10/

Harnessing epigenetics to improve chick quality with trace mineral zinc

Juxing Chen | juxing.chen@novusint.com
Novus International, Inc.
Mercedes Vázquez-Añón | mercedes.vazquez@novusint.com
Novus International, Inc.

JUXING CHEN, PhD., is the Senior Manager of Structure and Functional Biology at Novus International. Since joining Novus in 2012 Dr. Chen's research has included maternal feeding/epigenetic modification, gut health, structural integrity, and soybean meal quality. Dr. Chen's research in maternal feeding mainly focuses on delineating the mode of action of maternal supplementation of organic trace minerals on the growth and development of progeny in poultry, swine and ruminants. Her research findings have been published in the book, Nutrition of Hyperprolific Sows; the Journal of Animal Science, and various industry publications.

MERCEDES VÁZQUEZ-AÑÓN, PhD., is the Senior Director of Strategic Initiatives and Account Collaboration at Novus International. She received her bachelor's degree in Agriculture Engineer and Animal Science from Universidad Politécnica- Madrid, Spain. Her master's degree was earned at Penn State University in the U.S. Her doctorate degree in Nutritional Science is from the University of Wisconsin-Madison in the U.S. Dr. Vázquez-Añón joined Novus in 1996 as a research manager where she developed the application of several Novus products across species, became a Fellow Scientist in 2006, the director of animal nutrition research in 2007, and in 2012 became responsible for global animal research and facilities. Her research expertise encompasses amino acid, trace mineral, antioxidant, enzyme, and lipid nutrition and metabolism in ruminants and monogastrics, holds patents on different technologies, has published more than 40 refereed scientific publications and numerous conference proceedings. She is bilingual in English and Spanish.

Summary

The poultry industry seeks ways to improve efficiency and reduce the cost of production. One way to improve efficiency is to optimize the nutrition and management of the breeder hen by optimizing its reproduction and the performance of the progeny. Historically, more attention has been on the nutrition of the hen to improve reproduction and the progeny was an afterthought.. However, new research has provided evidence showing how some micronutrients in the breeder hen diet can boost the immune system of the progeny, providing new opportunities to help the chick develop its immune system, particularly in the face of an antibiotic-free environment. Of the trace minerals, zinc (Zn) and especially in its chelated form when fed to the breeder hen can improve the livability of the young chick by modulating the cellular and humoral immune response to pathogenic challenges. With recent advances in epigenetics, the underlying mechanism by which maternal Zn can modulate gene expression of the chick *in vivo* and improve immune development are better understood. Maternal Zn is stored in the egg yolk, yolk sac, and albumen prior to its delivery to the embryo's liver from where it is distributed to the developing organs as needed during embryo development and after hatch. Increasing level of Zn in the breeder diet - and in the form of chelates – is shown to improve storage of Zn in the egg yolk and albumen, so more Zn is available for embryo development. In the gut, Zn improves gut barrier function, mucosal and systemic immunity, and reduces gut inflammation of the progeny chicks by epigenetic modification of anti-inflammatory proteins.

In conclusion, feeding Zn, especially in the chelated form, to broiler breeders improves the chance for better livability of the chicks due to a more developed immune system, and provides a way to better prepare the chick for optimal performance in an antibiotic-free environment.

1. Introduction

The poultry industry is always looking for ways to improve efficiency and reduce the cost of production. In broiler production, the cost of hatching the egg or a day-old chick is very small compared to the cost of kilograms of live bird (Callini and Sirri, 2007). These low-cost periods provide opportunities to optimize the nutrition and management of the breeder hen to improve its performance and that of the progeny. However, most of the attention until now has been on broiler breeder nutrition and research to improve productivity in terms of saleable chicks produced, egg fertility, and hatchability and less attention has been given to the effect of maternal nutrition on the offspring broiler performance.

Breeder nutrition

Kidd (2003) and Callini and Sirri (2007) summarized the breeder nutrition studies

published in the literature. The concept of feeding the hen to improve progeny performance is not new but sometimes the goals of breeder nutrition - maximizing reproductive performance - are at odds with improving progeny development. In spite of limited information in the literature, there are two aspects of progeny development that can be improved via maternal nutrition. One is growth performance and carcass yield, and the other is the health and immunity of the progeny (Callini and Sirri, 2007). Reducing dietary protein levels in a breeder's diet can negatively affect the size of the egg, but does not appear to affect progeny performance (Kidd, 2003). Optimizing the level and type of energy in the breeder diet improves the carcass yield of the progeny. Brake *et al.* (2003) reported that progeny performance, especially in males, was improved when increasing protein and energy in the breeder diet. Lesson and Summer (2001), and Peebles *et al.* (1999, 2002a) showed that feeding fat sources with linoleic and linolenic acid content, such as corn oil, provided better progeny performance, carcass yield, and reduced embryo mortality than feeding poultry fat or lard. This effect was not observed when fed to younger breeders (Peebles *et al.*, 2002 b). Later, Kidd *et al.* (2005) reported that L-carnitine supplementation in high energy hen diets improved performance and carcass yield, especially in males, suggesting carnitine helps with the utilization of energy in the hen and embryo to support rapid growth in the male offspring. It has been postulated that hen nutritional programmes that increase yolk weight over egg weight provide more energy and protein to the chick posthatch and support faster growth of the offspring, especially of male broilers.

Supplementation of vitamins and minerals in breeder hen diets has been found to be more effective than other nutrients in improving liveability and immunity of the progeny (Callini and Sirri, 2007). Because of the high content of polyunsaturated fatty acids in egg yolk, supplementation with selenium and high levels of vitamin E in breeder diets has been reported to increase their content in yolk, protecting the unsaturated fatty acids from oxidation and improve the antioxidant and humoral immune status of the progeny (Surai, 2000). As an example, maternal supplementation of 25-hydroxy vitamin D3 increased hatchability and *in vitro* chick innate immunity towards *E. coli* (Saunders-Blades and Korver, 2015). Adequate levels of calcium (Ca) and phosphorus (P) were reported to improve skeleton ossification of the embryo and progeny at hatch (Triyuwanta *et al.*, 1992), and the addition of vitamin D improved calcification of the eggshell (Plaimast *et al.*, 2015). Supplementation of Zn has been shown to improve humoral and cellular immunity and to enhancethe progeny response to pathogenic challenges (Kidd, 1992, 1993). The combination of Zn and Mn improved the livability of the progeny (Virden *et al.*, 2003).

Factors during prenatal period that can affect the offspring

More recent work compiled by Dixon *et al.* (2016) identified factors in the prelay period and incubation environment that can have lasting effects on how the offspring responds to its environment, beyond genetics. During the prelay period, hen age, nutrition, and management can impact the performance of the offspring. It is well known that progeny

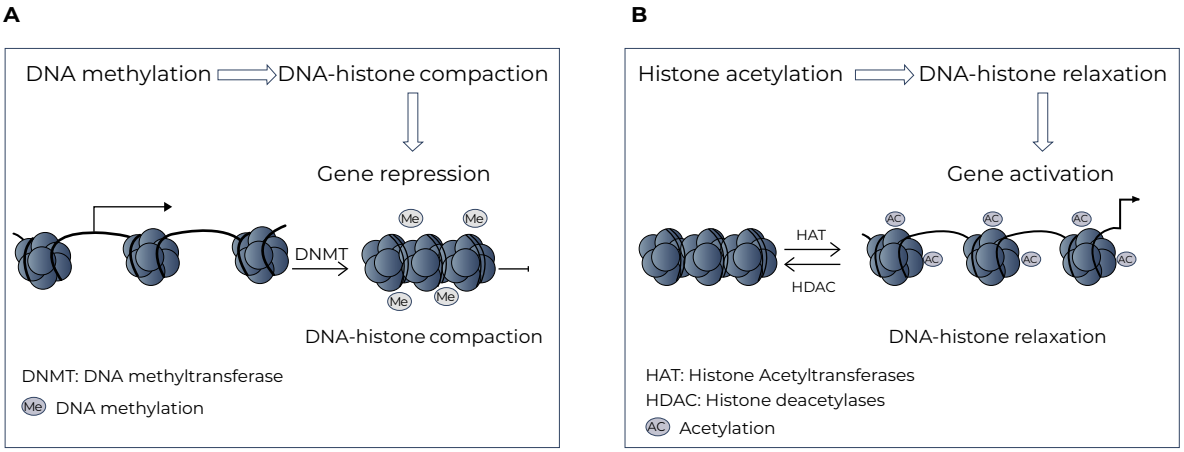

Figure 1. Diagram of DNA methylation (A) and histone H3K9 acetylation (B) regulating gene expression. (Modified from Moresi *et al.,* 2015)

from middle-aged breeders have higher performance than those from young or older breeders. As mentioned earlier, energy type and the content of the hen diet can impact the performance of the offspring, whereas vitamins and minerals can affect progeny health. Developing the offspring's immunity can be achieved by exposing the breeder to disease-associated pathogens through vaccination of attenuated live vaccines to enhance transmission of immunity via antibodies in the egg yolk (Hassan and Curtis, 1996).

Epigenetics

New advances in epigenetics have led us to better understand the mechanism by which maternal nutrition and management can impact progeny development. Epigenetics is an important regulatory mechanism of gene expression independent of the DNA sequence. It can be viewed as a life-course strategy for the offspring to help it meet the demands of the predicted environment in later life (Dixon *et al.*, 2016). Changing this life course is possible through epigenetics. DNA methylation, which causes DNA-histone compaction and represses gene expression (Figure 1A), and acetylation of lysine 9 on histone protein 3 (H3K9), which causes DNA-histone relaxation and activates gene expression (Figure 1B, are two common ways of epigenetic modification (Moresi *et al.*, 2015).

Epigenetics and immunity of the offspring with Zn supplementation

Recent studies have focused on understanding the mechanisms by which maternal nutrition could impact the health of progeny via epigenetic modification(s) (Berghof *et al.,* 2013). In chickens, the adaptive immune system is functionally immature and newly hatched birds are dependent on maternal antibodies transferred through the egg yolk. In addition, the intestine is

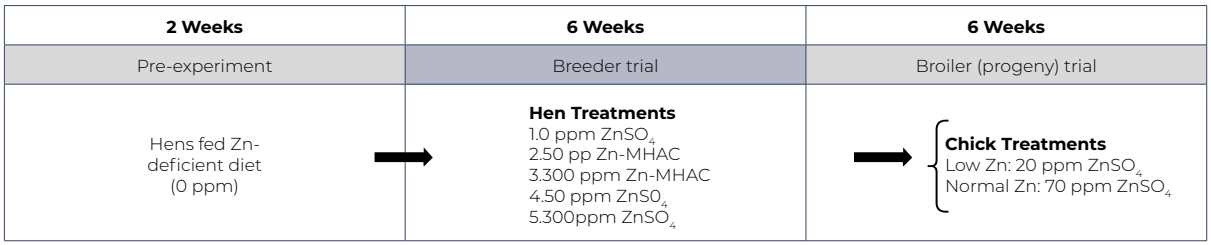

Figure 2. Diagram of experimental design. (Adapted from Li *et al.*, 2015)

the largest immune system of the body that plays a major role in resistance to infection. However, the functional development of gut-associated lymphoid tissues in neonatal chickens is slow and the functional maturation occurs in the second week of life (Bar-Shira and Friedman, 2006). Zinc (Zn) has been associated with different aspects of the immune system and maternal Zn can improve the development of the immune system of the offspring. Kidd *et al.* (1992, 1993) showed supplementation of organic Zn in the breeder diet increased embryonic bone weight and enhanced cellular immune response and primary antibody titers to Salmonella pullorum antigen. Virden *et al.* (2003) fed breeders with a combination of Zn and Mn in organic and inorganic forms and found improvements in progeny livability with higher response from the organic form. More recent work has focused on the mechanisms by which maternal Zn supplementation modulates the immune system of the offspring. Zhang *et al.* (2012) documented how Zn prevented Salmonella enteric-induced loss of intestinal mucosal barrier function by increasing expression of the main proteins responsible for maintaining gut integrity, including occludin and claudin-1. Zhu *et al.* (2017) showed

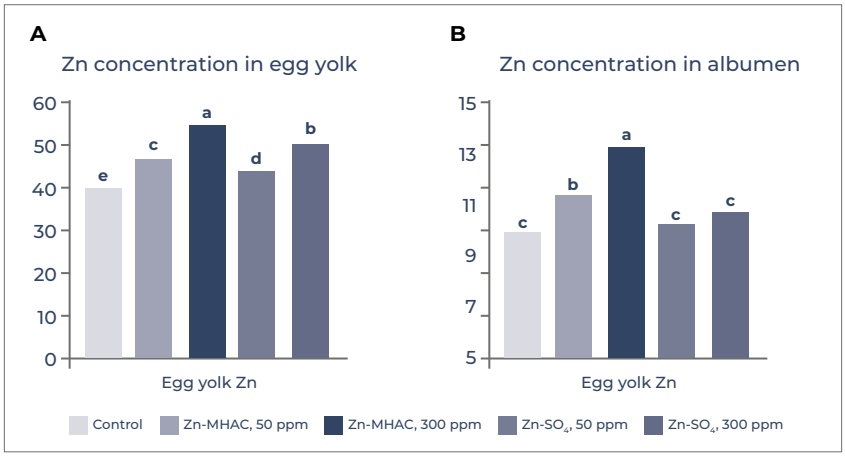

Figure 3. Zn concentration in egg yolk and albumen in breeder hens fed the same basal diets supplemented with 0, 50, 300 ppm ZnSO4 or 50, 300 ppm Zn-MHAC. Treatments with different letters are significantly different ($P < 0.05$). (Adapted from Li *et al.,* 2015)

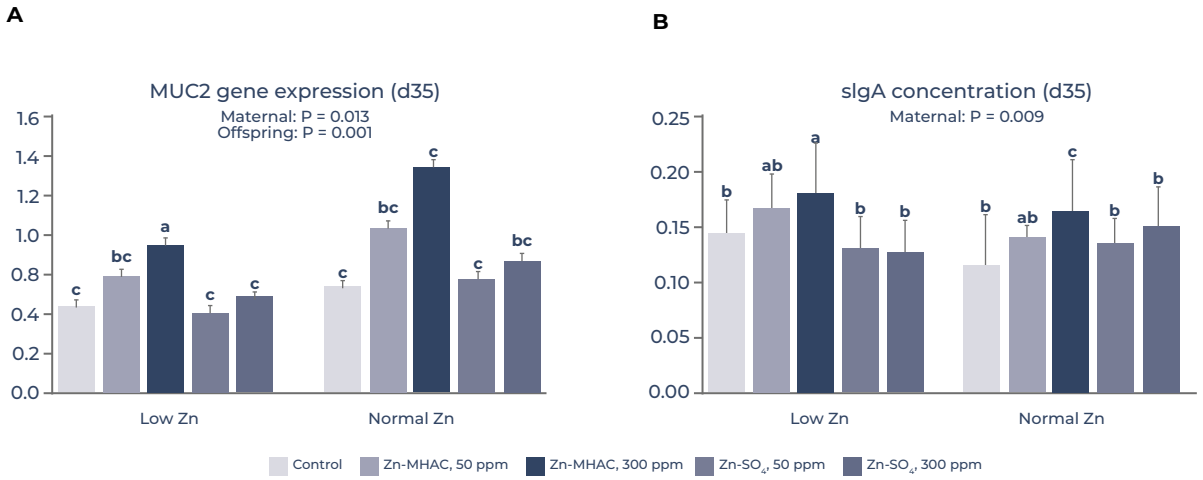

Figure 4. Jejunal secretory IgA (sIgA) production and mucosal MUC2 gene expression in offspring chickens at 35 d of age hatched from breeder hens supplemented with 0, 50, 300 ppm ZnSO4 or 50, 300 ppm Zn-MHAC. Treatments with different letters were significantly different (P < 0.05). (Adapted from Li *et al.,* 2015)

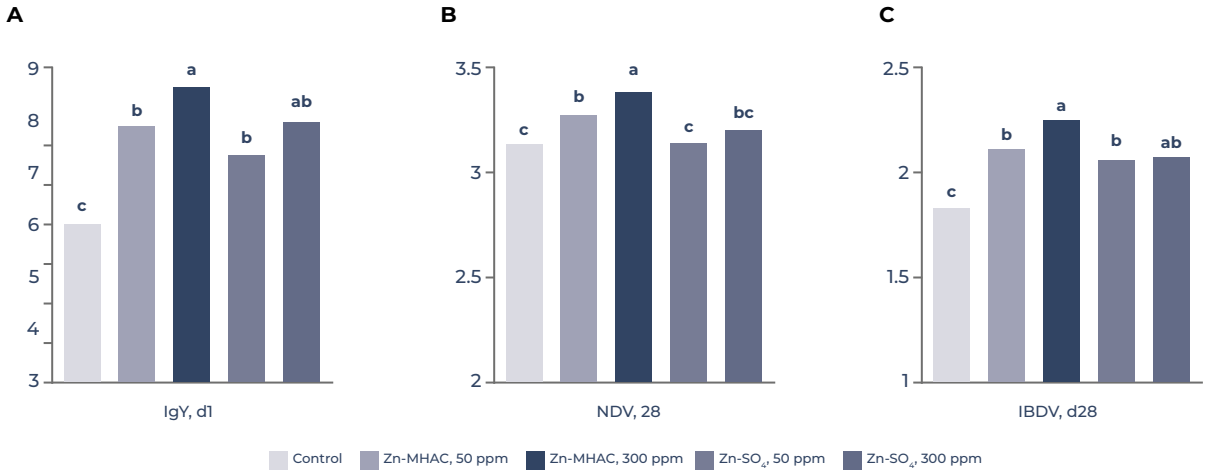

Figure 5. Concentration of IgY in offspring chicks at 1 d of age, NDV and IBDV in offspring chickens at 28 d of age hatched from breeder hens fed the same basal diets supplemented with 0, 50, 300 ppm ZnSO4 or 50, 300 ppm Zn-MHAC. Treatments with different letters were significantly different (P < 0.05). (Adapted from Li *et al.,* 2015)

that maternal dietary Zn supplementation, regardless of inorganic or organic source, enhanced the antioxidant ability of chick embryos from maternal normal and high temperatures. This was reflected in increased mRNA and protein expressions of metallothionein IV in the embryonic liver by reducing DNA methylation and increasing H3K9 acetylation of the metallothionein IV promoter. Li *et al.* (2015) evaluated the role of maternal Zn on intestinal immunity of the offspring and the underlying epigenetic mechanisms in broiler chickens. In this study, broiler breeder hens (45 weeks old) were

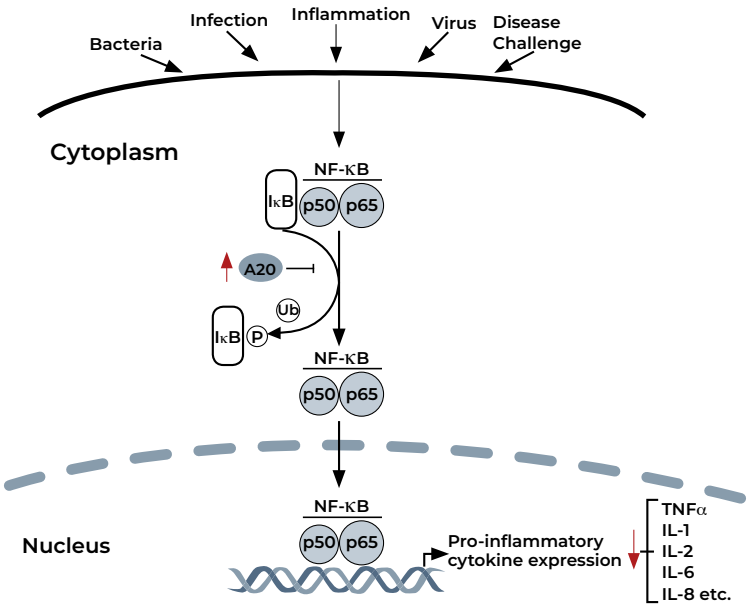

Figure 6. NF-kB signaling pathway in intestinal inflammation. (Modified from Neurath *et al.*, 1998)

first provided a Zn-deficient diet (0 ppm supplemental Zn) for 2 weeks to exhaust maternal Zn and then fed commercial (50 ppm) and high (300 ppm) levels of two sources of Zn, chelated (Zn methionine hydroxy analogue chelate, MHAC-Zn) and inorganic (ZnSO4); or 0 ppm supplemental Zn for 6 weeks (Figure 2). The F1 progeny chicks from those hens were fed either low (20 ppm) or normal (70 ppm) levels of inorganic Zn for 6 weeks.

Zn content in the egg yolk and albumen gradually increased with supplementation and was higher in the yolk than albumen in the chelated Zn treatments, suggesting more delivery of Zn to the progeny by the chelated Zn (Figure 3). Zn must first be stored in the yolk before it can be delivered to the embryo. The hen delivers Zn to the yolk via the vitellogenin transport protein, where it is stored and bound to lipovitellin and phosphovitin. The stored Zn is transferred to the yolk sac for temporary storage before it´s delivered to the liver of the embryo where it is distributed to the rest of the organs when needed via metallothionine protein (Richards, 1997). The levels of Zn in the yolk and yolk sac are gradually depleted during the incubation period as they are transferred to the embryo (Richards, 1997).

Zinc and modulation of intestinal epithelium immunity

Li *et al.* (2015) investigated several aspects of maternal Zn supplementation on gut immunity in offspring broilers. The authors measured jejunal secretory IgA (sIgA) production as the first line of defense in protecting the intestinal epithelium from enteric pathogens, jejunal mucin 2 (MUC2) gene expression as the main intestinal mucin of the mucus layer that protects the gut epithelial cells, and serum immunoglobulin

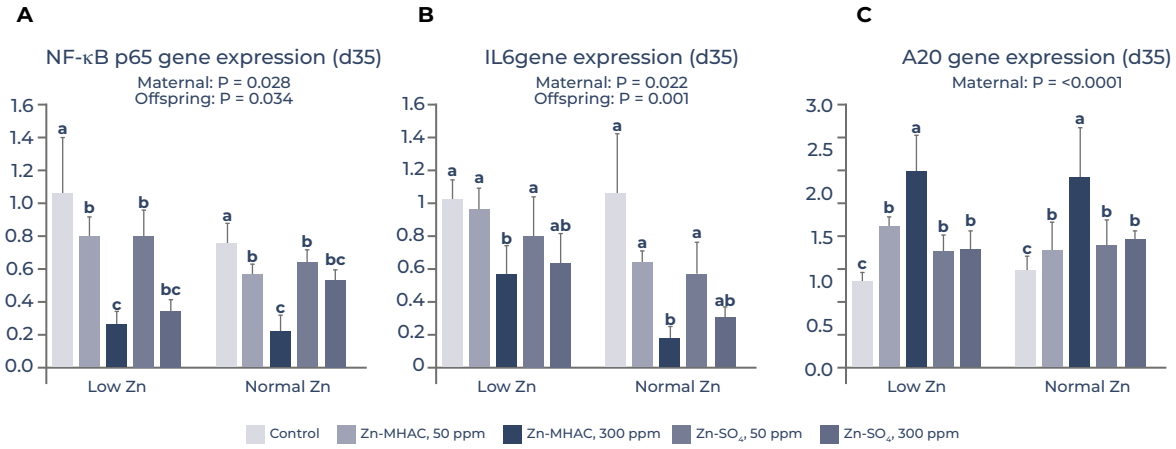

Figure 7. Zn supplementation in broiler breeder hens and offspring chickens increased A20 gene expression and reduced NF-κB and IL6 gene expression in the jejunum of offspring chickens at 35 d of age hatched from breeder hens supplemented with 0, 50, 300 ppm ZnSO4 or 50, 300 ppm Zn-MHAC. Treatments with different letters were significantly different (P < 0.05). (Adapted from Li *et al.,* 2015)

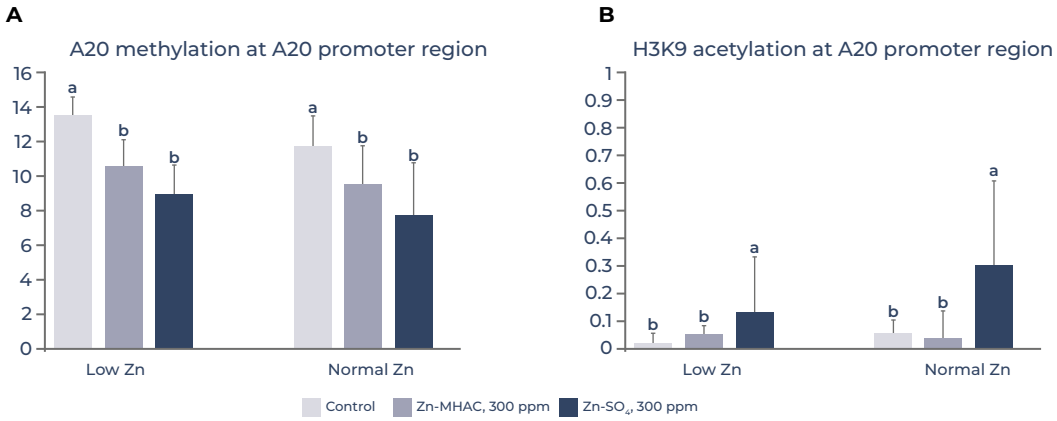

Figure 8. Zn supplementation in broiler breeder hens and offspring birds reduced DNA methylation at A20 promoter region and increased H3K9 acetylation at A20 promoter region in the jejunum of offspring birds at 35 d of age hatched from breeder hens supplemented with 0, 300 ppm ZnSO4 or 300 ppm Zn-MHAC. Treatments with different letters were significantly different (P < 0.05). (Adapted from *Li et al.*, 2015)

Y (IgY) and antibody titers responses against Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV). Compared to Zn-deficient or inorganic Zn treatments, chelated Zn supplementation in breeder hens 1) enhanced mucosal immunity by increasing sIgA production in progeny chickens at 35 days of age fed either a low or normal Zn diet (Figure 4A); 2) improved gut barrier function by increasing MUC2 gene expression (Figure 4B); and 3) enhanced systemic immunity in progeny chicks by increasing concentration of IgY in the offspring at 1 d of age (Figure 5A), and antibody titer of NDV (Figure 5B) and IBDV (Figure 5C) in offspring chickens at 28 d of age. These results suggest that Zn

supplementation in the breeder diet improves gut barrier function, mucosal immunity and systemic immunity of progeny chicks and that Zn in the chelated form is more effective than in an inorganic form.

Zinc and modulation of inflammatory signaling pathway via epigenetics

Li *et al.* (2015) also studied the underlying molecular epigenetic mechanism by which maternal Zn modulates gut immunity of the progeny. Evidence in human literature postulated that Zn suppresses inflammation via induction of A20-mediated inhibition of nuclear factor-κB (NF-κB) signaling pathway (Prasad *et al.*, 2011). Zn finger protein A20 can negatively regulate the inflammatory response by deubiquiting ubiquitin-dependent factors of NF-κB signaling cascades (Ma and Malynn, 2012; Catrysse *et al.*, 2014). NF-κB signaling pathway is one of the major pathways to regulate inflammation induced by external stimuli such as bacteria, infection, and inflammation (Figure 6, modified from Neurath *et al.*, 1998). A20 blocks the phosphorylation and activation of NF-κB, thereby inhibiting translocation of NF-κB and suppressing inflammation cascade. Therefore, up-regulation of A20 would down-regulate NF-κB pathway and reduce inflammation (Figure 6).

Li and colleagues (2015) measured the expression of A20, NF-κB and IL6 genes, DNA methylation and histone acetylation at A20 promoter region. Chelated Zn supplementation to breeder hens showed a greater effect than $ZnSO_4$ in 1) reducing gene expression of NF-κB p65 (Figure 7A) as well as its downstream inflammatory cytokines such as IL6 (Figure 7B); and 2) increasing A20 gene expression in the jejunum of progeny (Figure 7C). As A20 negatively regulates NF-κB pathway, increase of A20 is consistent with reduction of NF-κB p65 gene expression. These results suggest that Zn supplementation in breeder diets reduced gut inflammation in progeny birds by up-regulation of A20 expression.

To determine the molecular mechanism by which Zn regulates A20 gene expression, DNA methylation and histone H3K9 acetylation at A20 promoter region was measured in the jejunum of progeny birds at 35 d of age. Both chelated Zn and $ZnSO_4$ significantly reduced DNA methylation at A20 promoter (Figure 8A). Chelated Zn but not $ZnSO_4$ significantly increased histone H3K9 acetylation at A20 promoter (Figure 8B). Because methylation represses gene expression and histone acetylation activates gene expression, lower DNA methylation and greater histone H3K9 acetylation at A20 promoter region suggests that breeder Zn supplementation activates A20 gene expression. This is consistent with the increase of A20 mRNA levels shown in Figure 7C.

In summary, Zn supplementation in breeder hens improved gut barrier function, mucosal immunity and systemic immunity, and reduced gut inflammation of progeny chicks by epigenetic modification of A20 promoter. The higher levels of Zn tested as a chelated source appeared to have the most consistent effect in epigenetic modulation of A20 promoter.

Conclusions

Based on new research, the impact of breeder nutrition, management, and environment on progeny development becomes more evident. The nutrition of breeder hens provide a tool to prepare the immune system of the chicken post-hatching, especially under an antibiotic-free environment. Zn supplementation in the chelated form in breeder hen diets is an example of how maternal nutrition can modulate several aspects of the immune system of the progeny to help the chick to overcome pathogenic insults. The mechanism by which Zn improves the immune system of the chick has been elucidated thanks to the new developments in epigenetics. Maternal Zn transferred to the embryo epigenetically modulates A20 promoter, which upregulates A20 gene expression thereby down-regulating NF-kB gene expression, hence reducing gut inflammation, and improving gut barrier function, mucosal immunity, and systemic immunity of progeny chicks. More work in this area would allow for breeder hen nutrition to be used to further prepare the chick for optimal growth performance and meat yield.

References

Bar-Shira, E. and A. Friedman. 2006. A development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Developmental and Comparative Immunology 30: 930-941.

Berghof, T.V., Parmentier, H.K. and A., Lammers. 2013. Transgenerational epigenetic effects on innate immunity in broilers: An underestimated field to be explored? Poultry Science 92: 2904-2913.

Brake, J., Jenfestey, B.A. and P.W. Plumstead. 2003. Broiler performance to 21 days as affected by cumulative broiler breeder pullet nutrition during rearing. Poultry Science 14: 81-85.

Calini, F. and F. Sirri. 2007. Breeder nutrition and offspring performance. Brazilian Journal of Poultry Science 9: 77-83.

Catrysse, L., Vereecke, L., Beyaert, R. and G. van Loo. 2014. A20 in inflammation and autoimmunity. Trends in Immunology 35: 22-31.

Dixon, L.M., Sparks, N.H. and K.M. Rutherford 2016. Early experiences matter: a review of the effects of prenatal environment on offspring characteristics in poultry. Poultry Science 95: 489-499.

Hassan J. O. and R. Curtiss. 1996. Effect of vaccination of hens with an avirulent strain of salmonella typhimurium on immunity of progeny challenged with wild-type salmonella strains. Infection and Immunity 64: 938-944

Kidd, M.T., Anthony, N.B. and S.R. LEE,. 1992. Progeny performance when dams and chicks are fed supplemental zinc. Poultry Science 71: 1201-1206.

Kidd M.T., Anthony, N.B., Newberry, L.A. and S.R. Lee. 1993. Effect of supplemental zinc in either a corn-soybean or a milo and corn-soybean meal diet on the performance of young broiler breeders and their progeny. Poultry Science 72: 1492-1499.

Kidd M.T. 2003. A treatise on chicken dam nutrition that impacts on Progeny. World's Poultry Science Journal 59: 475-494

Kidd, M.T., McDaniel, C.D., Peebles, E.D., Barber, S.J., Corzo, A., Branton, S.L., Woodworth, J.C. 2005. Breeder hen dietary L-carnitine affects progeny carcase traits. British Poultry Science 46: 97-103.

Leeson, S. and J.D. Summers. 2001. Nutrition of the Chicken University Books, Guelph, Ontario, Canada.

Li., C, Guo, S., Gao, J., Guo, Y., Du, E., Lv, Z. and B. Zhang. 2015. Maternal high-zinc diet attenuates intestinal inflammation by reducing DNA

methylation and elevating H3K9 acetlylation in the A20 promoter of offspring chicks. Journal of Nutritional Biochemistry 26: 173-183

Ma, A. and B.A. Malynn. 2012. A20: linking a complex regulator of ubiquitylation to immunity and human disease. Nature Reviews Immunology 12:774-785.

Moresi, V., Marroncelli, N., Coletti, D. and S. Adamo. 2015. Regulation of skeletal muscle development and homeostasis by gene imprinting, histone acetylation and microRNA. Biochimica et Biophysica Acta 1849: 309-316.

Neurath, M.F., Becker, C. and K. Barbulescu. 1998. Role of NF-kappaB in immune and inflammatory responses in the gut. Gut. 43: 856-860

Peebles, E.D., Doyle, S.M., Pansky, T., Gerard, P.D., Latour, M.A., Boyle, C.R. and T.W. Smith.1999. Effects of breeder age and dietary fat on subsequent broiler performance. 2. Poultry Science 78: 512-515.

Peebles, E.D., Zumwalt, C.D., Gerard, P.D., Latour, M.A. and T.W. Smith. 2002. Market age live weight, carcass yield and liver characteristics of broiler offspring from breeder hens fed diets differing in fat and energy contents. Poultry Science 81: 23-29.

Peeble, E.D., Zumwalt, C.D., Smith, T.W., Gerard, P.D. and M.A. Latour. 2002b. Poultry fat and corn oil may be used to adjust energy in the diets of young breeder hens without affecting embryogenesis and subsequent broiler growout performance. Journal of Applied Poultry Research 11: 146-154.

Plaimast, H., Kijparkorn S. and P. Ittitanawong. 2015. Effects of Vitamin D3 and Calcium on Productive Performance, Egg quality and Vitamin D3 Content in Egg of Second Production Cycle Hens. The Thai Journal of Veterinary Medicine. 45: 189-195.

Prasad, A. S, Bao, B., Beck, F.W. and F.H. Sarkar. 2011. Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-kappaB. Nutrition 27: 816-823

Richards, M.P. 1997. Trace mineral metabolism in the avian embryo. Poultry Science 76:152-164

Saunders-Blades, J.L. and D.R. Korver. 2015. Effect of hen age and maternal vitamin D source on performance, hatchability, bone mineral density, and progeny in vitro early innate immune function. Poultry Science 94: 1233-1246.

Surai, P.F. 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. British Poultry Science 41: 235-243.

Triyuwanta, C.L., Leterrier, C. and Y. Nys. 1992. Dietary phosphorus and food allowance of dwarf breeders affect reproductive performance of hens and bone development of their progeny. British Poultry Science 33: 363-379.

Virden, W.S., Yeatman, J.B., Barber, S.J., Zumwalt, C.D., Ward, T.L., Johnson, A.B. and M.T. Kidd. 2003. Hen mineral nutrition impacts progeny livability. Journal Applied Poultry Research 12: 411-416.

Zhang, B., Shao, Y., Liu, D., Yin, P., Guo, Y. and J. Yuan. 2012. Zinc prevents Salmonella enterica serovar typhimurium-induced loss of intestinal mucosal barrier function in broiler chickens. Avian Pathology 41: 361-367.

Zhu, Y., Liao, X., Lu, L., Li, W., Zhang, L., Ji, C., Lin, X., Liu, H.C., Odle, J. and X. Luo. 2017. Maternal dietary zinc supplementation enhances the epigenetic-activated antioxidant ability of chick embryos from maternal normal and high temperatures. Oncotarget 8: 19814-19824.