19.51) while lowest gross profit was recorded in T_1 (12.48) notwithstanding the low production cost. Feeding cost in probiotic fed broiler chickens was found lower (Anjum *et al.*, 2005; Doley, 2011) and higher profit on using synbiotic (Saiyed *et al*., 2015) were reported. An improvement in the economic efficiency of the synbiotic fed broilers was also eminent (Narasimha *et al*., 2015). The high gross profit in all the *Lactobacillus* fed groups might be because of the higher body weight which generated economic benefit.

Conclusion

Better overall productive performances were observed in the broilers which received dietary *L. reuteri* PIA16 at 10^8cfu level along with mannan oligosaccharide. *L. reuteri* PIA16 can be a promising probiotic strain whose beneficial effect can be improved further when supplemented along with prebiotic, mannan oligosaccharide. The study also revealed no variation between the *Lactobacilli* showing singularity and non site-specificity. Furthermore, feeding dietary *L. reuteri* PIA16 with MOS to commercial broiler chickens generated high gross profit despite higher production cost. The Broiler Performance Efficiency Index (BPEI) reflected an increase in the index in all the *Lactobacillus* fed groups showing a positive impact on the economy.

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