Review

Transgenerational epigenetic effects on innate immunity in broilers: An underestimated field to be explored?

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ABSTRACT Transgenerational epigenetics is becoming more and more important for understanding the variation of physiological responses of individuals to the environment and the inheritance of these responses based on all mechanisms other than the actual DNA nucleotide sequence. Transgenerational epigenetics is the phenomenon that the information of the environment of (usually) a female animal is translated into memory-like responses preparing the offspring. As a consequence, individuals of the next generation may show different phenotypic traits depending whether their mothers were kept under different environmental conditions. This may result in either positive or negative effects on the next-generation individuals, which is different from individuals from mothers that have been kept in a different environment. Transgenerational epigenetic effects have been proposed and indicated for specific immune (T cell and antibody) responses (especially in mammals, but also in birds) and innate immunity (nonvertebrates), but surprisingly very little is known of transgenerational effects on innate immunity in chickens. Given the short lifespan of the chicken and therefore the likely dependence of chicken on innate immune mechanisms, more attention should be given to this arm of immunity and mechanisms of inheritance including transgenerational effects that can be initiated in the breeder generation. In addition, it is becoming evident that innate immunity also underlies metabolic disorders in broilers. In the current paper, we will argue that although very little is known of transgenerational effects of innate immunity in poultry, more attention should be given to this type of study. We will illustrate examples of transgenerational epigenetics, and finally propose strategies that should reveal the presence of transgenerational epigenetic effects on innate immunity in chickens and strategies to modulate breeder birds such that these effects positively affect innate immunity of broilers. It is suggested that a mismatch between breeder environment and broiler environment may account for unwanted effects of innate immunity in the broiler.

Key words: broiler, broiler breeder, transgenerational epigenetic effect, innate immunity, microbiota

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INTRODUCTION

Currently the broiler production sector is facing various health challenges including immune-related disorders (Knowles et al., 2009) and enhanced disease susceptibility (Huff et al., 2002). As a consequence of prolonged selection for enhanced broiler growth through broiler breeding programs, broilers face reduced immune functioning (Leshchinsky and Klasing, 2001), and likely, in combination with the type of housing, this may underlie the high prevalence of infectious diseases and consequently the frequent use of antibiotics. On the other hand it is feasible to breed broilers for enhanced innate immune reactivity that may combat infection (Swaggerty et al., 2009).

Transgenerational epigenesis is a promising area of interest. However, in poultry, transgenerational effects on (especially innate) immunity have not been explicitly investigated in depth before. Here we investigated literature for evidence of these effects and whether these effects have potential to maintain or improve health of broilers.

In this literature study, we address the following 4 topics. First, we define epigenesis and transgenerational epigenesis. Second, we summarize literature on transgenerational epigenetic mechanisms, with emphasis on immune responses. Third, we illustrate transgenerational epigenetic phenomena. Finally, we propose types of studies that could be performed to 1) identify transgenerational effects on innate immunity and 2) modulate the parental (especially mother) birds such that

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transgenerational epigenetic effects could enhance the health status of broilers. We speculate that a mismatch between housing and feeding management of the broiler breeder and that of the broiler may underlie health problems of the broiler based on misdirected transgenerational epigenetic information. This literature study will provide a definition of transgenerational epigenetic effects and transfer mechanisms, including an overview of reported examples. Based on these findings, we propose future directions, with a focus on innate immunity.

Avian Innate Immune System and Its Importance

Roughly 98% of all multicellular organisms possesses an innate immune system (Kogut, 2009). It is hypothesized that chickens use the innate immune system to a greater degree than the adaptive immune system based on specific T cells and specific antibodies (Lee, 2006; Klasing, 2007). In addition, the short life of most broilers harvested for food (approximately 6 wk) limits the time available for full adaptive immune system development. This underscores the importance of the innate immune system in broilers.

The 3 hallmarks of the innate immune system are: rapid action, limited duration, and limited specificity (Klasing, 2007; Kogut, 2009). The innate immune system consists of various cell types: monocytes/macrophages, natural killer (**NK**) cells, heterophils, and various soluble components such as natural antibodies (**NAb**), defensins, and various complement cascades (He et al., 2003; Parmentier et al., 2004; Bar-Shira and Friedman, 2006; Rogers et al., 2008; Nerren and Kogut, 2009; Sjöberg et al., 2009). Although there are many cells involved in innate immunity of vertebrates, they can all be functionally triggered by microbe-associated molecular patterns (**MAMP**). Microbe-associated molecular patterns are life-cycle essential (and thus conserved) structures of microbes. These structures are recognized by pattern recognition receptors, which are expressed by all (innate) immune cells. This enables the host to respond quickly to a wide range of microbes/pathogens (Kumar et al., 2011).

The chicken's innate immune system is already present at hatch, but is not fully functional yet. For example, heterophils become only fully functional after 2 to 3 wk, but are also suggested to play an important protective role in the neonate (Friedman and Bar-Shira, 2005; Swaggerty et al., 2009). The NK cells were suggested to be important in mucosal immunity where they are almost solely located (Rogers et al., 2008).

During the development of the innate immune system, and the functional absence of a specific immune system, maternal antibodies (**mAb**) transferred via yolk and albumen are present to protect the hatchling (Klipper et al., 2004; Friedman et al., 2012). After 2 wk of life, mAb are no longer detectable in significant levels (Grindstaff et al., 2003) and adaptive immunity by the chick itself is being developed (Bar-Shira et al., 2003). However, it takes at least 3 to 4 additional weeks for the adaptive immune system to become fully functional (Lammers et al., 2010). Therefore it is believed that during the first 6 wk of life the chicken mainly relies on its innate immune system and mAb, although currently applied vaccination strategies in ovo and in the first week of life indicate that protective adaptive responses can be induced. Additionally, direct and indirect influences of mAb on the development and functionality of the innate immune system (immunological imprinting) in chicken are suggested (Klipper et al., 2004), as is also proposed previously for mammals (Lemke et al., 2004). These aspects are further reviewed by Hasselquist and Nilsson (2009).

One of the key stimulators of immune and intestinal development in the chick is physical exposure to feed (Noy et al., 2001; Friedman and Bar-Shira, 2005). Feed withholding (up to 72 h) resulted in delayed development of several physiological and immune parameters. Feed-withheld chickens needed at least 2 wk for full immunological recovery compared with chickens that were not feed-withheld (Bar-Shira et al., 2005).

Gut Microbiota

Immediately posthatch, a complex intestinal bacterial population will develop (Bar-Shira et al., 2003). This intestinal microbiota is derived from the feed and the environment. As these bacteria produce hundreds of proteins that contribute to host physiology, but that cannot be produced by the host itself (Costello et al., 2009), the host and its microbiota are regarded as a superorganism in which energy and metabolites can be exchanged and homeostasis is maintained by the immune system (Cerf-Bensussan and Gaboriau-Routhiau, 2010). This interplay between intestinal microbiota, (innate) immunity, health, and production has been investigated extensively. For instance, the cecal microbiota composition was associated with feed efficiency expressed by the feed conversion ratio (Dibner et al., 2008; Torok et al., 2008; Stanley et al., 2012). Next to this, the gut microbiota plays an important role in preventing colonization of pathogenic bacteria by nutrient and space competition and by secretion of antimicrobials (Barnes, 1979).

In the first 2 wk of life, enterococci and lactobacilli are the dominant species in all segments of the broiler gastrointestinal tract (van der Wielen et al., 2000; Snel et al., 2002). Coliforms are also present in high numbers in the ceca (van der Wielen et al., 2000). In the subsequent 3 to 4 wk of age, the microbial composition in the chickens intestines is unstable and fluctuating (den Hartog, 2013). This unstable period is accompanied with signs of mild inflammation. Multivariate analyses revealed that the intestinal microbiota composition correlated with mRNA levels of several pro-inflammatory cytokine genes. These data strongly suggest interplay

between microbiota composition and activity of the innate immune system. In the adult chicken, a more or less stable composition is established, where lactobacilli are mainly present in the crop, duodenum, and ileum. Many unspecified obligate anaerobic bacteria are found in the ceca (Apajalahti, 2005; Gabriel et al., 2006).

The gut microbiota likely plays an essential role in the ontogeny of the vertebrate immune system (Macpherson and Harris, 2004), and also gut development was found to be highly associated with the presence and the composition of the microbiota (Maisonnier et al., 2003). For instance, development of the (adaptive) immune system and growth of the chicken hindgut (with high loads of microbes) was more sensitive to feed deprivation than the development of the foregut immune components (Bar-Shira et al., 2003, 2005; Bar-Shira and Friedman, 2006). Examples of effects of gut microbiota on innate immunity in vertebrates are an enhanced activity of NK cells, granulocytes, macrophages, and cytokine responsiveness (Mandel et al., 1989; Granholm et al., 1992; Tlaskalová-Hogenová et al., 2004; Farnell et al., 2006; Gabriel et al., 2006). However, many aspects of the interplay between microbiota and the immune system are still unknown.

TRANSGENERATIONAL EPIGENETIC EFFECTS

In the next section we will 1) define transgenerational epigenesis/epigenetic effects, 2) summarize examples and mechanisms of transgenerational epigenetic effects, and 3) provide examples of such effects.

Definition

Waddington (1957) was the first to define the term epigenetics. As a developmental/molecular biologist, Waddington focused on how gene expression patterns are modified during differentiation and development (Youngson and Whitelaw, 2008). However, evolutionary biology uses the term epigenetics as well to study the transfer of nongenetic information across generations (Youngson and Whitelaw, 2008), which has led to the formulation of several definitions and interpretations of epigenetics. For the sake of clarity, we define our working definition of transgenerational epigenetic effects based on a combination of definitions from Kouzarides (2007) and Youngson and Whitelaw (2008), with some minor adjustments as follows: transgenerational epigenetic effects are effects based on information 1) carried by a cell/individual, but 2) that is not encoded by changes in the nucleotide sequence of the DNA and 3) that is transferred to successive generation(s) 4) without the necessity of the original environmental stimulus. Transgenerational epigenetic effects provide thus a life-course strategy for offspring, which is mapped by the parents, to meet the demands of the predicted environment in later life (Gluckman et al., 2007; Godfrey et al., 2007). As a consequence of this definition, the environment of the offspring may be such that the transgenerational epigenetic effects may also negatively affect the offspring.

Worldwide, roughly 60 billion broilers are kept on a yearly basis (Aviagen Group, Huntsville, AL). Approximately 460 million broiler breeders are used to produce these numbers (calculated from Aviagen Group, Huntsville, AL), which means that one broiler breeder hen produces around 100 broilers per year. Therefore, even a small transgenerational epigenetic improvement of the broilers health via environmental modulation of the breeders can have a major impact on broilers. In addition, some transgenerational epigenetic effects last for more than one generation, thereby making the grandparents also key factors in the epigenetic process.

Mechanisms and Examples of Transgenerational Epigenetic Effects

Mechanisms or routes of transfer of both transgenerational and nontransgenerational epigenetic information (i.e., within one generation) are summarized in Table 1. For readability, these mechanisms will from now on be referred to as epigenetic mechanisms regardless of inheritance.

Molecular epigenetic mechanisms are DNA modifications (e.g., DNA methylation, DNA acetylation), histone modifications (e.g., acetylation), and exchange associated with altered chromatin structure and noncoding RNA and microRNA (**miRNA**). These mechanisms are all related with the accessibility to DNA introns and generally involved in cell differentiation during ontogeny and tissue development. These epigenetic mechanisms have been studied extensively, though are not always understood completely. Some basic principles have been clarified: unmethylated CpG-islands (DNA containing many C and G nucleotides) at promoter regions were found to be accessible for transcription, whereas heavily methylated CpG-islands were found to be inaccessible for transcription (Korte et al., 2005), indicating that the degree of methylation determines the degree of transcription (Jones and Takai, 2001). The degree of transcription of CpG-islands was also associated with structural chromatin changes: euchromatin and heterochromatin, respectively (Delcuve et al., 2009; Carson et al., 2011).

These molecular mechanisms are suggested to be underlying mechanisms in epigenetics/meiotic transfer of information (parent to offspring), but the majority of research is focused on epigenesis/mitotic transfer of information within an individual (cell to cell). As mentioned before, we will use the term transgenerational epigenetic effects only for information transferred from parent to offspring. However, epigenesis (cell to cell transfer within one generation) does illustrate that molecular epigenetic mechanisms do occur in innate immune cells as well. Epigenesis regulates gene expression

Table 1. Information transferring epigenetic mechanisms of transgenerational epigenetic effects

Category	Origin	Process	References
DNA modifications	Parental	Molecular alteration of DNA affects its accessibility for transcription. Modifications include methylation, acetylation, and phosphorylation.	Delcuve et al. (2009), Gluckman et al. (2007)
Chromatin structural changes (by histone variants or modifications)	Parental	Molecular alteration of the chromatin structure by histone modifications affects DNA accessibility for transcription. Modifications include methylation, acetylation, and phosphorylation.	Carson IV et al. (2011), Delcuve et al. (2009), Gluckman et al. (2007)
RNA (noncoding RNA and microRNA)	Parental	Small RNA molecules (21 to 23 nucleotides) encoded in the genome alter gene expression by binding to target mRNA, thereby downregulating the stability, translation, or both of mRNA. Also microRNA is thought to directly influence DNA modification and chromatin structural changes.	Delcuve et al. (2009), Gluckman et al. (2007), Godfrey et al. (2007), Nätt et al. (2012)[1]
Self-sustaining loops	Parental	Gene activity is autoregulated via self-sustaining loops by their protein products.	Ho and Burggren (2010)[1]
Mitochondria	Parental	Quality and quantity of the mitochondria present in sperm cell and oocyte.	Gluckman et al. (2007), McConnell (2006), Sutovsky et al. (1999)
Sperm properties	Paternal	Altered sperm concentration, motility, morphology, seminal fluid, and other quality factors.	Aitken et al. (2004), Du Plessis et al. (2010), Ng et al. (2010)
Paternal factors	Paternal	Several factors (e.g., transcription factors, signaling molecules) are involved in various embryonic developmental processes.	Hsu and Schulz (2000), Shalgi et al. (1994)
Maternal antibodies	Maternal	Transfer of immunologically relevant environmental information.	Grindstaff et al. (2003),[1] Klipper et al. (2004),[1] Lemke et al. (2004)
Hormones and other egg components	Maternal	Maternal hormones and other egg components present in the egg.	Chin et al. (2009),[1] Groothuis et al. (2005),[1] Surai et al. (2003)[1]
Gametal RNA	Parental	Parental RNA present in sperm cell and oocyte.	Gluckman et al. (2007), Ho and Burggren (2010),[1] Rassoulzadegan et al. (2006)
Microbiota	Maternal	Maternal and environmental microbiota present on eggshell.	Cook et al. (2005),[1] Grönlund et al. (1999), Soler et al. (2011)[1]

[1]Study performed (partly) in birds.

independently of DNA nucleotide sequence in healthy individuals as often investigated in murine or human cells, for example to keep intestinal immune homeostasis, to generate oral tolerance, or to acquire trained immunity (Takahashi et al., 2009; Kleinnijenhuis et al., 2012; Martino and Prescott, 2013). Kleinnijenhuis et al. (2012) showed that stimulation of monocytes resulted in nonspecific protection from reinfection via epigenetic reprogramming, known as trained immunity. Consequently, trained immune cells have a memory for innate host defense (Netea et al., 2011). Additionally, epigenesis is also involved in diseases [i.e., allergies, asthma, airway inflammation, cancer, metabolic, and autoimmune disease (Godfrey et al., 2007; Zhu et al., 2010; Carson IV et al., 2011; Brand et al., 2012)].

Next to the molecular routes of epigenetic mechanisms on the DNA level, information from one generation to the other can also be transferred by soluble components present in the uterus or the egg, such as maternal antibodies (Grindstaff et al., 2003; Lemke et al., 2004), hormones, and other components (Groothuis et al., 2005; Nätt et al., 2009; Ho et al., 2011).

In addition, sperm properties (Du Plessis et al., 2010; Ng et al., 2010), oocyte and sperm RNA (Rassoulzadegan et al., 2006; Gluckman et al., 2007) are routes for transferring information. Also quantity and quality of maternal mitochondria can transfer information from one generation to the next generation (Gluckman et al., 2007). Paternal mitochondria are likely not a route because they are degraded during spermatogenesis or are lost in the fertilized oocyte (McConnell, 2006).

Although the maternal microbiota is not necessarily transferred to the next generation (microbes can die or loose attachment to the eggshell), the maternal gut microbiota can act as a transfer mechanism as well (Cook et al., 2005; Soler et al., 2011).

The listed epigenetic transfer mechanisms also show us why poultry are good models for transgenerational epigenetic studies. The eggs can be isolated and the hatchling can be raised without direct influence of parents (Gluckman et al., 2007; Nätt et al., 2009). Although the egg provides an easier model for studying epigenetics, it should be realized that the composition of eggs differs between chicken breeds, between hens of the same breed, and even between eggs of the same hen (Vieira and Moran, 1998; Silversides and Scott, 2001; Friedman et al., 2012). Maternal diets were also found to influence egg composition (Surai et al., 2003; Karadas et al., 2005).

Noteworthy to mention is the suggestion that transgenerational epigenetic effects, especially methylation of DNA, were essential in the domestication and selection of the modern chicken (Nätt et al., 2012).

Examples of Transgenerational Epigenetic Effects

Only a very limited number of articles describe transgenerational epigenetic effects in birds and even fewer describe transgenerational epigenetic effects on innate immunity in poultry.

A notable observation that suggests transgenerational epigenetic effects was done by Lindqvist et al. (2007) and later repeated by Nätt (2008) and Nätt et al. (2009): 2 groups of laying hens were raised with an unpredictable or predictable light regimen. Birds with an unpredictable regimen showed different feeding behavior than birds with a predictable regimen. The offspring of these 2 groups showed the same directed feeding behavior as their parents, even though both groups experienced a normal predictable light regimen. The offspring of birds that underwent the unpredictable light regimen was also found more competitive and grew faster than the control group. Immunoglobulin gene expression was found to be downregulated in birds with the unpredictable light regimen as well as in their descendants (Nätt et al., 2009). Regulation of these genes, which are involved in both neural development (Huh et al., 2000) and immunity, suggests that also immune parameters can be epigenetically transferred to the next generation (Nätt et al., 2009). The estradiol levels in the eggs from hens that underwent the unpredictable light regimen were higher than in the eggs from birds that underwent the predictable light regimens (Nätt, 2008; Nätt et al., 2009). Estradiol levels have been related to feeding behavior (Eckel, 2004), and therefore, when transferred between generations, may be an explanation for these observations (Nätt, 2008; Nätt et al., 2009).

During life, broiler breeders experience chronic hunger. They receive 50 to 80% feed restriction during their growth period and up to 50% feed restriction during adulthood (Mench, 2002). In line with this, a study was done on broiler breeders by Van der Waaij et al. (2011). Normal feed-restricted broiler breeders were found to produce offspring that were significantly lighter at slaughter than offspring of breeder hens fed ad libitum (Van der Waaij et al., 2011). Offspring of ad libitum fed hens had also relatively less abdominal fat at slaughter (van der Waaij et al., 2011), which was also observed in other animals (Dwyer et al., 1994; Zhu et al., 2004). However, it cannot be determined whether observed differences are due to an undernutritional state of the parental birds or due to nutrient deficiencies in the diet. In mammals, undernutrition promotes the dominance of T helper 2 cells over T helper 1 cells and reduces the development of regulatory T cells (De Rosa et al., 2007; Kau et al., 2011). Neutrophil, monocyte, and macrophage activation are also negatively influenced by under nutrition (La Cava and Matarese, 2004). Unfortunately, no immune parameters were measured in the study of Van der Waaij et al. (2011), but their results suggested detrimental effects of broiler breeders kept under normal restricted feeding and also suggested a transgenerational epigenetic effect on broilers.

In another study, broiler breeders were fed a control diet or an improved diet (control diet supplemented

with vitamins and minerals), whereas offspring of these 2 groups received the same control diet. It was found that offspring of the improved diet group showed altered gene expression in intestinal development and immune functioning (Rebel et al., 2004, 2006). However, no differences in performance were found between the 2 offspring groups (Rebel et al., 2006). A similar experiment, where broiler breeders were fed a control diet or a deteriorated diet (control diet supplemented with mycotoxins of the fungus *Aspergillus*), reported altered activity of macrophages and different blood levels of specific antibodies in the offspring (Qureshi et al., 1998). These studies highlight the opportunities to epigenetically influence the immunity of offspring via parental nutrition.

Maternal antibodies, although their presence in broilers is limited to mainly the first 2 wk of life (Hamal et al., 2006), may exert a lifelong determinative influence on the neonatal immune system, which can dominate over seemingly genetic predispositions (Grindstaff et al., 2003; Lemke et al., 2004). The specific anti-idiotypic binding of the mAb may have strong immunoregulatory properties and influence the emergence of the available specific neonatal B cell binding repertoire (Wikler et al., 1980; Montesano et al., 1999; Fink et al., 2008), and likely also the neonatal T cell repertoire (Martinez et al., 1986; Rubinstein et al., 1982). Maternal antibodies can therefore be seen as nongenetic, information-bearing molecules that transfer knowledge about the immunologically relevant environment (antigenic specificities from microbes, feed components, and so on) gathered by the mother (Lemke and Lange, 1999; Lemke et al., 2004) to her offspring. As an example for poultry, BSA-specific mAb induced in mother layer hens blocked the induction of oral tolerance to BSA in newly hatched chicks (Klipper et al., 2004). Besides mAb, antimicrobials and other egg components are likely to be (partly) functional in the neonatal intestine for several days. One of these antimicrobials, avidin, was suggested to guide the gut microbiota composition (Klasing, 2007). Avidin is an acute phase protein, which is present in the egg albumen (Cucco et al., 2010). It is bacteriostatic and inhibits pathogenic bacterial growth (Klasing, 2007). A second transgenerational epigenetic mechanism for microbiota inheritance is the transfer of microbiota via the eggshell to young chickens (Grönlund et al., 1999; Cook et al., 2005). Soler et al. (2011) investigated the relation between bacterial load on shells and the innate immune system in 29 bird species. A negative association between (parental titers of) natural antibodies (**NAb**) and bacterial density was found in 19 bird species (including poultry), revealing that a strong parentally derived immune system (in this case NAb) benefits the reduction of the vertical transmission of pathogens (Soler et al., 2011). Finally, transgenerational epigenetic effects on immune responses can be indirectly interpreted from studies on the heritability of NAb levels and specific antibody (**SpAb**) responses that were estimated in 2 chicken lines divergently selected for specific antibody levels against SRBC because maternal environmental effects significantly influenced the heritability of both NAb and SpAb levels in subsequent generations (Wijga et al., 2009).

As indicated above, evidence for transgenerational epigenetic effects on innate immunity is mostly lacking in broilers, but well provided by studies with insects. Insects lack an adaptive immune system and therefore rely completely on innate immunity, which is proposed to be the evolutionary root of the vertebrate's immune system, based on several immunological homologies (Pölkki et al., 2012; Arvantis et al., 2013). Transgenerational epigenetic effects on the offspring's innate immune response were found in various insect species and resulted in better immunological functioning after parental exposure to pathogens or MAMP, but the molecular mechanisms of transgenerational epigenesis remain to be elucidated (Little et al., 2003; Sadd et al., 2005).

Taken together, although many aspects are still unknown, based on the abovementioned studies, it is likely that transgenerational epigenetic mechanisms will affect innate immune responses in broilers also. Note that innate responses are frequently generated via or are related to dietary components and their effects on the microbiota.

CONCLUSIONS AND FUTURE DIRECTIONS

Transgenerational epigenetic effects are gaining increased awareness in the field of broiler husbandry as a new tool to maintain or improve health, or alternatively to prevent health problems. They may explain variation within treatment groups in various experimental and observational studies. However, hardly any study actually investigates these effects. We performed this literature study as a first step to summarize what has been described previously about transgenerational epigenetic effects and to examine their potential as a tool to improve innate immunity in broilers. Transgenerational epigenetic effects are of major interest because one broiler breeder hen produces approximately 100 broilers, thereby potentially having a large impact on the broiler generation. With respect to transgenerational epigenetic effects on innate immunity, the immune strategy of broiler may depend on innate responses. Genetic selection of parental broilers with improved innate immune responsiveness was proposed to reduce infections by foodborne pathogens (Swaggerty et al., 2009). Innate immunity, however, is not only an important first line of defense and a prerequisite for good specific immune responses, but on the other hand also may underlie various metabolic and autoimmune diseases that seemingly are not related with innate immunity but are characterized by a status of low chronic inflammation such as obesity and type II diabetes (Tanti et al., 2013) and type I diabetes (Wen et al., 2008) in humans. It has been postulated that broilers are also characterized by a status of low chronic inflammation in the absence of infections as well (Balog et al., 2000; Niewold, 2007).

In broilers, inflammation appeared to affect or underlie processes such as growth and ascites, lameness (Caplen et al., 2013), and white striping of breast muscles (Kuttappan et al., 2013). Antiinflammatory intervention could increase growth (Niewold, 2007) and reduce local and systemic innate immunity in the absence of infections (Munyaka et al., 2012), but was not always consistently successful (Balog et al., 2000). It is tempting to postulate that the mismatching of breeder and broiler management (e.g., restricted versus ad libitum feeding) underlies health problems of the broiler. The broiler may not be transgenerational-epigenetically prepared for its environment. In addition, transgenerational epigenetic transfer of the maternal microbiota to the broiler is impeded due to artificial brooding and treatment of eggs with formalin, which may affect the development of innate immunity and the establishment of the gut microbiota in a negative fashion.

To further elaborate transgenerational epigenetic effects on innate immunity in poultry, we propose the following research strategies: 1) identification of innate (and specific) immune responses prone to transgenerational epigenetic effects, 2) modulation of transgenerational epigenetic effects on the innate immune system in broilers, for instance by dietary intervention or, more indirectly, by intervention of the microbiota composition of breeders, 3) unravelling mechanisms of the interrelationships between innate immunity, diet, and intestinal microbiota in both breeder and broiler birds, and the consequences of intervention for broilers' health and production. Experimental approaches would include 2 × 2 factorial designs to measure the effects of modulation of maternal innate immunity on innate immunity and health of broilers kept in the same and another environment (including diet and microbiota). Innate parameters to be studied are levels of natural (and specific) maternal antibodies in the egg and neonate, levels of complement components from all cascades, inflammatory and anti-inflammatory cytokines, hormones, antimicrobials and defensins in breeders, eggs, and hatchlings, but also the composition of the maternal intestinal microbiota in and on the egg, and the consequences of modulation of the breeder hen for (innate) immune reactivity of the broiler. We suggest (dietary) interventions to modulate the innate immune system of breeders as a likely start for investigation. Dietary innate immune-stimulating (i.e., an inflammatory environment in the breeder) or inflammation-suppressing treatments of broiler breeders should reveal effects on type and levels of innate immune responses and microbiota composition of the offspring broiler generation and their consequences for resistance to both infectious diseases as well as metabolic disorders.

Transgenerational epigenetic effects have often been mentioned in the broiler production sector, but there are only few scientific studies about these effects. In this study, we mainly focused on broilers, but the scarcity of information forced us to also use studies of other species. Based on these results, we conclude that much more knowledge and therefore studies addressing transgenerational epigenetic effects on innate immunity in chickens is desired, as there are precedents that indicate that such mechanisms do also operate in birds and may account for current health problems in broilers. Improving innate immunity of broilers by modulating breeders will lead to less use of antibiotics and due to the interplay with specific immunity will likely improve specific immunity (vaccine responses) as well. In this perspective, innate vaccination programs in broiler breeders with vaccines (containing pathogens) and adjuvants (often mimicking MAMP) may have a significant impact on the immune functioning of broilers. On the other hand, it should be taken into account that an enhanced status of the innate immune system may account for current health problems in broilers.

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