

Harnessing epigenetics to improve chick quality with trace mineral zinc

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.

by **Juxing Chen, Nestlé Purina Petcare, and Mercedes Vázquez-Añón, Novus International, Inc.**
www.novusint.com

These low-cost periods provide opportunities to optimise 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) summarised 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 – maximising 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

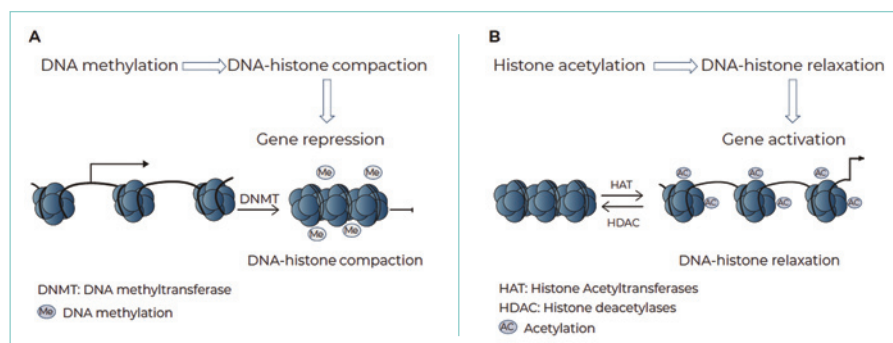


Fig. 1. Diagram of DNA methylation (A) and histone H3K9 acetylation (B) regulating gene expression. (Modified from Moresi et al., 2015).

maternal nutrition. One is growth performance and carcass yield, and the other is the health and immunity of the progeny. 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. Optimising 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. 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 utilisation 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. 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.

As an example, maternal supplementation of 25-hydroxy vitamin D3 increased hatchability and in vitro chick innate immunity towards E. coli. Adequate levels of calcium (Ca) and phosphorus (P) were reported to improve skeleton ossification of the embryo and progeny at hatch, and the addition of vitamin D improved calcification of the eggshell. Supplementation of Zn has been shown to improve humoral and cellular immunity and to enhance the progeny response to pathogenic challenges.

The combination of Zn and Mn improved the livability of the progeny.

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

| Two weeks | Six weeks | Six weeks |
|-----------------------------------|--|--|
| Pre-experiment | Breeder trial | Broiler (progeny) trial |
| Hens fed Zn-deficient diet (0ppm) | Hen treatments: 0, 50, and 300ppm ZnSO ₄ 50 and 300ppm ZnSO ₄ and Zn-MHAC | Chick treatments: Low Zn: 20ppm ZnSO ₄ Normal Zn: 70ppm ZnSO ₄ |

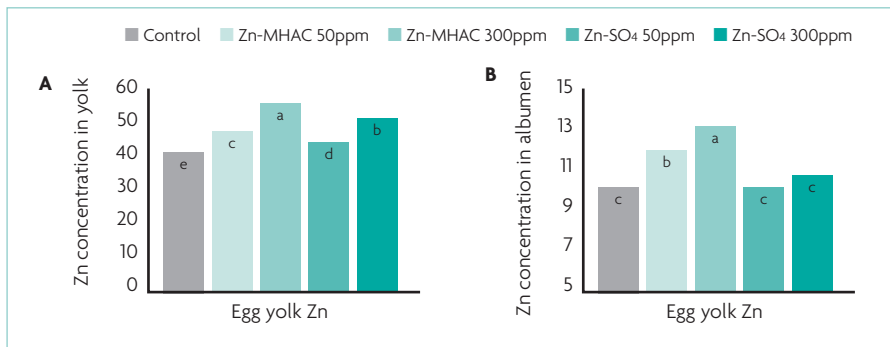


Fig. 3. Zn concentration in egg yolk and albumen in breeder hens fed the same basal diets supplemented with 0, 50, 300ppm ZnSO₄ or 50, 300ppm Zn-MHAC. Treatments with different letters are significantly different. (P<0.05). (Adapted from Li et al., 2015).

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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 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.

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. Changing this life course is possible through epigenetics. DNA methylation, which causes DNA histone compaction and represses gene expression (Fig. 1A), and acetylation of lysine 9 on histone protein 3 (H3K9), which causes DNA-histone relaxation and activates gene expression (Fig. 1B, are two common ways of epigenetic modification).

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). 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 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.

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.

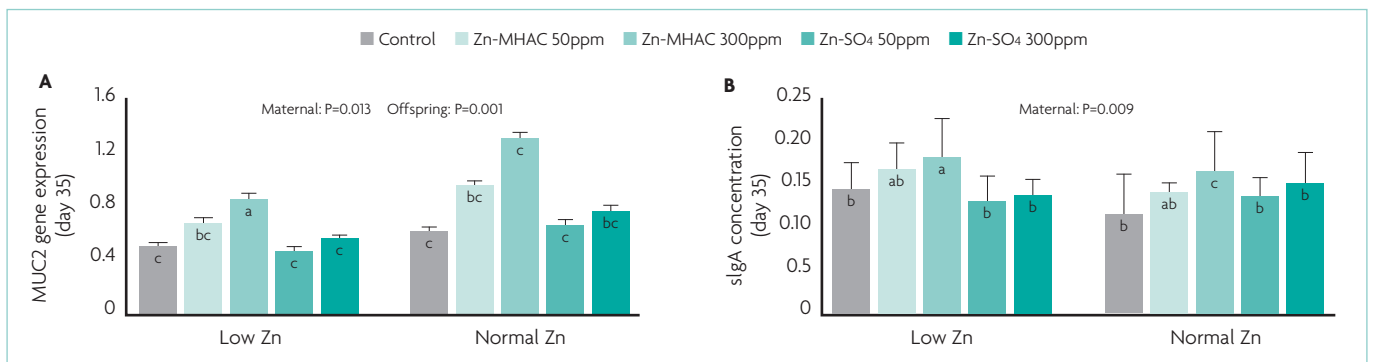
Zhu et al. (2017) showed that maternal dietary Zn supplementation, regardless of inorganic or organic source, enhanced the antioxidant ability of chick embryos from maternal normal and high temperatures.

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 first provided a Zn-deficient diet (0ppm supplemental Zn) for two weeks to exhaust maternal Zn and then fed commercial (50ppm) and high (300ppm) levels of two sources of Zn, chelated (Zn methionine hydroxy analogue chelate, MHAC-Zn) and inorganic (ZnSO₄) or 0ppm supplemental Zn for six weeks (Fig. 2).

The F1 progeny chicks from those hens were fed either low (20ppm) or normal (70ppm) levels of inorganic Zn for six weeks. Zn content in the egg yolk and albumen gradually increased with supplementation and was higher in the yolk than albumen in

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



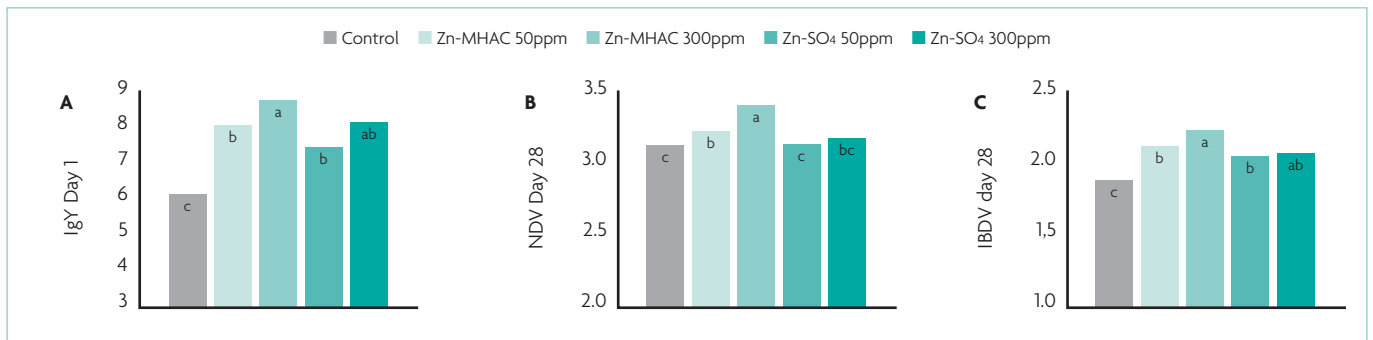


Fig. 5. Concentration of IgY in offspring chicks at 1 day of age, NDV and IBDV in offspring chickens at 28 days of age hatched from breeder hens fed the same basal diets supplemented with 0, 50, 300ppm ZnSO₄ or 50, 300ppm Zn-MHAC. Treatments with different letters were significantly different (P<0.05).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

the chelated Zn treatments, suggesting more delivery of Zn to the progeny by the chelated Zn (Fig. 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 is delivered to the liver of the embryo where it is distributed to the rest of the organs when needed via metallothionein protein.

The levels of Zn in the yolk and yolk sac are gradually depleted during the incubation period as they are transferred to the embryo.

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 defence 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 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 (Fig. 4A); 2) improved gut barrier function by increasing MUC2 gene expression (Fig. 4B); and 3) enhanced systemic immunity in progeny chicks by increasing concentration of IgY in the offspring at 1 day of age (Fig. 5A), and antibody titer of NDV (Fig. 5B) and IBDV (Fig. 5C) in offspring chickens at 28 days 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 signalling pathway

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- κ B (NF- κ B) signalling pathway.

Zn finger protein A20 can negatively regulate the inflammatory response by deubiquitinating ubiquitin-dependent factors of NF- κ B signalling cascades. NF- κ B signalling pathway is one of the major pathways to regulate inflammation induced by external stimuli such as bacteria, infection and inflammation (Fig. 6, modified from Neurath et al., 1998).

A20 blocks the phosphorylation and activation of NF- κ B, thereby inhibiting translocation of NF- κ B and suppressing inflammation cascade. Therefore, up-

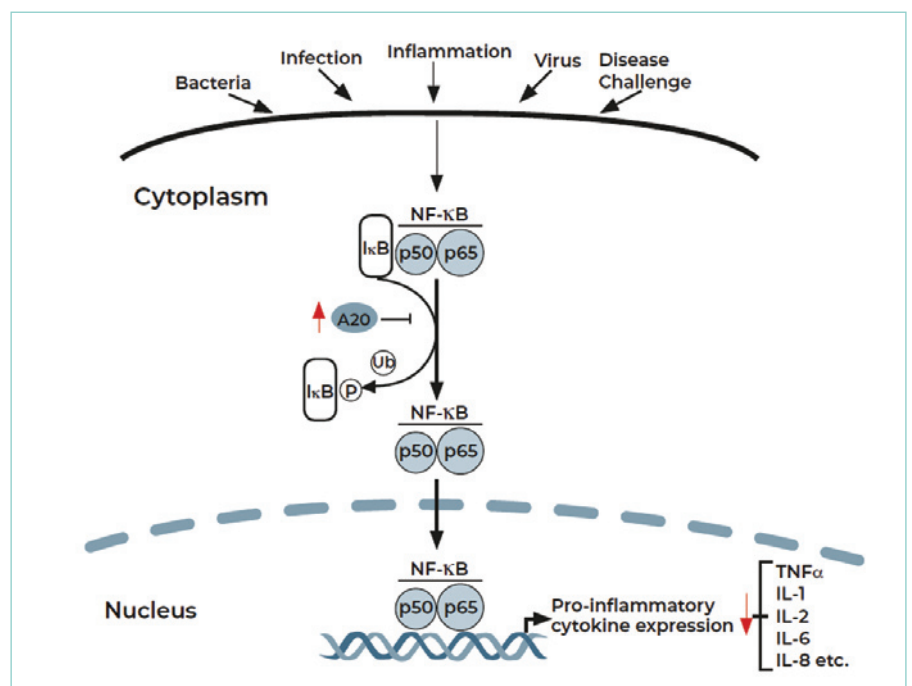
regulation of A20 would down-regulate NF- κ B pathway and reduce inflammation (Fig. 6). Li and colleagues (2015) measured the expression of A20, NF- κ B and IL6 genes, DNA methylation and histone acetylation at A20 promoter region.

Chelated Zn supplementation to breeder hens showed a greater effect than ZnSO₄ in 1) reducing gene expression of NF- κ B p65 (Fig. 7A) as well as its downstream inflammatory cytokines such as IL6 (Fig. 7B); and 2) increasing A20 gene expression in the jejunum of progeny (Fig. 7C). As A20 negatively regulates NF- κ B pathway, increase of A20 is consistent with reduction of NF- κ B 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

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Fig. 6. NF- κ B signalling pathway in intestinal inflammation. (Modified from Neurath et al., 1998).



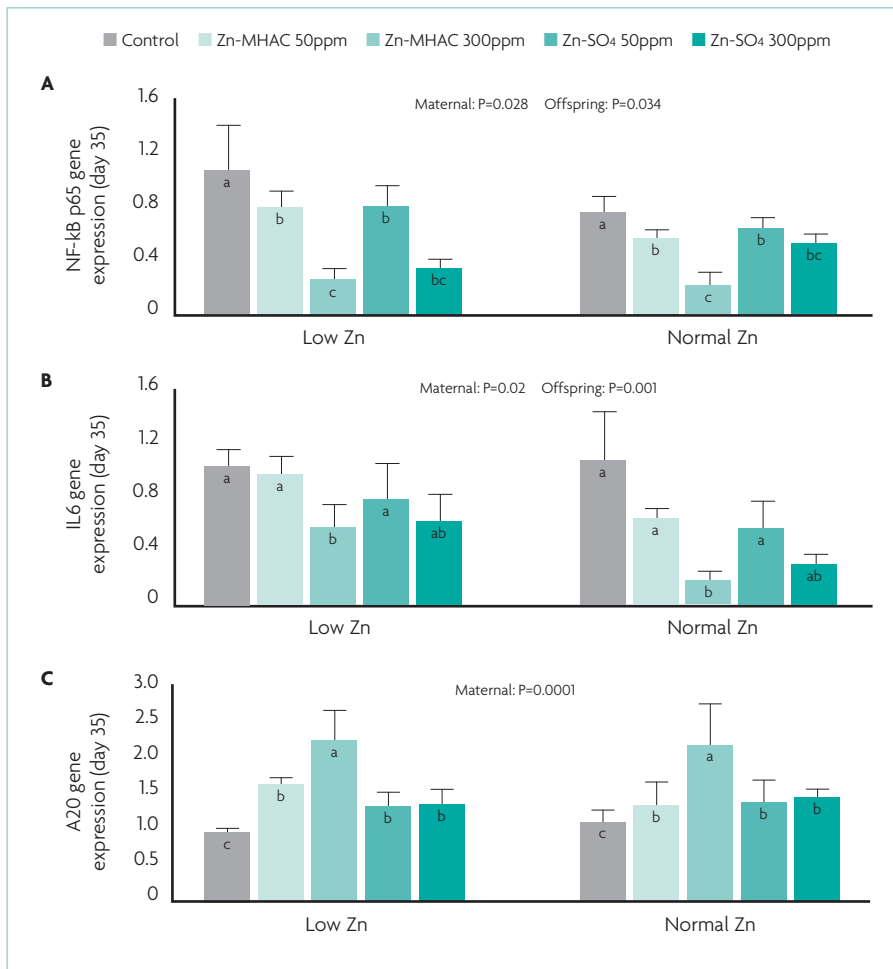


Fig. 7. Zn supplementation in broiler breeder hens and offspring chickens increased A20 gene expression and reduced NF- B and IL6 gene expression in the jejunum of offspring chickens at 35 days of age hatched from breeder hens supplemented with 0, 50, 300ppm ZnSO₄ or 50, 300ppm Zn-MHAC. Treatments with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

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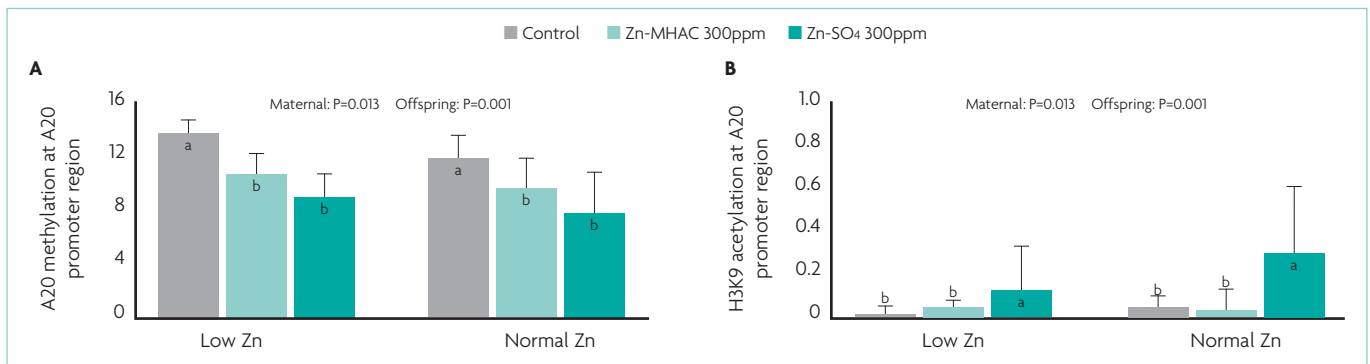
methylation and histone H3K9 acetylation at A20 promoter region was measured in the jejunum of progeny birds at 35 days of age.

Both chelated Zn and ZnSO₄ significantly reduced DNA methylation at A20 promoter (Fig. 8A). Chelated Zn but not ZnSO₄ significantly increased histone H3K9

acetylation at A20 promoter (Fig. 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

Fig. 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 days of age hatched from breeder hens supplemented with 0, 300ppm ZnSO₄ or 300ppm Zn-MHAC. Treatments with different letters were significantly different (P<0.05). (Adapted from Li et al., 2015).



increase of A20 mRNA levels shown in Fig. 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-κB 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 are available from the author on request
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