Effect of In Ovo Feeding of Probiotics on Intestinal Cell Populations in Chickens

Emily Meluch, Dr. Eric Wong, Sara Cloft, and Sydney Kinstler

School of Animal Sciences, Virginia Tech, Blacksburg, VA 24061

INTRODUCTION

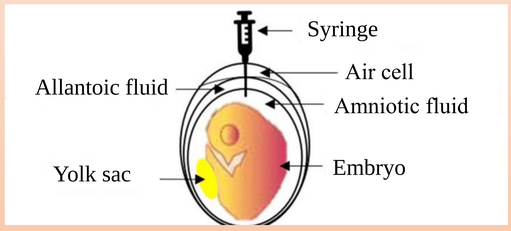
- With the concern of antimicrobial resistance and the rise of antibiotic free market demand, there has been a need within poultry production for alternatives to antibiotics.
- The poultry industry invests in new alternatives to antibiotics to promote a healthy immune response and to enhance growth and laying performance.
- Probiotics are live bacteria that can colonize the gut and enhance gut health and development.
- Probiotics may stimulate an early immune response in the gut.
- In ovo feeding introduces compounds into the amniotic fluid of a late-stage embryonic chick.
- The chick swallows amniotic fluid with the compounds introduced during in ovo feeding, entering the chick intestine prior to hatch.

OBJECTIVE

- To determine the effects of administering probiotics through in ovo feeding on intestinal immune-related gene expression of broiler chicks during the first 7 days post-hatch.

IN OVO FEEDING (IOF)

- Can stimulate an early immune response and provide the growing gut with intestinal microflora prior to chick hatch.
- Allows chicks to put more resources/energy towards growth, maintenance, and physical activity during production.
- Probiotics are manually injected into the amniotic sac of an embryonic chick at embryonic day 17.5.
- At days 18-20 of incubation, the embryonic chick begins to swallow some of the amniotic fluid containing injected compounds.



Goel, A., Ncho, C. M., Jeong, C. M., & Choi, Y. H. (2022). Embryonic thermal manipulation and in ovo gamma-aminobutyric acid supplementation regulating the chick weight and stress-related genes at hatch. Frontiers in veterinary science, 8, 807450. Figure 2

METHODS

Incubation:

1. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Virginia Tech.
2. Approximately 325 fertile broiler chicken eggs (Cobb 500) were received from a local hatchery and incubated.
3. On embryonic day (e) 12 all eggs were candled to assess viability. Six eggs were removed due to embryo death or infertility.
4. On e17.5, IOF treatments were applied to all eggs, with approximately 100 eggs per treatment.

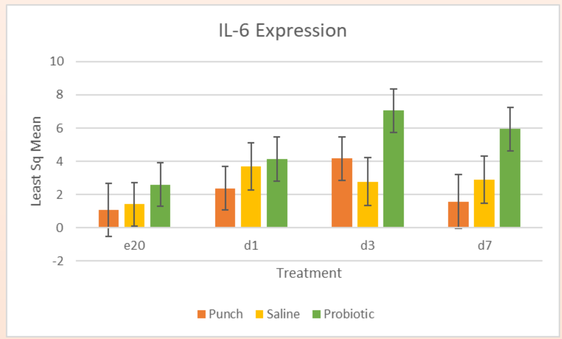
In Ovo Feeding:

1. Eggs were punched with a 20-gauge syringe needle creating a hole in the shell.
2. Then individual eggs were manually injected with 0.3 ml of either 0.75% Saline, or Primalac W/S Probiotic.
3. One group of eggs had a hole punched but were not injected with any solution to serve as the non-injected controls (Punch).
4. After treatment application, eggs were transferred into a hatcher basket separated by treatment.
5. On e20, 6 eggs per treatment were randomly selected from hatcher baskets for sampling.
6. Chicks were individually weighed and placed in 3 battery cages (n = 20/cage) per treatment.
7. Each cage was provided access to water and a starter diet ad libitum.
8. The middle section of the small intestine, jejunum, was removed intact and rinsed with cold phosphate buffered saline.
9. The jejunum was then diced and snap frozen in liquid nitrogen before being placed at -80℃ awaiting gene expression analysis.
10. On 1, 3, 5, and 7 days (d) post-hatch, 2 chicks per cage (n=6/treatment) were randomly selected and weighed and then euthanized by cervical dislocation for collection of the jejunum.
11. No mortalities occurred during this study.

Analysis:

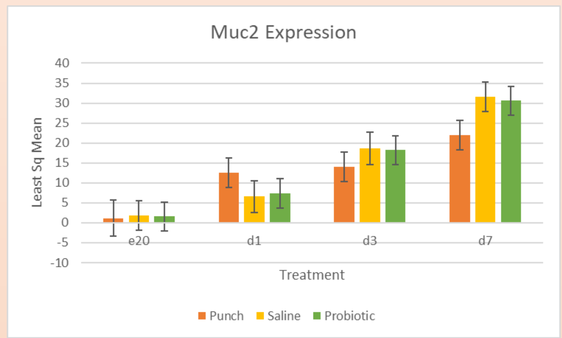
1. RNA was collected from tissue samples, and quantitative PCR was conducted to measure mRNA expression.
2. Statistical Analysis consisted of two-way ANOVA considering Age and Treatment using JMP software.

RESULTS



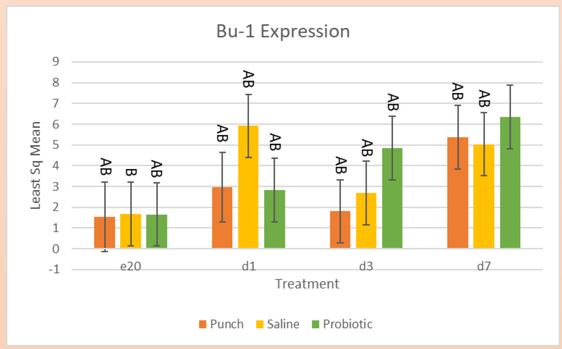
IL-6:

- Proinflammatory cytokine
- Functions in inflammation and maturation of B cells
- IL-6 mRNA was upregulated with Probiotic compared to Punch and Saline
- Probiotic initiated an inflammatory response



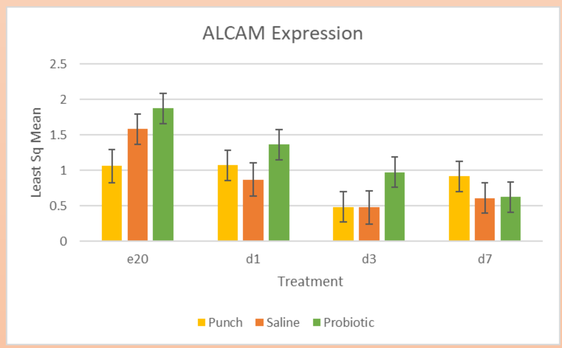
Muc2

- Produces mucus that lines the intestine
- Mucus creates protective layer in lumen
- Muc2 mRNA increased by day
- Muc2 mRNA was not affected by Probiotic treatment



Bu-1

- Immune response factor related to B-cells, which promotes immune response
- Bu-1 mRNA increased by day
- Bu-1 mRNA was not affected by Probiotic treatment



ALCAM

- T-cell marker, helps the body fight off infection
- ALCAM mRNA was increased by Probiotic treatment
- ALCAM mRNA was decreased with age

ACKNOWLEDGEMENTS

I would like to thank The John Lee Pratt Scholarship Internship for funding this opportunity for me to promote undergraduate research in animal nutrition. This has allowed me to explore research I am passionate about and has accelerated my understanding of animal sciences outside of the classroom setting. I would also like to thank Dr. Wong, my advisor, Sara Cloft, and Sydney Kinstler, for engaging me in undergraduate research, as I have gained valuable experience and found my interest in animal research. I am grateful for their dedicated support during my project, and for their help in and out of the lab.

REFERENCES

Beck, C. N., McDaniel, C. D., Wamsley, K., & Kiess, A. S. (2019). The potential for inoculating Lactobacillus animalis and Enterococcus faecium alone or in combination using commercial in ovo technology without negatively impacting hatch and post-hatch performance. Poultry science, 98(12), 70507062.https://doi.org/10.3382/ps/pez441

Cloft, S. E. (2022). Outlining a balance-point model of homeostasis in the small intestine of broiler chickens (Doctoral dissertation, Virginia Tech).

Duan A-y, Ju A-q, Zhang Y-n, Qin Y-j, Xue L-g, Ma X, Luan W-m and Yang S-b (2021) The Effects of In Ovo Injection of Synbiotics on the Early Growth Performance Intestinal Health of Chicks. Front. Vet. Sci. 8:658301. doi:3389/fvets.2021.658301

Sneeringer, S. MacDonald, J., Nigel, N., McBride, W., and Mathews, K.. Economics of Antibiotic Use in U.S. Livestock Production, ERR-200, U.S. Department of Agriculture, Economic Research Service, November 2015.

CONCLUSIONS

- The mRNA for IL-6, which is a proinflammatory cytokine, was upregulated with Probiotic compared to Punch and Saline.
- The mRNA for the B-cell marker Bu-1 increased with age, and was not affected by Probiotic treatment.
- The mRNA for the T-cell marker ALCAM decreased with age and was increased by Probiotic treatment.
- The mRNA for Mucin 2, which produces mucus that lines the intestine, increased with age, but was not affected by Probiotic treatment.
- In conclusion, the in ovo feeding of probiotics stimulated an inflammatory response.