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Short Communication

Influence of *in ovo* Inoculation of Probiotic Strains on the Jejunal Goblet Cell Counts and Morphometry in Peri- and Post-hatching Chicks

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Abstract

The objective of the present study was to evaluate the effects of *in ovo* inoculation of different probiotic strains (*Bacillus subtilis, Enterococcus faecium*, and *Pediococcus acidilactici*) on jejunal goblet cells counts and morphometry in chicken. Probiotics were inoculated into the amniotic fluid of 480 eggs (day 17 of incubation), with four treatments and five replicates. At days 21 of incubation and 3 post-hatch, counts of goblet cells were 30% and 16% higher in the jejunum of group inoculated *Bacillus subtilis* as compared with the control group, respectively. Inoculation of *Enterococcus faecium,* and *Pediococcus acidilactici* had no effect (P>0.05) on goblet cells counts. Inoculation of *Bacillus subtilis* and *Pediococcus acidilactici* resulted in an increase of villus height, a decrease in crypt depth and a decrease in ratio of villus height to crypt depth compared with the control group (P>0.05), at days 8 and 28 of age. As a conclusion, various effects of different probiotic strains on goblet cells count and intestinal morphometry were observed. Among probiotic strains evaluated in this study, *Bacillus subtilis* has higher benefit effect on goblet cells counts in the early of life and morphometry of jejunum.

Keywords: *Incubation, Intestine, Morphology, Goblet cell, Probiotic*

Kuluçkadan Çıkış Öncesi ve Sonrası Civcivlerde Probiyotik Suşlarının *in ovo* Inokulasyonunun Jejunal Goblet Hücre Sayısı ve Morfometrisi Üzerine Etkisi

Özet

Bu çalışmanın amacı tcivcivlerde değişik probiyotik suşlarının (*Bacillus subtilis, Enterococcus faecium*, ve *Pediococcus acidilactici*) *in ovo* inokulasyonunun jejenum goblet hücre sayısı ve morfometri üzerine etkilerini araştırmaktır. Probiyotikler beş tekrar olmak üzere dört farklı uygulama olarak (uygulamanın 17. günü) 480 yumurtanın amniyotik sıvısı içine inokule edildi. Inokulasyonun 21. günü ve 3 post-yumurtadan çıkma, jejenum goblet hücre sayıları kontrol grubu ile karşılaştırıldığında *Bacillus subtilis* inokule edilenlerde sırasıyla %30 ve %16 daha yüksekti. *Enterococcus faecium* ve *Pediococcus acidilactici* inokulasyonlarının goblet hücre sayıları üzerine etkisi gözlenmedi (P>0.05). *Bacillus subtilis* ve *Pediococcus acidilactici* inokulasyonlarının 8 ve 28. günlerde villus boyunu artırdığı, kript derinliği ile villus boyu: Kript derinliği oranını ise kontrol grubuyla karşılaştırdığında azalttığı (P>0.05) belirlendi. Sonuç olarak, farklı probiyotik suşlarının goblet hücre sayıları ve barsak morfometrisi üzerine etkileri gözlemlendi. Çalışmada denenen probiyotik suşlarından *Bacillus subtilis*'in erken yaşta goblet hücre sayısı ve jejenum morfometrisi üzerine daha fazla yararlı etkiler sunduğu belirlendi.

Anahtar sözcükler: *İnkubasyon, Barsak, Morfoloji, Goblet hücresi, Probiyotik*

INTRODUCTION

In the modern poultry production, the contact between newborn chicks and hens is excluded, and colonization of bacteria in the gut depends on the type of bacteria present in the hatchery environment [1]. This condition exposes chicks to pathogenic bacteria colonization in the gut and causes a delay in desirable bacteria colonization [2]. The first contact of chicks with hatchery environment may include pathogen bacteria and leave gut colonization

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open to them, so it is necessary to inoculate desirable bacteria named probiotic. Different routes of early delivery of probiotics were examined, for example *in ovo* inoculation, immersion of eggs in probiotic medium, oral gavage, vent lip, and spraying of chick with probiotic solution [3-6]. In the previous studies that examined *in ovo* administration, probiotic solutions were injected into the air cell of eggs. This route of inoculation results in low hatchability. Chicken embryo swallow amniotic fluid during the last period of incubation; therefore, intra-amniotic inoculation of probiotic strains enables chicks to received desirable bacteria in the early of life without affecting the hatchability rate. Moreover, to our knowledge, in the literature the effect of intra-amniotic inoculation of different probiotic strains on intestinal characteristics of chicks was not evaluated. Therefore, the main objective of this study was to evaluate the effects of *in ovo* inoculation of different probiotic strains on the goblet cells counts and jejunal morphometry of broiler chicken at the peri- and post-hatch periods.

MATERIAL and METHODS

Chicks used in this study received human care based on criteria outlined in the Guide for the Care and Use of Laboratory Animals [7], and the experimental protocol was approved by the Research Committee of Islamic Azad University, Science and Research Branch (Approval date: 05.05.2013; No: 23874).

Fertile Cobb chicken eggs were obtained from a commercial hatchery from a flock with the 38 weeks of age. Eggs were incubated in a single-stage setter under the same condition of 37.6°C and 60% relative humidity and turned once per h. On day 17 of incubation, eggs with live embryo (No: 480, average weight of 58±1.1 g) were selected and weighed. In a completely randomized design, eggs were assigned to four experimental groups and five replicates of twenty four eggs per each. The four treatment groups that received *in ovo* 0.5 mL of sterile distilled water or probiotic mediums (10^7 cfu) into the amniotic fluid were: 1) sterile distilled water as control group, 2) B*acillus subtilis*, 3) *Enterococcus faecium* and 4) *Pediococcus acidilactici*. The *in ovo* inoculation procedure was performed as described by Tako et al.[8]. Solution was inoculated with a suitable needle inserted into the amniotic fluid, which was identified by candling. After inoculation, the hole in egg wall was sealed with cellophane tape, and eggs were placed in hatching trays. Upon hatching the chickens were allocated to related floor pens and raised for 6 weeks. Chickens management (water, feed, light program and pen environment) were based on Cobb 500 broiler chickens [9].

On days of 19 and 21 peri-hatch and days 1, 3, 8 and 28 post-hatch, two birds per each replicate were randomly selected, anesthetized with diethyl ether and caecal removed. The entry of caecal was sealed, removed and placed in ice and used for microbial assays. Also, samples of jejunum (3 cm) were taken and placed in buffered formalin solution (10%) for intestinal morphometry and goblet cells count. Histo-preparation was done according to the method described by Iji et al.[10]. Goblet cell count was determined by double-stained of samples with Periodic Acid-Schiff and hematoxylin according to the method of Horn et al.[11]. The goblet cells were counted in scale of 300 µm of epithelium length.

The normality of data was evaluated using Kolmogorov-Smirnov test. Then data were analyzed using the GLM procedure of SAS for Windows, version 9.1 (SAS Institute Inc., Cary, NC). Means were separated using Duncan's Multiple Comparison test (P<0.05).

RESULTS

There was a difference (P<0.05) between chicks received *Bacillus subtilis* and other treatments for goblet cells counts on day 21 of incubation and day 3 post-hatch *(Table 1)*. Differences among treatment for goblet cells count on day 19 peri-hatch and days 8 and 28 post-hatch were not significant statistically (P>0.05).

The means of jujunal villus height, crypt depth, and villus height: Crypt depth ratio are presented in *Table 2*. There were no differences (P>0.05) among treatment for mentioned traits on days 1 and 3 post-hatch, but on days 8 and 28 post-hatch differences were appeared among treatment (P<0.05). Inoculation of *Bacillus subtilis* and *Pediococcus acidilactici* resulted in increase of villus height and decrease in crypt depth and their ratio compared with the control group (P<0.05). There were no differences (P>0.05) for these traits between *Enterococcus faecium* and the control group.

DISCUSSION

Inoculation of probiotic bacteria via oral feeding is now recognized as a suitable route to reduce the risk of

Table 1. *Goblet cells counts (n per 300 µm of epithelium length) in the jejunum of chicks at different ages*

Tablo 1. *Farklı yaşlardaki civcivlerde jejenum goblet hücre sayıları (epitel uzunluğunun 300 µm'da bir n)*

Treatments	Peri-hatch		Post-hatch		
	19	21	3	8	28
Control	10.1	11.5 ^{b}	15.95 ^{b}	20.6	22.9
Bacillus subtilis	14.2	16.5 ^{a}	19.0 ^{a}	22.7	25.1
Enterococcus faecium	12.3	12.9 ^{b}	17.1 ^{ab}	22.1	23.2
Pediococcus acidilactici	12.4	13.4 ^{b}	17.8 ^{ab}	22.5	24.0
P value	0.258	0.001	0.033	0.744	0.463
SEM	1.43	1.85	0.62	1.38	2.01

^{a,b} Means with different superscripts within the same column differ significantly (P ≤ 0.05)

Table 2. *Villus height, crypt depth and their ratio in the jejunum of chicks at different ages*
Tablo 2. *Farklı yaşlardaki civcivlerde jejenum villus boyu, kript derinliği ve oranları*

Treatments	Days Post-hatch											
	Villus Height (µm)				Crypt Depth (µm)				Villus Height/Crypt Depth			
	1	3	8	28	1	3	8	28	1	3	8	28
Control	267	401	675 ^{b}	870 ^{b}	64	125	184 ^{a}	201 ^{a}	4.17	3.21	3.67 ^{b}	4.33^{b}
*BS**	276	408	850 ^{a}	985 ^{a}	61	113	152 ^{b}	164 ^{b}	4.52	3.61	5.59 ^{a}	6.01^{a}
EF	282	391	721 ^{ab}	880 ^{ab}	59	115	165 ^{ab}	169 ^{b}	4.78	3.40	4.38 ^{b}	5.21^{a}
PA	274	405	832 ^{a}	985 ^{a}	65	104	155 ^{b}	169 ^{b}	4.22	3.89	5.37^{a}	5.83^{a}
SEM	26.7	56.3	45.6	34.7	4.8	6.3	8.2	6.5	0.405	0.525	0.330	0.266
P value	0.985	0.997	0.032	0.045	0.792	0.427	0. 054	0.004	0.753	0.778	0.002	0.002

*^{a,b} Means with different superscripts within the same column differ significantly (P≤0.05) ***BS:** Bacillus subtilis; **EF:** Enterococcus faecium; **PA:** Pediococcus acidilactici*

intestinal infection by pathogenic bacteria [2]. An interesting study demonstrated that the time of initial intestinal colonization by desirable bacteria play an important role on the colonization of pathogens [1]. In the previous studies [4-6,8,12], the protection effects of *in ovo* inoculation or other route administration of probiotics against Salmonella infection were investigated, but the effects of inoculation of different probiotic strains on intestinal morphometry, and goblet cells count have not been attended. The main objective of this study was to evaluate the effect of three probiotic strains, *Bacillus subtilis, Pediococcus acidilactici* and *Enterococcus faecium,* on intestinal characteristics.

Chicks received *Bacillus subtilis* had higher goblet cells counts than the control group and those received other probiotic strains. An interesting study [13] showed that dietary factors and microbiota could affect goblet cell numbers. Feeding probiotic to the turkey poults has been reported to increase the goblet cell number in the small intestine, which can protect epithelia from pathogenic bacteria [14]. Mucin production is correlated with the goblet cells number and if a pathogen enters via the digestive tract, a thick mucus layer produced by goblet cells, will block the pathogen from penetrating the host's cells.

There were no differences among treatment for mentioned traits on days 1 and 3 post-hatch. In agreement to our finding, Santin et al.[15] with feeding *Saccharomyces cerevisiae* and Sieo et al.[16] with six Lactobacillus strains reported no differences in the small intestine morphometry. Probiotics strains were inoculated at day 17 of incubation and it seems that probiotics needs more times to affect the proliferation of intestinal cells.

Inoculation of *Bacillus subtilis* and *Pediococcus acidilactici* resulted in the increase of villus height and decrease in crypt depth and their ratio compared with control group on days 8 and 28 post-hatch. The increase in villus height due to the probiotic inoculation could be considered important and beneficial for the absorptive capacity of jejunum. An increase in the villus height suggests increase in the surface area capable of higher absorption of nutrients. *Enterococcus faecium* had no effect on intestinal morphometry parameters. In contrast, Chichlowski et al.[17] and Samli et al.[18] reported that inclusion of *Enterococcus faecium* increased the jejunal villus height and decreased the villus crypt depth as compared with the control group.

As a conclusion, various effects of different probiotic strains on goblet cells count and intestinal morphometry were observed. Among probiotic strains evaluated in this study, *Bacillus subtilis* has higher benefit effect on goblet cells counts in the early of life and morphometry of jejunum.

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REFERENCES

1. Sterzo E, Paiva J, Penha-Filho R, Berchieri-Junior A: Time required to protect the intestinal tract of chicks against *Salmonella enterica* serovar *Enteritidis* using competitive exclusion. *Braz J Poult Sci*, 7, 119-122, 2005. DOI: 10.1590/S1516-635X2005000200009

2. Cukrowska B, Lodinova-Zadnikova R, Enders C, Sonnenborn U, Schulze J, Tlaskalova-Hogenova H: Specific proliferative and antibody responses of premature infants to intestinal colonization with non-pathogenic probiotic *E. coli* strain Nissle 1917. *Scand J Immunol*, 55, 204-209. 2002. DOI: 10.1046/j.1365-3083.2002.01005.x

3. Schneitz C, Nuotio L, Mead G, Nurmi E: Competitive exclusion in the young bird: Challenge models, administration and reciprocal protection. *Int J Food Microbiol*, 15, 241-244, 1992. DOI: 10.1016/0168-1605(92)90055-8

4. Hashemzadeh Z, Karimi-Torshizi MA, Rahimi S, Razban V, Zahraei-Salehi T: Prevention of *Salmonella* colonization in neonatal broiler chicks by using different routes of probiotic administration in hatchery evaluated by culture and PCR techniques. *J Agric Sci Tech*, 12, 425-432, 2010.

5. Hosseini-Mansoub N, Vahdatpour T, Arjomandi M, Vahdatpour S: Comparison of different methods of probiotic prescription against *Salmonella* infection in hatchery broiler chicken. *Adv Environ Biol*, 5, 1857-1860, 2011.

6. Yamawaki RA, Milbradt EL, Coppola MP, Rodrigues JCZ, Andreatti Filho RL, Padovani CR, Okamoto AS: Effect of immersion and enoculation *in ovo* of *Lactobacillus* spp. in embryonated chicken eggs in the prevention of *Salmonella Enteritidis* after hatch. *Poult Sci*, 92, 1560-1563, 2013. DOI: 10.3382/ps.2012-02936

7. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF: Special Report: The 1996 Guide for the Care and Use of Laboratory Animals. *ILAR J*, 38, 41-48, 1997.

8. Tako E, Ferket PR, Uni Z: Effects of *in ovo* feeding of carbohydrates and beta-hydroxy-beta-methylbutyrate on the development of chicken intestine. *Poult Sci,* 83, 2023-2028, 2004. DOI: 10.1093/ps/83.12.2023

9. Cobb Broiler Performance & Nutrient Supplement Guide: Cobb-Vantress Inc., Siloam Springs, AR, USA, 2012. http://cobb-vantress.com/academy/product-guides; *Accessed:* 2 July 2015.

10. Iji PA, Saki AA, Tivey DR: Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. *Anim Feed Sci Technol*, 89, 175-188, 2001. DOI: 10.1016/s0377-8401(00)00223-6

11. Horn NL, Donkin SS, Applegate TJ, Adeola O: Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. *Poult Sci*, 88, 1906-1914, 2009. DOI: 10.3382/ps.2009-00009

12. de Oliveira JE, van der Hoeven-Hangoor E, van de Linde IB, Montijn RC, van der Vossen JMBM: *In ovo* inoculation of chicken embryos with probiotic bacteria and its effect on posthatch *Salmonella* susceptibility. *Poult Sci*, 93, 818-829, 2014. DOI: 10.3382/ps.2013-03409

13. Sharma R, Schumacher U: Morphometric analysis of intestinal mucins under different dietary conditions and gut flora in rats. *Dig Dis Sci*, 40, 2532-2539, 1995. DOI: 10.1007/BF02220438

14. Rahimi S, Kathariou S, Grimes JL, Siletzky RM: Effect of direct-fed microbials on performance and *Clostridium perfringens* colonization of turkey poults. *Poult Sci,* 90, 2656-2662, 2011. DOI: 10.3382/ps.2011-01342

15. Santin E, Maiorka A, Macari M, Grecco M, Sanchez J, Okada T, Myasaka A: Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. *J Appl Poult Res*, 10, 236-244, 2001. DOI: 10.1093/japr/10.3.236

16. Sieo CC, Abdullah N, Tan W, Ho Y: Influence of beta-glucanase-producing *Lactobacillus* strains on intestinal characteristics and feed passage rate of broiler chickens. *Poult Sci*, 84, 734-741, 2005. DOI: 10.1093/ps/84.5.734

17. Chichlowski M, Croom WJ, Edens FW, MacBride BW, Qiu R, Chiang CC, Daniel LR, Havenstein GB, Koci MD: Microarchitecture and spatial relationship between bacteria and ileal, cecal and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac, and salinomycin. *Poult Sci*, 86, 1121-1132, 2007. DOI: 10.1093/ps/86.6.1121

18. Samli HE, Senkoylu N, Koc F, Kanter M, Agma A: Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and microbiota. *Arch Anim Nutr*, 61, 42-49, 2007. DOI: 10.1080/17450390601106655