Characterization of intestinal microbiota of newly hatched ducklings

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Objectives of Project: 1. Characterize microbiota from the gut of ducklings from different breeder flocks on day of hatch (DOH). Compare gut microbiota of DOH ducklings from poor-performing (PPF) and good-performing breeder flocks (GPF). Compare gut microbiota of DOH ducklings with and without probiotics application in hatcher cabinet

Start Date: June 28, 2019

End Date: July 26, 2019

Experimental Design

Fertile eggs from two different flocks were evenly distributed among six incubators each on day 25 of incubation.

Treatments:

Treat	Fertile Egg Source	Reps	Number of Eggs	Probiotic LAB
T1	GPF[1]	3	14	
T2	GPF	3	14	$9.6x10^7$
T3	PPF[2]	3	13	
T4	PPF	3	13	$9.6x10^7$

([1] – Good Performing Flock; [2] – Poor Performing Flock)

Timeline & Samples:

Day 1 of incubation, Thursday, June 27– All eggs placed into incubator at the turkey farm

Day 7 of incubation, Wednesday, July 3 – Eggs candled and checked for fertility

Day 25 of incubation, Monday, July 22 – Transferred all eggs to hatcher cabinets at the laboratory and grew lactic acid bacteria (LAB) under aerobic conditions

Day 26 of incubation, Tuesday, July 23 – Applied spray treatment to pipping/hatching ducklings in spray groups every 4 hours

Day 27 Wednesday, July 24 – Weighed all ducklings and aseptically collected GI tract from 6 birds per hatcher cabinet

Samples: Collection of GI tract (duodenum to ceca)

- Lactic acid bacteria (LAB) and Gram-negative enumeration by plating GI tract contents on MacConkey's, MRS, and BD CHROMagar Orientation
- DNA extraction for Next-Generation Sequencing
- Blood collection for glucose level comparison

Methods:

The eggs were transferred to smaller hatcher cabinets on day 25 for monitoring and treatment preparation. When the eggs were determined to be ~40% pipped a treatment of five sprays of $9.6x10^7$ CFU/ml of Lactic acid bacteria (LAB) based probiotic inoculum were administered to the good and poor performing treatment groups every four hours over the course of sixteen hours. Over the course of hatching the ducks were marked for hatch order. All ducklings were then weighed, and GI tracts (duodenum to ceca) and blood was collected from the six birds. The samples were then plated on MRS, MacConkey's, and BD CHROMagar to verify and compare LAB and gram-negative colonies.

To determine differences in body weight and bacterial colony counts were compared using Student's *t*-test ($p \leq 0.05$) (JMP Software, SAS Inc., 2018). The significant differences were distinguished by different superscripts within columns.

Results

The Bacterial Recovery in the different treatments is shown in Figures 1 and 2.

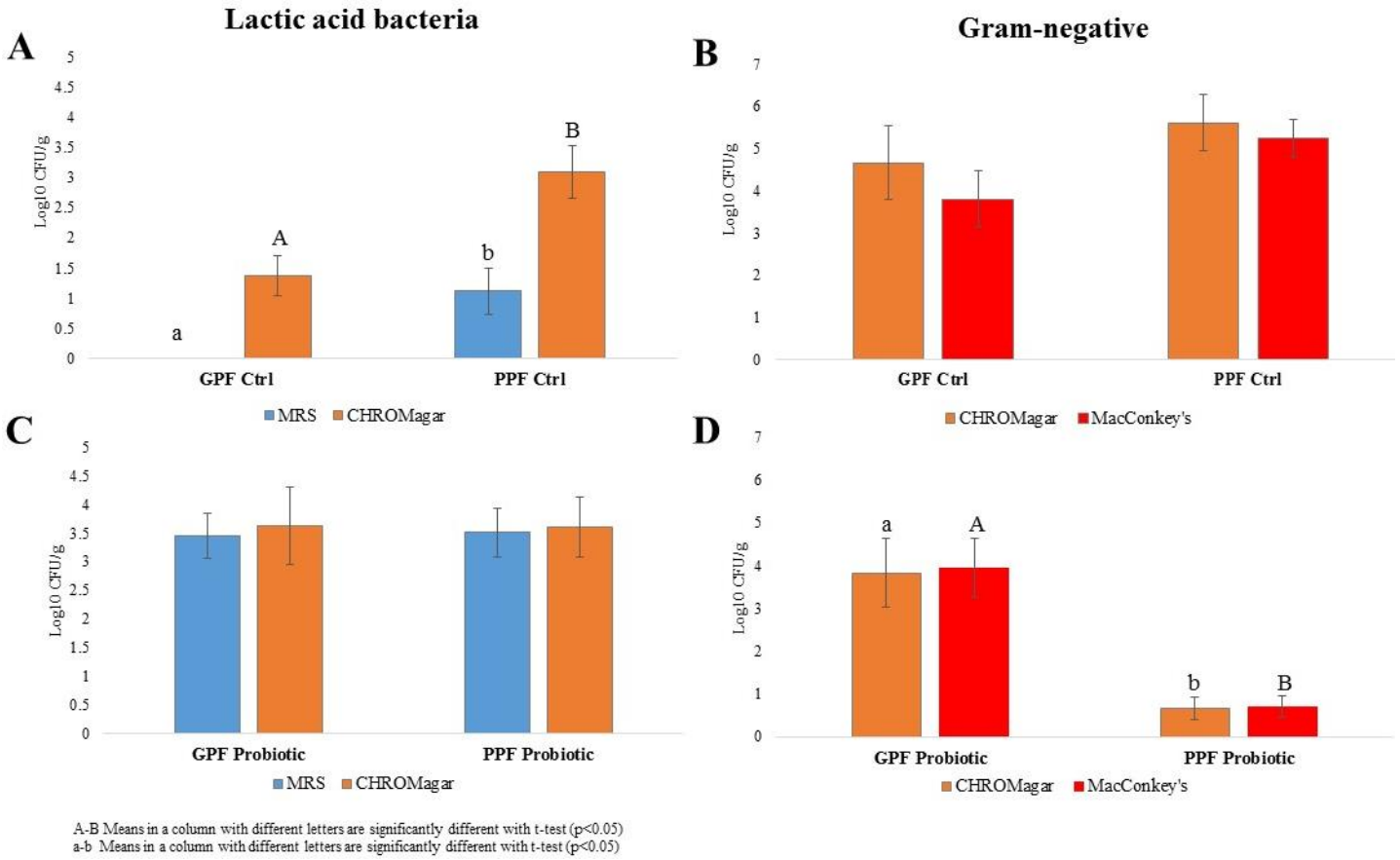

Figure 1. Intestinal Lactic acid bacteria(LAB) and Gram-negative recovery from duckling at day of hatch (DOH) in control negative and positive treatments. (**A, B**) The comparisons were made between control good performing flock (GPF) and poor performing flock (PPF). (**C,D**) Bacterial recovery in the probiotic-treated groups.

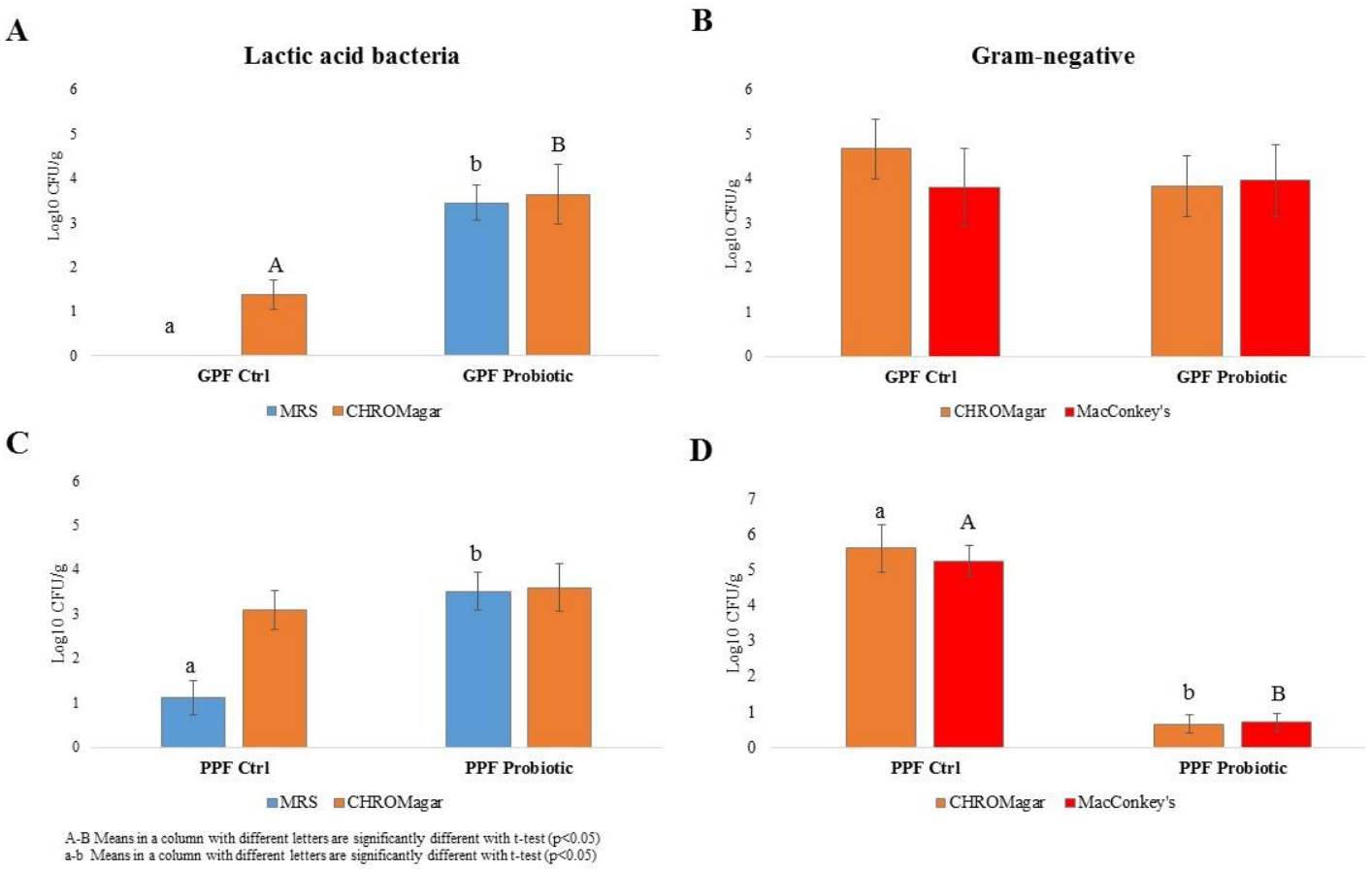

Figure 2. Differences between intestinal Lactic acid bacteria(LAB) and Gram-negative recovery in control and probiotic-treated groups in duckling at day of hatch. (**A**) LAB counts from two different agars and (**B**) Gram-negative recovery from MacConkey's and BDCHROMagar in good performing flock (GPF) treatment. (**C,D**) Similar analyses were carried out in poor performing flock (PPF).

To compare gut microbiota of DOH ducklings from the two breeder flocks, a quantitative and qualitative evaluation of bacterial recovery were performed using different selective agars. There was a higher population ($p<0.05$) of LAB (both MRS ($p=0.007$) and CHROMagar ($p=0.0036$).) in control PPF than GPF treatment (Fig. 1 A). Interestingly, both control GPF and PPF had similar counts of gram-negative bacteria (Fig. 1 B). However, when the probiotic was applied in PPF eggs, the population of gram-negative bacteria was significantly lower compared to GPF at DOH (Fig. 1 D).

LAB recovery between the GPF and PPF control groups was shown to be significant for both. However, there were no significant differences between gram-negative ($p=0.081$) and E. coli ($p=0.3963$) recovery in the GI tracts of the two flocks.

To evaluate the effect of probiotics application on intestinal counts of LAB and gram-negative bacteria, a bacterial recovery comparison between control treatments and probiotic treated-groups was performed. Recovery of LAB from the intestine of both the GPF and PPF was significantly higher ($p<0.0001$ and $p<0.0002$, respectively, Fig. 2 A, C) in the treated groups

with probiotics as compared to the control treatment at DOH. Interestingly, gram-negative bacteria recovery were greatly reduced ($p<0.0001$ for both agars) in the PPF (Fig. 2 D), whereas there was a parallel (Table 3, supplementary material) but no significant reduction ($p=0.8822$ and $p=0.4844$, respectively) in the GPF treatment (Fig. 2 B).

Weights among individual hatchers of both flocks varied, but differences between hatchers were not statistically significant, with the GPF having a $p=0.4867$ and PPF having a $p=0.2815$ (Table 1).

Table 1. Body weight of ducklings at day of hatch

Treatment	BW(g)	p-Value
GPF Ctrl	577.45 ± 9.32	0.486
GPF Probiotic	568.56 ± 7.62	
PPF Ctrl	583.77 ± 11.00	0.281
PPF Probiotic	598.02 ± 9.31	

Table 2. Total hatchability

Flock	Fertile Eggs/ Total Eggs	Viable/Deceased at Time of Sampling	Hatchability of Fertile Eggs (%)
GPF	84/100	61/23	72.6
PPF	77/100	55/22*	71.4

(* - One egg ruptured while still in the incubator)
Good Performing Flock (GPF); Poor Performing Flock (PPF

Hatchability between the fertile eggs of both flocks was only shown to have a small difference of 1.2%, although the difference in overall hatchability when including infertile eggs, jumps to a difference of 6% in favor of the GPF (Table 2).

Conclusion

The commercial hatching system for poultry has been automated to maximize production and limit disease transmission. However, as a consequence of the limited contact with hens' microbiota, the assembly of the intestinal microbiome in newly hatched birds has the predominant influence of the hatchery environment. The pioneer colonization in the intestine becomes ultimately important because it will serve as the basis from which the intestinal microbial communities will settle at a later age. As shown in the previous studies reported by this lab (Supplementary material), the pioneer colonization in chicks drives the course of microbial community composition and diversity over time, in which providing probiotics before chicks have hatched supported colonization of a greater heterogeneity of symbiotic populations affecting growth metabolism and immune response. Here, in this study, we showed that the early

manipulation of pioneer colonizers in ducklings, probiotics sprayed into hatcher cabinets, increased the intestinal population of LAB, which has been widely reported as playing a critical role in driving high performance and immune system development of poultry. Besides, the exposure to probiotics decreased the intestinal colonization of gram-negative bacteria that is highly associated with pathogens as *Escherichia coli*.

Interestingly, it was also found different colonization pattern of LAB based on the source of the eggs suggesting that the maternal flock may affect the microbiota of the ducklings at DOH. In addition, this study showed that the application of probiotics significantly reduced the gram-negative bacteria colonization in the gut of PPF birds. Given that enteric inflammation is one of the biggest poultry concerns for the industry, these results revealed a valuable potential strategy to decrease the intestinal population of gram-negative and increase commensal bacteria in ducklings at DOH.

Supplementary material

Table 3: Bacterial Recovery from the intestine of ducklings at day of hatch

Treatment	Lactic acid bacteria (MRS)	Gram-negative	Lactic acid bacteria (CHROMagar)	E. coli
GPF Control	0 ± 0	3.81 ± 0.67	1.37 ± 0.32	4.67 ± 0.88
GPF Spray	3.45 ± 0.39	3.95 ± 0.68	3.63 ± 0.68	3.82 ± 0.81
p-Value	<0.0001	0.8822	0.0058	0.4844
PPF Control	1.12 ± 0.39	5.24 ± 0.44	3.09 ± 0.44	5.61 ± 0.66
PPF Spray	3.51 ± 0.43	0.69 ± 0.25	3.6 ± 0.52	0.65 ± 0.27
p-Value	<0.0002	<0.0001	0.4865	<0.0001

Good Performing Flock (GPF); Poor Performing Flock (PPF)