Haryana Vet. (June, 2021) **60(1),** 137-139

Short Communication

ALTERATION OF LACTIC ACID BACTERIA PROFILE IN PIGLETS AFTER DIETARY SUPPLEMENTATION OF PROBIOTICS: A COMPARATIVE STUDY

ARUP KALITA*, P.C. KALITA, P. ROYCHOUDHARY[1], O.P. CHOUDHARY and P.J. DOLEY
Department of Veterinary Anatomy and Histology, [1]Department of Veterinary Microbiology,
College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (I), Selesih, Aizawl-796014, India

Received: 23.06.2020; Accepted: 07.08.2020

SUMMARY

The present study was carried out on 18 healthy Large White Yorkshire piglets, irrespective of sex obtained from three litters at different stages of development as age-group of 20, 30 and 60 days. They were divided into control group fed with basal diet and treatment group fed with probiotics ($1.25x10^9$ cfu/d orally for 10 days) along with the basal diet. The piglets were weaned at 28 days of age. After sacrificing the animals at day 20, 30 and 60 from both the groups, abdominal cavity was opened and intestinal contents were collected in sterile tube separately from three different segments of small intestine. The population of lactic acid bacteria (LAB) was counted by pour plate method and the numbers were determined as colony-forming unit per mL (cfu/mL) in intestinal content. The cultivable cell counts increased from proximal to distal part of small intestine and towards the advancement of age in both the groups. These cell counts were non-significantly higher in the treatment group of piglets as compared to control group of animals in all segments of small intestine and in all age-groups. However, they were significantly higher ($P<0.05$) in the treated piglets at day 20 in jejunum and, at day 30 and day 60 in ileum.

Keywords: Lactic acid bacteria, Piglet probiotic, Zinc

How to cite: Kalita, A., Kalita, P.C., Choudhary, P.R., Choudhary, O.P. and Doley, P.J. (2021). Alteration of lactic acid bacteria profile in piglets after dietary supplementation of probiotics: A comparative study. *Haryana Vet.* **60(1)**: 137-139.

The gut microbiota provides both nutritional and protective functions by fermentation of the food materials and other secreted products (Snel *et al.*, 2002), induce host immunity (Kelly and King, 2001), and prevent colonization of pathogenic bacteria. Early colonization of commensal microbial population provides progressive development and maturity of digestive and immune system in neonatal piglets (Gebert *et al.*, 2011). During the post-weaning period, the piglets are susceptible to overgrowth of pathogenic bacteria due to changes in structural, functional, and barrier function in the intestine, which can lead to post-weaning diarrhoea (Hopwood and Hampson, 2003). This pathogenic microbiota can reduce the capacity for digestion and absorption of nutrients resulting poor growth and diarrhoea (Williams *et al.*, 2001). In various studies, it has been revealed that administration of probiotics improves growth and feed conversion ratio with decreased pathogenic bacterial load, and increased beneficial microbial population in the intestinal tract of weaned piglets (Chiang *et al.*, 2015). Hence, probiotic supplementation could be a protective measure to prevent post-weaning diarrhoea (Prieto *et al.*, 2014). Therefore, the present investigation was carried out to evaluate the colonization of orally administered probiotic quantitatively in different stages of pre and post-weaning period of piglets.

The present investigation was conducted from February, 2017 to May, 2019 on 18 healthy Large White piglets, irrespective of sex from three litters at different stages of development as age-group of 20, 30 and 60 days. The piglets were maintained in the pig farm of College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India. From each litter, 6 (six) numbers of piglets were selected and were divided into control group (C) fed with basal diet and treatment group (T) fed with probiotic oral supplement along with the basal diet. A mixture of probiotic consisting of *Lactobacillus acidophilus* (650 million), *Lactobacillus rhamnosus* (400 million) and *Bifidobacterium longum* (200 million) was orally administered to the treatment group of piglets @ 1.25×10^9 CFU (1 gm powder dissolved in 3 ml of sterilized saline solution) once a day from birth to 10 days of age (Liu *et al.*, 2014). The piglets of the control group were given the same volume of sterilized saline solution. The piglets were weaned at 28 days of age. The Institutional Animal Ethics Committee (IAEC) ethically approved the animals used for the experiment vide Approval No. 770/ac/CPCSEA/FVSc/AAU/IAEC/17-18/490 dated 09.08.2017.

The experimental animals were first anesthetized using diazepam @ 2mg/kg body weight followed by ketamine @ 10 mg/kg body weight intravenously and then exsanguinated. The animals were sacrificed at day 20, 30 and 60 from both the groups. Scarification of animals was done because this particular study was only a part of complete immunohistological and immunohistochemical study in different parts of small intestine. Subsequent to sacrifice, the abdominal cavity of the animal was exposed by reflecting the skin, fascia, abdominal muscles and peritoneum and parts of the small

*Corresponding author: arup.kalita@gmail.com

intestine were observed. The abdominal cavity was opened and the parts of the small intestine were then dissected out as per the method of Habel (1964). The intestinal digesta were collected in the sterile tube for lactic acid bacteria count and the excess was removed by a cold saline infusion wash (Ciro *et al.*, 2013).

The population of lactic acid bacteria (LAB) was determined in the intestinal content from different segments of the small intestine (duodenum, jejunum and ileum) by pour plate method (Pieper *et al.*, 2006). Numbers were determined as colony-forming unit per mL (cfu/mL) in intestinal content. Briefly, the samples were serially diluted 10-fold in sterile de Man, Rogosa and Sharpe (MRS) agar broth (Hi-media) up to 10^{-6} and were plated from the highest three dilutions in duplicate to MRS agar (Hi-media), which was specific for lactic acid bacteria. Culture plates were then incubated aerobically at 37 ºC for 24 hours. The LAB colonies were counted from the three plates and the average values were expressed as log10 cfu/mL. The intestinal tissue samples were prepared for scanning electron microscopy as per the standard method and observed for colonization of bacteria.

The data obtained were analyzed using statistical package SPSS version 20. General Linear Model of two way ANOVA based on Fisher's Least Significant Difference method was used to determine the significant difference among days (20, 30 and 60 days) for control and treatment groups. The significant values in the ANOVA were further tested through the Duncan multiple range test. Results are presented as mean ± SEM and differences were considered significant when $P<0.05$. An independent sample t-test has been applied between groups (Control and treatment) at different days to see the significant changes.

In the present investigation, the lactic acid bacterial load was determined in the intestinal content from different segments of small intestine by pour plate method (Fig. 1). These bacteria were found to be colonized on the

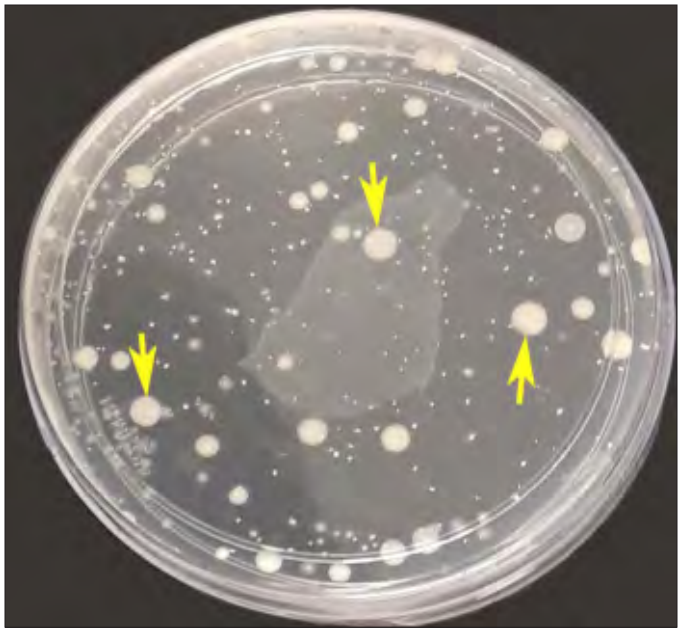

Fig. 1. Photograph of MRS agar plate showing colonies of lactic acid bacteria (arrow) in diluted small intestinal content from experimental piglet

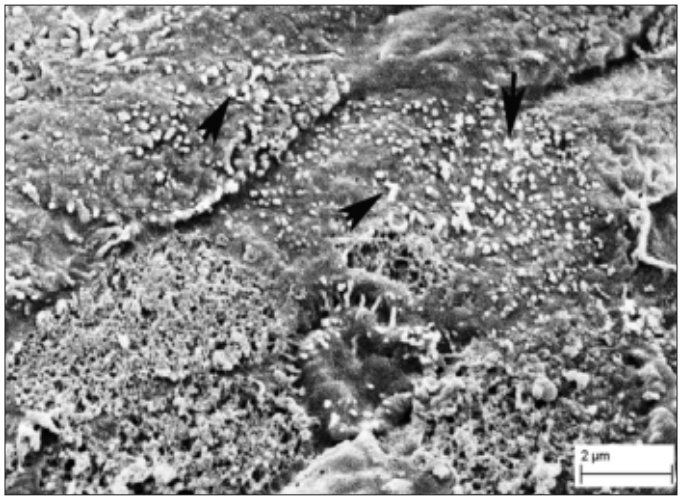

Fig. 2. Scanning electron micrograph showing colonies of lactic acid bacteria (arrow) in the luminal wall of jejunum at 20 days old treated piglet

Table 1

Comparison of lactic acid bacteria (LAB) count in the small intestinal content of control and treatment group of piglets

Parameter	Intestinal segment	Pre-weaning		Post-weaning				p-value	
		Day 20 (n=6)		Day 30 (n=6)		Day 60 (n=6)			
		Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
LAB count (log 10 cfu/ml)	Duodenum	5.86 ± 0.18^{a}	6.33 ± 0.41	6.20 ± 0.09^{ab}	6.26 ± 0.13	6.47 ± 0.04^{b}	6.55 ± 0.05	0.03	0.72
	Jejunum	6.04 ± 0.16^{ar}	7.28 ± 0.39^{ds}	6.06 ± 0.04^{ab}	6.26 ± 0.05^{e}	6.41 ± 0.03^{b}	6.87 ± 0.29^{de}	0.09	0.11
	Ileum	7.66 ± 0.47	7.95 ± 0.24^{d}	7.87 ± 0.03^{r}	8.10 ± 0.04^{ds}	7.88 ± 0.24^{r}	8.69 ± 0.07^{es}	0.85	0.03

Data are presented as LAB count in log10 cfu/mL intestinal content (Mean ± SEM) of different age-groups. a,bMeans with different superscripts between control groups significantly differ ($P<0.05$); d,eMeans with different superscripts between treatment groups significantly differ ($P<0.05$); r,sMeans with different superscripts within groups significantly differ ($P<0.05$).

luminal wall of small intestine (Fig. 2). The numbers of lactic acid bacteria were determined as colony forming unit per mL (cfu/mL) in intestinal content are presented in table 1.

The cultivable cell counts of lactic acid bacteria (LAB) in the present study increased from proximal to distal part of small intestine and towards the advancement of age in both the groups. The increased number of cultivable microflora from proximal to distal part of the gastrointestinal tract has also been reported by Pieper *et al.* (2006) in piglets, due to velocity of digesta flow being higher in the proximal intestine compared to more distal segments.

In the current study, dietary inclusion of probiotic revealed higher number of colony forming units in the treatment group of piglets than the control group of animals in all age-groups and in all segments of small intestine. The cultivable cell counts were significantly higher ($P<0.05$) in the treated piglets at day 20 in jejunum and, at day 30 and day 60 in ileum. Siggers *et al.* (2008) also documented increased colonization of beneficial commensal microbiota after probiotic administration in piglets. Chiang *et al.* (2015) documented that feeding of probiotic could increase the numbers of lactobacilli and decrease the numbers of *E. coli* in the faeces of weaned piglets which have high potential to be used as feed additives in the pig industry. Davis *et al.* (2007) recorded increased microbial diversity in the gastrointestinal tract 10 days after weaning in pigs provided probiotic treatment. Similarly, Dowarah *et al.* (2017) observed significant ($P<0.001$) increase in population of lactic acid bacteria and bifidobacteria in the faeces after feeding *Lactobacillus acidophilus* in pigs. Post-weaning diarrhoea which was very common in piglets was characterized by reduction of healthy bacteria and increased pathogenic bacteria (Konstantinov *et al.*, 2006). In the present study, more number of beneficial microbiota observed in the treatment group of piglets might reduce the pathogenic bacterial load in the small intestine and provide a healthy environment for better digestion and immunity in this group of piglets especially in early post-weaning period.

From the present study, it is concluded that the cultivable cell counts of lactic acid bacteria was higher in piglets fed with probiotic in comparison to the control animals both during pre and post-weaned period. Further, it is also recorded that these bacterial counts increased from proximal to distal segments of small intestine and towards the higher age-groups in both control and treatment group of piglets.

REFERENCES

Chiang, M.L., Chen, H.C., Chen, K.N., Lin, Y.C., Lin, Y.T. and Chen, M.J. (2015). Optimizing production of two potential probiotic lactobacilli strains isolated from piglet feces as feed additives for weaned piglets. *Asian Australas. J. Anim. Sci.* **28(8)**: 1163-1170.

Ciro, J.A., Lopez, A., and Parra, J.E. (2013). Expresión molecular de la vilina en yeyuno de lechones posdestete que consumieron LPS de *E. coli. Ces. Med. Vet. Zootec*. **8**: 32-41.

Davis, M.E., Brown, D.C., Baker, A., Bos, K., Dirain, M.S., Halbrook, E., Johnson, Z.B., Maxwell, C. and Rehberger, T. (2007). Effect of direct-fed microbial and antibiotic supplementation on gastrointestinal microflora, mucin histochemical characterization, and immune populations of weanling pigs. *Livest Sci.* **108**: 249-253.

Dowarah, R., Verma, A.K.and Agarwal, N. (2017). The use of *Lactobacillus* as an alternative of antibiotic growth promoters in pigs: A review. *Anim. Nutr.* **3**: 1-6.

Gebert, S., Davis, E., Rehberger, T. and Maxwell, C.V. (2011). *Lactobacillus brevis* strain 1E1 administered to piglets through milk supplementation prior to weaning maintains intestinal integrity after the weaning event. *Benef. Microbes.* **2(1)**: 35-45.

Habel, R.E. (1964). Guide to the dissection of domestic ruminants. Edwards Brother Inc., Ann. Arbor, Michigan.

Hopwood, D.E. and Hampson, D.J. (2003). Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate post-weaning period. Wageningen Academic Publishers, Wageningen, Netherlands. pp. 199-218.

Kelly, D. and King, T.P. (2001). Luminal bacteria: regulation of gut function and immunity, Nottingham University Press, Nottingham, UK. pp. 113-131.

Konstantinov, S.R., Awati, A.A., Williams, B.A., Miller, B.G., Jones, P., Stokes, C.R., Akkermans, A.D.L., Smidt, H. and de Vos, W.M. (2006). Post-natal development of the porcine microbiota composition and activities. *Environ. Microbiol.* **8(7)**: 1191-1199.

Liu, H., Zhang, J., Zhang, S., Yang, F., Thacker, P.A., Zhang, G., Qiao, S. and Ma, X. (2014). Oral administration of *Lactobacillus* fermentum I5007 favors intestinal development and alters the intestinal microbiota in formula-fed piglets. *J. Agril. Food Chem.* **62**: 860-866.

Pieper, R., Janczyk, P., Schumann, R. and Souffrant, W.B. (2006). The intestinal microflora of piglets around weaning - with emphasis on lactobacilli. *Archiva Zootechnica*. **9**: 28-40.

Prieto, M.L., O'Sullivan, L., Tan, S.P., McLoughlin, P., Hughes, H., O'Donovan, O., Rea, M.C., Kent, R.M., Cassidy, J.P., Gardiner, G.E. and Lawlor, P.G. (2014). Evaluation of the efficacy and safety of a marine-derived *Bacillus* strain for use as an in-feed probiotic for newly weaned pigs. *PLOS one.* **9(2)**: e88599.

Siggers, R.H., Siggers, J., Boye, M., Thymann, T., Molbak, L., Leser, T., Jensen, B.B. and Sangild, P.T. (2008). Early administration of probiotics alters bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing enterocolitis in preterm pigs. *J. Nutr.* **138(8)**: 1437-1444.

Snel, J., Harmssen, H.J.M., van der Wielen, P.W.J.J. and Williams, B.A. (2002). Dietary strategies to influence the gastrointestinal microflora of young animals, and its potential to improve intestinal health. Wageningen Academic Publishers, Netherland. pp. 37-39.

Williams, B.A., Verstegen, M.W.A. and Tamminga, S. (2001). Fermentation in the large intestine of single-stomached animals and its relationship to animal health. *Nutr. Res. Rev.* **14**: 207-227.