EPIGENETIC ADAPTATIONS IN POULTRY: A CASE FOR IN OVO FEEDING STRATEGIES

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INTRODUCTION

There are a lot of things in nutritional sciences that cannot be clearly explained by simple cause and effect research observations. Indeed, nutritional treatments that respond a certain way in one experiment may respond completely differently in another experiment that uses animals of identical genetic make-up. Even within a heterogeneous population, growth and health characteristics can change from generation to generation as if something in the diet or environment of parents mysteriously affects the metabolism and physiology of future generations. Consider the incidental rise in certain health problems in the US and Canada, such as diabetes, chronic fatigue/fibromyalgia, ADD/ADHD, arthritis, allergies, cancer, bipolar depression, etc. Consider the obesity trends among adults in the USA recently reported by the US Centers for Disease Control and Prevention (http://www.cdc.gov/nccdphp/dnpa/obesity/trend/maps/). In 1985, only a few states had an incidence of obesity over 10%; whereas in 2007, the incidence of obesity is over 25% in all but a few states. Has the American lifestyle changed so much in the past 20 years to justify this obesity epidemic? Alternatively, can it be due to something inherited from the previous generation's lifestyle? Can the way we breed and manage our domestic animals alter genetic responses and disease resistance of their progeny? This paper will address these questions and discuss how it relates to commercial poultry production.

Modern animal agriculture constantly strives to maximize biological performance of food animals in an effort to optimize economic efficiency and profit potential. The significant advances best demonstrate this trend in production efficiency observed in the poultry industry during the past 50 years. Although improvements in growth performance and meat and egg production efficiency with each generation is the main incentive in our capitalist society, these advances are becoming more restrained by issues of social conscience, such as biosecurity, food safety, environmental stewardship, and animal welfare.

It is becoming more apparent that genetic selection for increasing production efficiency may not achieve the expected rewards in commercial practice. The theory of evolution proposed by Charles Darwin and Alfred Russell Wallace assumes that heritable traits, that increase an individual's chance of successfully reproducing, are passed from one generation to the next. But evolutionary rule is somewhat modified in commercial poultry production. Geneticists control the economically-important traits that are passed on to the next generation, and live production managers must adjust the bird's diet and environmental condition to assure successful reproduction and survival. Genetic selection of commercial poultry continually changes the "playing field" for those who raise poultry; and often the genetic expression changes in response

to environmental conditions, resulting in problems such as reduced hatchling survival, metabolic and skeletal disorders, and poor disease resistance.

Genetic selection for increased meat production efficiency has dramatically altered the physiological timeline of commercial broilers and turkeys. Growth performance and meat yield has improved linearly each year along with greater input efficiency in commercial broilers (Havenstein et al., 2003) and turkeys (Havenstein et al., 2007). This trend will likely continue in the future as new technologies in genetics, biotechnology, and developmental biology are introduced and adopted by the poultry industry. As the time it takes meat birds to achieve market size decreases, the period of embryonic development becomes a greater proportion of a bird's life. Today, the 21 day incubation period and the 10 day post-hatch period of the chick composes about 50% of a 2 kg broiler's lifespan. Anything that hinders or promotes growth and development during this neonatal period will have a marked effect on overall performance and health. Modern fast-growing strains of broilers and turkeys are more susceptible to aberrations in early growth and development than their ancestors because their metabolic demands are much greater for growth. Indeed, as selection for greater growth rate continues in commercial meat birds, they are becoming more altricial (birds like pigeons and songbirds that require parental feeding after hatch) and less precocial (birds like chickens and ducks that require little parental nutritional support).

A major difference between precocial and altricial birds is how they manage the constraints on growth, including the availability of nutrient resources, the capacity to utilize the available nutrient resources, and the compromise between somatic growth and tissue maturation and function (Ricklefs et al., 1969, 1979). In contrast to altricial birds, precocial birds quickly adapt to carbohydrate metabolism and seek food on their own to satisfy their nutrient requirements. Precocial birds have the capacity to digest and utilize complex dietary nutrients at hatch, whereas altricial birds need a more simple diet that requires little body resources to digest and absorb, leaving more resources for somatic growth. Finally, precocial chicks grow more slowly than altricial chicks because they partition more energy towards tissue maturation and maintenance than somatic growth. Although altricial chicks may hatch with a less mature digestive tract, they have the advantage over precocial chicks in growth rate. So the poultry meat industry has a dilemma: they select birds to become more altricial to take advantage of rapid growth rate and shorter days to market, but they want to manage these birds as if they are self-sufficient precocial animals. Consequently, early survival problems will increase as the poultry industry moves toward more fast-growing strains.

Modern poultry genetic selection is based on Darwin's theory of evolution and Mendel's fundamental laws of heritability. Based on his classic studies with garden peas, Gregor Mendel hypothesized that all animals within an inbred strain should be phenotypically indistinguishable. Centuries before Mendel's theory was accepted, Greek philosophers believed that the traits of individuals were acquired from contact with the environment, and that such acquired characteristics could be inherited by offspring. Mendel's contemporary, Jean Lamarck, was the most famous proponent of the inheritance of acquired characteristics. The so-called Lamarckism theory emphasizes the use and disuse of organs as the significant factor in determining the characteristics of an individual, and it postulates that any alterations in the individual could be transmitted to the offspring through the gametes. Despite many attempts, this inheritance of acquired characteristics has never been experimentally verified. Furthermore, many of

Lamarck's examples, such as the long neck of the giraffe, can be more satisfactorily explained by means of natural selection.

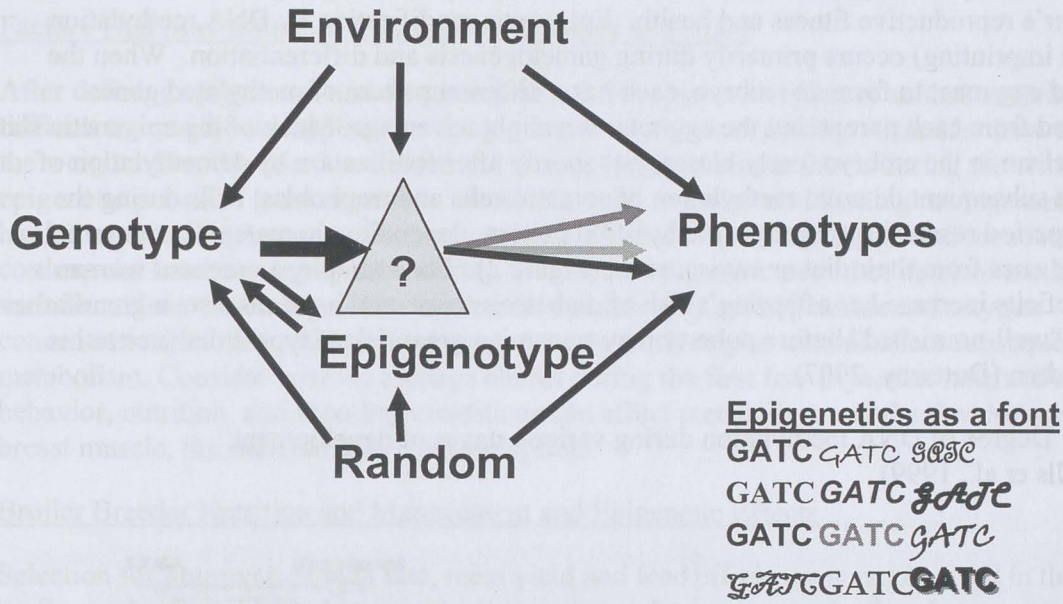
For over a century, the academic discipline of genetics has followed Mendel's basic concepts, and it was reinforced by the discovery and sequencing of DNA. Genetics describes the inheritance of information on the basis of DNA sequence. As the DNA sequence fragments were scientifically associated with certain biological traits, molecular biologists began to study gene expression by up-regulation of mRNA. Others have moved onto the study of proteomics and metabolomics. Many molecular geneticists now think gene expression in response to environmental cues can be passed on to future generations. The old Greek philosophers and Jean Larmarck may have been correct after all; they just did not have the scientific tools to prove it. Epigenetics describes the inheritance of information on the basis of gene expression.

What Is Epigenetics And How Is It Expressed?

I am the son of Dutch immigrants who came to Canada in 1956 to farm as did their ancestors before them. They left the Netherlands to be free from any possibility of experiencing what they had experienced when they were children during World War II. My parents often told me of how the Germans took nearly all the food their family farm produced to feed their soldiers, leaving barely enough of the food they toiled to produce to eat for themselves. Towards the end of World War II, a Nazi-imposed food embargo in the Netherlands, scarce food supplies because of war-torn agricultural lands, and an unusually harsh winter led to the starvation of over 30,000 people. Detailed birth records collected during that "Dutch Hunger Winter" have provided scientists with useful data for analyzing the long-term health effects of prenatal exposure to famine. The children of this famine to 3 generations have unusually high incidence of developmental and adult disorders, including low birth weight, short body height, diabetes, obesity, coronary heart disease, and cancer, (Pray, 2004). I have relatives and friends of our family that suffer many of these chronic illnesses. In another study, Kaati et al. (2002) correlated grandparent's prepubertal access to food with diabetes and heart disease. Remarkably, a pregnant mother's diet can affect the expression of her genes in such a way that not only her children, but her grandchildren and possibly great-grandchildren inherit the same health problems.

According to Dr. Denise Barlow, group leader of the Center for Molecular Medicine of the Austrian Academy of Science, "Epigenetics has always been the weird and wonderful things that can't be explained by genetics". Dr. Barlow is among a growing community of scientists who study epigenetics (http://epigenome-noe.net/). Today, a variety of illnesses, behaviors, and other health indicators are thought to have some link to epigenetic mechanisms, including cancers of almost all types, cognitive dysfunction, and respiratory, cardiovascular, reproductive, autoimmune, and neurobehavioral illnesses. Known or suspected epigenetic agents include heavy metals, pesticides, diesel exhaust, tobacco smoke, polycyclic aromatic hydrocarbons, hormones, radioactivity, viruses, bacteria, and basic nutrients (Weinhold, 2006). As illustrated by Figure 1, these environmental and random effects influence the genetic code defined by the genotype; not by altering the gene sequence, but by modifying nucleotide characteristics within a gene sequence (the Epigenotype) to produce various phenotypes. In other words, an epigenetic gene sequence is as a font of text: the letters are the same, but they may be interpreted differently.

Figure 1. Influence of environmental and random effects on genotype epigenotype on phenotype.

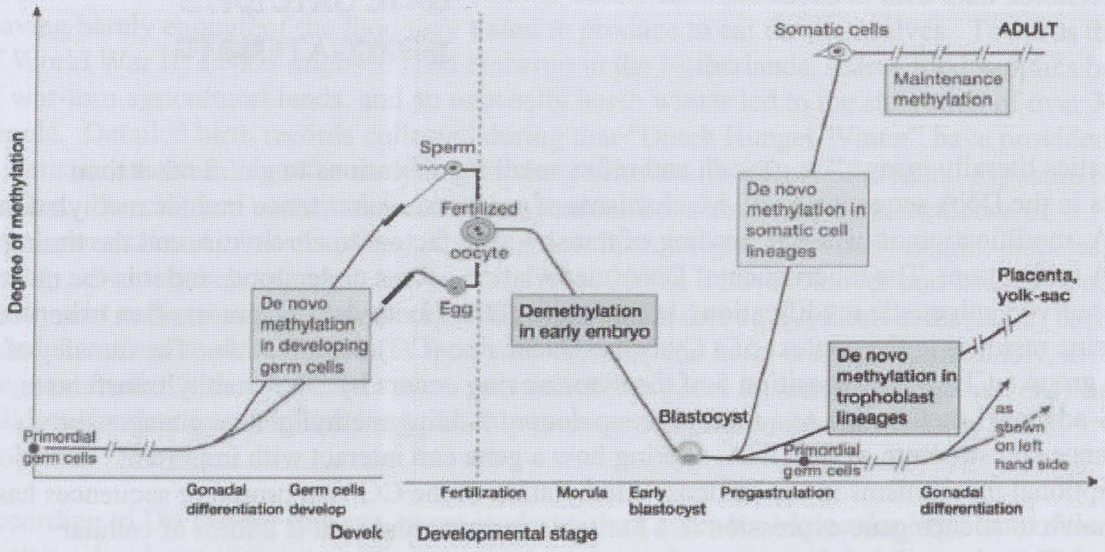


Epigenetics literally means "on genes", and refers to all modifications to genes other than changes in the DNA sequence itself. Mechanisms of epigenetic inheritance include methylation of DNA, modification of histones, binding of transcription factors to chromatin, and the timing of DNA replication. The inheritance of DNA methylation is best understood, and it is the most often observed epigenetic modification. In mammals, DNA methylation is most often observed at cytosine residues in the context of a Cytosine-Guanosine (CG) dinucleotide. The transfer of a methyl group (-CH3) to the position 5 of the cytosine ring occurs by DNA methyltransferases using S-adenosyl-methionine as a methyl group donor. Adding methyl groups changes the appearance and structure of the DNA, altering how a gene can interact with important transcriptional mechanisms in the nucleus. Methylation of the CG-rich promoter sequences has been shown to silence gene expression in a heritable manner, and so it is a form of cellular memory. This transcriptional silencing associated with 5-methylcytosine is required for many fundamental biological processes, such as embryonic development, protection against intragenomic parasites, X-inactivation in females, genomic imprinting and cognitive functions. Aberrant promoter methylation and inappropriate silencing of tumor suppressor genes has recently emerged as a major cause leading to cancer. Humans and other animals are susceptible to epigenetic changes because of an evolutionary trait to protect themselves against "junk" remnants of viral infections, called "transponsons" randomly inserted within the genome. The primary purpose of DNA methylation is to inactivate these junk transposons and prevent their replication, but sometimes a functional gene is also inadvertently methylated.

Epigenetic modifications often differ between males and females, and they are designed to assure the genes of the stronger individual are passed onto the next generation. Many paternally expressed genes favor cellular proliferation and increased growth of the embryo, even at the

expense of the transfer of nutrients from the mother (via the placenta in mammals and via the egg nutrients in oviparous species). In contrast, many maternally expressed genes tend to "fight" the paternally expressed genes to limit the size of the embryo or offspring in order to maintain the mother's reproductive fitness and health. Epigenetic modification by DNA methylation (genomic imprinting) occurs primarily during gametogenesis and differentiation. When the sperm and egg meet to form an embryo, each has a different pattern of methylated genes contributed from each parent, but the egg retains a slight advantage. Much of the epigenetic slate is wiped clean in the embryo (early blastocyst) shortly after fertilization by demethylation of DNA; but subsequent de novo methylation of somatic cells and trophoblast cells during the perinatal period resets the progeny's methylation pattern, depending on maternal or paternal epigenetic cues from their diet or environment (Figure 2). For example, a pregnant woman's dietary deficits increase her offspring's risk of diabetes, stroke, and heart disease; a grandfather who was "well-nourished" before puberty may transmit a great risk of type 2 diabetes to his grandchildren (Duttaroy, 2007).

Figure 2. Degree of DNA methylation during various stages of development.
(From Falls et al., 1999)



As discussed above, incidental exposure to anything that influences DNA methylation patterns during development can change the phenotypic response of an animal for a lifetime and subsequent generations. Nutrition is a key epigenetic modifier. The diet is the primary source of nutrients that supply methyl groups or affect their metabolism: more specifically folate, methionine, choline, betaine, vitamin B_{12}, selenium, zinc, vitamin D, and vitamin A. The epigenetic effects of these nutrients were demonstrated in a series of experiments conducted by Dr. Randy Jirtle, professor of radiation oncology at Duke University, and his postdoctoral student, Dr. Robert Waterland. They used fat yellow mice that carry the agouti gene, which makes them ravenous eaters and prone to cancer and diabetes. However, when the dams were fed diets rich in methyl donors and related cofactors, their offspring are slender, mousy brown, and do not display their parent's susceptibility to cancer, diabetes and short life-span. The mothers passed along the agouti gene to their offspring intact, but thanks to their methyl-rich

pregnancy diet, they had added to the gene a chemical switch that dimmed the gene's deleterious effects. Similar nutritional effects on epigenetic responses are likely possible in other species, including poultry.

Factors That May Influence Epigenetic Responses In Poultry

After defining epigenetics and discussing how it may occur in different animal models, it is not difficult to imagine the application of epigenetic responses in poultry. Consider how we manage the weight of broiler breeders before and during egg production: this is during the critical epigenetic period of gametogenesis. Broiler breeder nutrition and feeding management likely has an important epigenetic effect on progeny. Consider how we manage and incubate commercial hatching eggs: this is during the critical epigenetic period of *de novo* methylation of somatic cells in the embryo. Environmental conditions (*i.e.* temperature and oxygen concentration) in the incubator may program epigenetic responses that affect subsequent metabolism. Consider how we manage chicks during the first few days after hatch. Feeding behavior, nutrition, and brooding conditions can affect metabolism and the development of breast muscle, the skeleton, and immune system.

Broiler Breeder Nutrition and Management and Epigenetic Effects

Selection for improved growth rate, meat yield and feed efficiency is emphasized in the male line broilers, whereas sustained egg production is more of a concern in the female lines. In order to optimize reproductive capacity and fitness in both male and female lines at the grand-parent and parent generation levels, the weight gain must be controlled by limiting feed intake well below the ravenous appetite they are genetically selected to have. In effect, the limit-fed broiler parent stock may be sending an epigenetic message to their progeny that they will live in a world of limited nutrient resources. Of course this minimal nutritional condition usually does not occur for the commercial broiler stock, so much like those Agouti mice or those children of the "Dutch Winter Hunger" discussed above, the incidence of metabolic disorders associated with chronic disease may arise as a consequence.

There is little research on the effects of breeder nutrition and management on epigenetic effects on progeny in commercial poultry, but research on this topic is just starting to appear. Recently, Lindqvist et al. (2007) conducted an elegant experiment that demonstrated the epigenetic effects of stress among White Leghorn, selected for egg production, and the ancestral Red Junglefowl on the ability of progeny to learn a task. Fifteen males and 15 females of each strain were subjected to what the researchers called a chronic mild stressful treatment of unpredictable light-dark rhythm: controlling for total number of light hours per week, light and dark periods of 3, 6, 9, 12, 18 and 24 hours were randomly applied. The same number of control birds always had a 12:12 light:dark cycle. The total number of light hours per week was the same for each treatment group. In effect, birds subjected to the unpredictable light:dark regime were unable to predict for how long food and water would be available. Eggs were collected from each group, incubated in a commercial-type incubator, and the progeny placed in pens for observation. The nutrient resource limitation of the parental stock significantly increased progeny weight at hatch among the RJF strain and 8 day weights of the WL hens. However, feeding behavior as expressed by time occupying the feeder was increased only among progeny from stressed commercial hens (WL; Table 1). Evidently, genetic selection for productivity caused the progeny to be more epigenetically sensitive to the parent's "stress".

Table 1. Weight and food competition capacity in offspring of White Leghorn hens (WL) and Red Junglefowl (RJF). (From Lindqvist et al., 2007).

	White Leghorn (WL) Parents		Red Junglefowl (RJF) Parents	
	Stressed	Control	Stressed	Control
Hatching weight (g)	44.4 ± 0.5^{a}	43.7 ± 0.5^{a}	26.4 ± 0.5^{b}	24.5 ± 0.5^{c}
8 day Weight (g)	70.6 ± 1.2^{a}	66.0 ± 1.2^{b}	47.7 ± 1.2^{c}	44.8 ± 1.2^{a}
Percent time occupying feeder	58.2 ± 5^{a}	41.8 ± 5^{b}	51.0 ± 5^{c}	49.0 ± 5^{c}

Birds were weighed within an hour after hatching and at 8 days of age. Food competition capacity was estimated as the percentage of time in which each individual occupied the feeder in a pair-wise competition test. The data were analyzed with ANOVA, using breed and parental treatment as fixed independent variables.

Similarly, subjecting the commercial parent stock to "stress" adversely affected the progeny's learning behavior more than among the ancestral parent stock. A series of learning tests were conducted on each parental group at 133 days of age and their progeny at 33 days of age. The learning test was conducted in a T-maze, following 15 h of feed deprivation, of which 12 hours consisted of darkness. Learning ability was measured as a percentage of birds that successfully solved the task after a number of attempts. The "stress" treatment significantly reduced the learning ability of the parental stock in both strains, but the WL strain took many more repetitions to learn the task than the RJF strain. Likewise, progeny from the "stressed" WL hens required more attempts to solve the learning task, but there was no "stress" effect on the learning ability of progeny from RJF parents (Figure 3). In effect, eliminating the predictability of nutrient availability (light:dark schedule) makes commercial WL parents and their progeny more stupid than the ancestral RJF strain. Moreover, microarray evaluation revealed that correlations between the magnitude of differential expression of genes between parents and progeny to the parent's stress were considerably different among WL and RJF strains (Figure 4). This observation clearly indicates transgenerational epigenetic effects can differ significantly among different strains of poultry. Although the study of Lindqvist et al. (2007) may have limited application in commercial poultry production, it is does demonstrate that measurable epigenetic defects can be passed from parent stock to progeny.

Figure 3. Spatial learning in White Leghorn parents and their non-stressed offspring. Each panel shows cumulative proportion of tested birds which had solved the spatial learning task at successive test instances; the criterion for solving the task was five correct choices out of six successive tests, so the smallest number of required tests was five. (a) White Leghorn parents. (b) Red Junglefowl parents. (c) White Leghorn offspring. (d) Red Junglefowl offspring. The differences in cumulative proportions of birds from different treatments solving the task were tested with $\chi 2$ –analysis after five test rounds and onwards, and significant differences are indicated ($p<0.05$). (From Lindqvist et al., 2007).

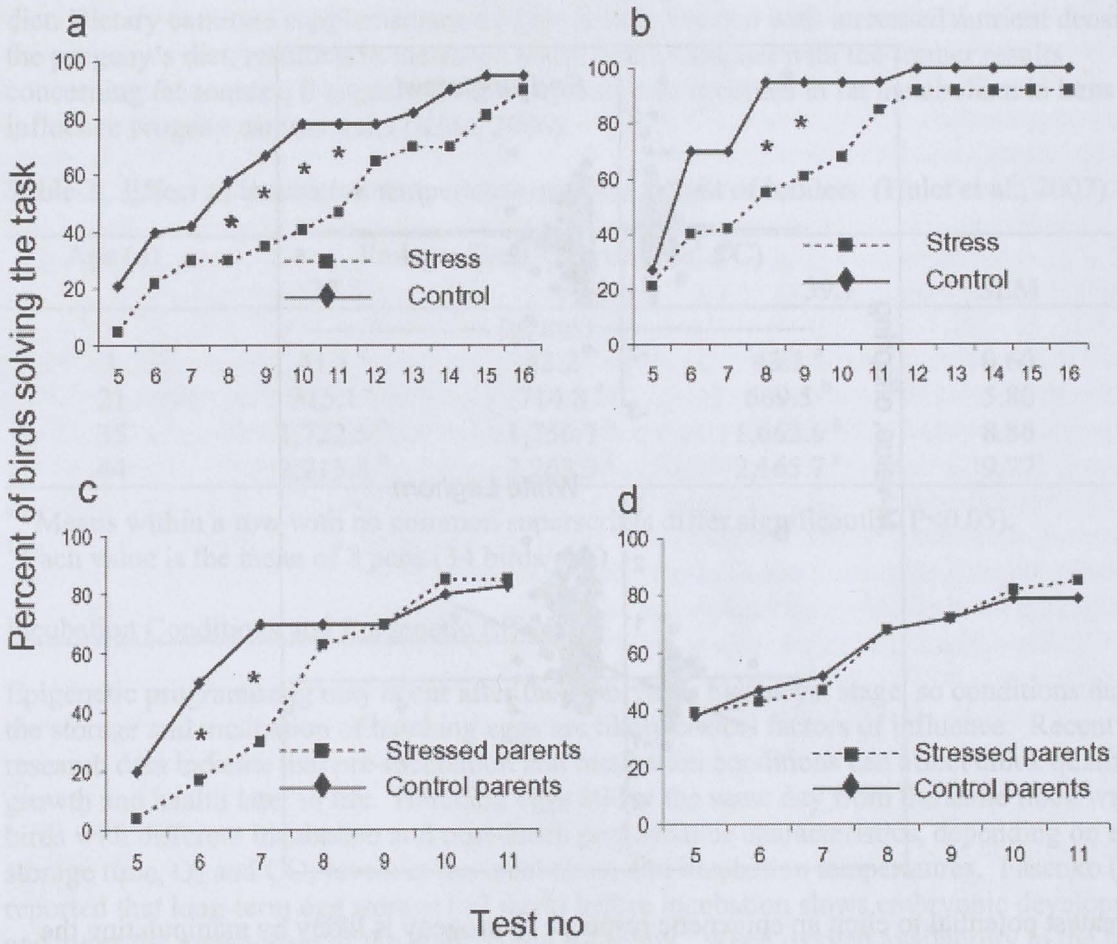
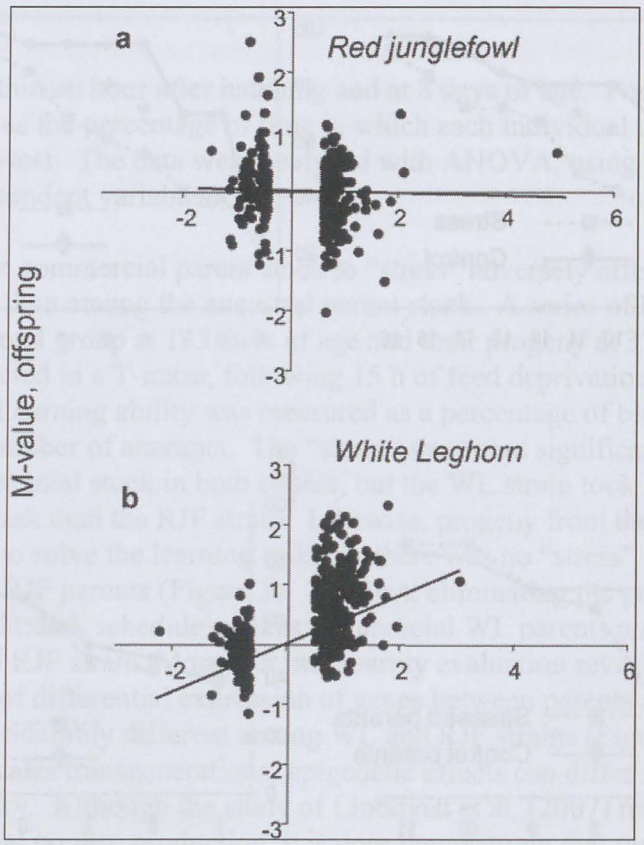


Figure 4. Correlations between magnitude of differential expression of genes between parents and offspring. Diagrams show M-values for the differential expression (comparing stressed vs. control parents, and offspring of stressed vs. offspring of control parents) of the 500 most differentially expressed genes (largest log2 difference caused by stress in parents) in (a) Red Junglefowl, and (b) White Leghorns. Each point represents one spot on the microarray. Positive M-values indicate upregulation and negative downregulation by stress (or by having stressed parents). The average correlation line is shown in both comparisons. (From Lindqvist et al., 2007).



The greatest potential to elicit an epigenetic response in progeny is likely by manipulating the diet of the maternal parent. In avian species, all the nutrients available to the developing embryo are included in the egg. These nutrients are deposited into the egg from the hen until it is laid. Thus, the hen's diet can have a marked effect on the egg's nutritional status, especially if some nutrients are limiting because of restricted feeding practices or marginal formulation. Kidd (2006) reviewed many aspects of improving chick quality *via* hen mineral nutrition, protein and energy nutrition, and vitamin nutrition. Among the minerals, selenium was the only mineral that positively affected progeny growth as a result of parental supplementation, and the effect lasted up to 14 days post hatch. Organic zinc and manganese supplementation of breeder hens improved immune function and infectious disease resistance of progeny, but did not affect their growth. Kidd (2006) reported that dietary protein intake of breeder hens had no effect on

progeny performance, but the type of dietary fat can. Peebles et al. (1999) observed that feeding corn oil to breeder hens increases 21-day body weights of their progeny in comparison to those breeders fed poultry fat, and it also improved slaughter weight in comparison to equal levels of poultry fat or lard (Peebles et al., 2002). Perhaps more polyunsaturated fats in the diet of broiler breeders have some epigenetic effect on progeny growth and carcass characteristics. Kidd and co-workers recently fed broiler breeder hens (beginning at 21 weeks of age) diets with and without L-carnitine (0 of 25 mg/kg of diet). Progeny performance and carcass traits were evaluated in three hatches (30, 35, and 37 weeks). Dietary carnitine supplementation of the broiler breeders reduced abdominal fat pad in progeny at processing, irrespective of progeny diet. Dietary carnitine supplementation of the hens interacted with increased nutrient density of the progeny's diet, resulting in increased breast meat. Coupled with the former results concerning fat sources, it appears that dietary nutrients involved in fat metabolism in hens can influence progeny carcass traits (Kidd, 2006).

Table 2. Effect of incubation temperature on body weight of broilers. (Hulet et al., 2007).

Age (d)	Embryo Shell Temperature[1] (°C)			
	37.5	38.6	39.7	SEM
	---------- (grams) ----------			
1	41.1 [c]	42.2 [b]	43.1 [a]	0.60
21	715.1 [a]	714.8 [a]	669.5 [b]	5.80
35	1,722.5 [b]	1,756.7 [a]	1,663.6 [c]	8.86
44	2,213.8 [b]	2,263.3 [a]	2,165.7 [c]	9.77

[a-c] Means within a row with no common superscripts differ significantly ($P<0.05$).
[1] Each value is the mean of 8 pens (34 birds/pen)

Incubation Conditions and Epigenetic Effects

Epigenetic programming may occur after the embryonic blastocyst stage, so conditions during the storage and incubation of hatching eggs are likely critical factors of influence. Recent research data indicate that pre-incubation and incubation conditions can affect chick quality and growth and health later in life. Hatching eggs laid at the same day from the same flock will yield birds with different incubation and post-hatch performance characteristics, depending on egg storage time, O_2 and CO_2 levels in the incubators, and incubation temperatures. Fasenko (2007) reported that long-term egg storage (>7 days) before incubation slows embryonic development and alters the metabolism of the embryo and hatchling. When oxygen availability to the late-term embryo is limited by low egg conductance or poor incubator ventilation, the embryos and hatchlings may suffer a low glycogen status (Christensen et al., 2000ab) and impaired enteric development (Christensen et al., 2003). Excessive temperature during the plateau stage of oxygen consumption of the late-term embryo will impair intestinal and cardiac development in poults (Christensen et al., 2004ab) and chicks (Wineland et al., 2006). High yield strains are most adversely affected by excessive incubation temperature (Decuypere and Bruggeman, 2006). Hulet et al. (2007) incubated eggs from high-yielding broiler breeder flocks (Cobb x Cobb) at 2 ages (29 and 57 wk of age) for 16 d at 37.5°C dry bulb and 29.4°C wet bulb. After candling on d 16, one-third of the fertile eggs from both breeder flocks were transferred into separate hatchers that maintained shell temperatures of 37.5°C (low, **L**), 38.6°C (middle, **M**), and 39.7°C (high, **H**). The hatchlings were then raised under the same conditions and growth performance was

measured. Body weights of day-old chicks increased as the incubation temperature increased, but growth performance after 21 days of age was depressed by the H and L incubation temperatures (Table 2). According to Oviedo-Rondón et al. (2006a), heat stress during the perinatal period of poultry adversely affects hormonal control of bone growth, namely thyroid hormones, and may cause epigenetic defects that last the lifetime of the bird.

Effect of In Ovo Feeding and Early Nutrition (Perinatal Nutrition)

The nutrients deposited into the egg by the hen are the only source of nutrients available to the embryo, and this may be the last chemical means by which the hen may transfer an epigenetic message to its offspring. Although the digestive capacity begins to develop after the embryo consumes the amniotic fluid, most of the development occurs post-hatch when the neonatal chick begins consuming feed. Any delay in feed intake initiation will suppress gastro-intestinal development and cause early malnutrition (Uni et al., 2003), suppress thyroid activity (Reyns et al., 2002), and inhibit satellite cell proliferation and muscle growth potential (Mozdiak et al., 2002; Halevy et al., 2003; and Moore et al., 2005). Considering these lasting effects of early nutrition on subsequent growth characteristics, it is possible that epigenetic responses may also persist. However, I am not aware of any research that confirms this hypothesis.

An alternative means of manipulating the epigenetic response to nutrients deposited in the egg by the hen is to inject nutrients into the egg at a time when the embryo is most sensitive to epigenetic programming. By injecting an isotonic in ovo feeding (IOF) solution into the embryonic amnion, the embryo can naturally consume supplemental nutrients orally before hatching (Uni and Ferket, 2003; US Patent No. 6,592,878). In ovo feeding "jump-starts" or stimulates the adaptation to external feeding to begin earlier than would otherwise occur after the birds hatch. Improving the nutritional status of the neonate by in ovo feeding may yield several advantages: greater efficiency of feed nutrient utilization; reduced post-hatch mortality and morbidity; improved immune response to enteric antigens; reduced incidence of developmental skeletal disorders; and increased muscle development and breast meat yield. These benefits will ultimately reduce the production cost of poultry meat by alleviating the growth constraints of "altricial" broilers selected for rapid growth rate.

The benefits of in ovo feeding on early growth and development of broilers and turkeys have been demonstrated by several experiments in our laboratory (Uni and Ferket, 2004). In ovo feeding has increased hatchling weights by 3% to 7% ($P<.05$) over controls, and this advantage is often sustained at least until 14 days post-hatch. The degree of response to in ovo feeding may depend upon genetics, breeder hen age, egg size, and incubation conditions (i.e. the epigenotype). Above all, IOF solution formulation has the most profound effect on the neonate. Positive effects have been observed with IOF solutions containing NaCl, sucrose, maltose, and dextrin (Uni and Ferket, 2004; Uni et al., 2005), β-hydroxy-β-methyl butyrate, egg white protein, and carbohydrate (Foye et al., 2006ab), Arginine (Foye et al., 2007), and zinc-methionine (Tako et al., 2005). In addition to the increased body weights typically observed at hatch, the positive effects of in ovo feeding may include increased hatchability (Uni and Ferket, 2004; Uni et al., 2005); advanced morphometic development of the intestinal tract (Uni and Ferket, 2004; Tako et al., 2004) and mucin barrier (Smirnov et al., 2006); enhanced expression of genes for brush border enzymes (sucrase-isomaltase, leucine aminopeptidase) and their biological activities,

along with enhanced expression of nutrient transporters, SGLT-1, PEPT-1, and NaK ATPase (Tako et al., 2005; Foye et al., 2007); increased liver glycogen status (Uni and Ferket, 2004; Uni et al., 2005; Tako et al., 2004; Foye et al., 2006a); enhanced feed intake initiation behavior (de Oliveira, 2007); and increased breast muscle size at hatch (Uni et al., 2005; Foye et al., 2006a). In ovo feeding clearly advances the digestive capacity, energy status, and development of critical tissues of the neonate by about 2 days at the time of hatch.

In ovo feeding enhances gut development and digestive capacity. This is the goal of *in ovo* feeding: the sooner the neonate develops the functional capacity to digest and absorb nutrients, the more likely it is able to grow according to its genetic potential. Digestive capacity is a function of both the gut muscosa surface area and the brush border enzyme activity per unit of tissue mass. Development of the mucosal surface area and brush border enzyme activity is determined by the rate of enterocyte proliferation and differentiation. *In ovo* feeding has been demonstrated to significantly increase the absorptive surface area in several segments of the gut of newly hatched poults by increasing villus height and villus apical and basal width (Bohórquez *et al.*, 2007), and this was associated with increased early growth rate.

Foye *et al.* (2007) also demonstrated *in ovo* feeding enhanced enteric brush border enzyme activity of turkeys. Turkeys were *in ovo* fed at 23 days of incubation with 1.5mL of A) 0.1% HMB + 0.7% Arginine in 0.4% saline (HMB + ARG); B)18% Egg white protein + 0.1% HMB + 0.7% Arginine in 0.4% saline (EWP + HMB + ARG); or C) a non-injected control. *In ovo* feeding of ARG + HMB significantly enhanced sucrase, maltase and LAP brush border activity within 48 hours of nutrient administration. Additionally, *in ovo* fed poults of the ARG + HMB treatment group had increased sucrase, maltase and LAP activity at 14-day post-hatch. These results imply that *in ovo* feeding HMB and ARG may positively affect intestinal brush border enzymes for up to two weeks. As with the broiler experiments, the increased brush border enzyme activity corresponded with improvements in post-hatch growth. In addition to providing nutrients to fuel the development of the late-term embryo, *in ovo* feeding affects the expression of genes that control the development of digestive capacity. Foye *et al.* (2007) observed in turkeys that the increase in brush border enzyme activity and nutrient transporters by *in ovo* feeding was preceded by a corresponding increase in the expression of related genes (mRNA). *In ovo* feeding may also enhance the protective function of enteric mucosa. Hatchlings are very susceptible to the colonization of enteric pathogens due to minimal competitive exclusion by symbiotic microflora that populate the mucin layer of the gut mucosa. The mucus gel layer of the intestinal epithelium is the first barrier to enteric infection. Smirnov *et al.* (2006) observed the proportion of goblet cells containing acidic mucin increased 50% over controls at 36 h after *in ovo* feeding, which corresponded to enhanced expression of the mucin mRNA. Using scanning electron microscopy, Bohórquez *et al.* (2008) observed that *in ovo* feeding significantly increased functional maturity and mucus secretion of goblet cells of villi of ileum and ceca of turkey poults. Associated with these goblet cells was the colonization of lactobacilli. Therefore, *in ovo* feeding may help improve the colonization resistance of enteric pathogens of neonatal chicks and poults.

In ovo feeding improves glycogen status: Glycogen reserves in the avian embryo provide the critical energy needed for hatching. In turkeys, extensive embryonic mortality occurs toward the end of the incubation period when hatching-related events occur, such as pipping of the egg membrane and shell, beginning of pulmonary respiration, and the actual egg emergence

(Christensen *et al.*, 2000a). Glycogen reserves in the embryo are significantly depleted during the peri-hatch period in order to meet the high energy demand during the process of emergence

(Freeman, 1965, 1969; Freeman and Manning, 1971). Hepatic and muscle glycogen reserves are depleted due to carbohydrate utilization for muscular activity during the hatching process (Bakhuis, 1974; John *et al.*, 1987, 1988) and for post-hatch growth, activity and maintenance (Warriss *et al.*, 1988).

Uni and Ferket (2003) demonstrated that turkey poults *in ovo* fed HMB had approximately a 40% increase in hepatic glycogen over the injected and non-injected controls. Moreover, hatchability rates were positively correlated with liver glycogen content of turkey embryos before hatch (Uni and Ferket, 2003). Foye *et al.* (2006a) observed that *in ovo* feeding saline solutions containing egg white protein and HMB increased liver glycogen at hatch and breast muscle glycogen content through to 7 days post-hatch. In another experiment, Foye *et al.* (2006b) observed poults *in ovo* fed saline solutions of .1% HMB and/or .7% arginine had over 75% greater total liver glycogen content and hepatic glucose-6-phosphatase activity than controls. Using focused gene array technology, de Oliveira *et al.* (2007) confirmed that *in ovo* feeding up-regulates the expression of critical enzymes associated with glycogen deposition and its utilization during pipping and hatching. *In ovo* feeding clearly enhances glycogen status as indicated by hepatic gluconeogenic activity and hepatic glycogen reserves, which provide the fuel needed to support the hatching process, thermal regulation, and rapid growth during the critical post-hatch period until sufficient energy resources are consumed upon feed intake initiation.

In conclusion, *in ovo* feeding offers promise of sustaining the progress in production efficiency and welfare of commercial poultry. Although selection for fast growth rate and meat yield may favor the modern broiler to become a more altricial, proper early nutrition and *in ovo* feeding may help these birds adapt to a carbohydrate-based diet and metabolism typical of a precocial bird at hatch. Our research on *in ovo* feeding has established a new science of neonatal nutrition, and we are gaining greater understanding of the developmental transition from embryo to chick. However, much more work must be done before *in ovo* feeding can be adopted for commercial practice.

TAKE-HOME MESSAGE

The science of epigenetics is a new and exciting field of research that will gain much attention in the near future. As new molecular biology tools, such as microarray gene expression analysis, become more accessible and affordable, the study of factors that affect epigenetic response will gain greater attention. Epigenetic research may yield great dividends and affect several economically important aspects of poultry production, including breeder management, incubation, chick management, early nutrition, disease resistance, performance efficiency, reproductive efficiency, and animal behavior. Among the most interesting areas of epigenetic research on the horizon is "personalized" nutrition that accommodates genetic X environmental interactions. For example, in ovo feeding may be customized to epigenetically program a specific strain of bird to express a specific performance characteristic. Epigenotypes of poultry may be developed to satisfy a unique management system, feed regimen, or market niche.

Poultry scientists like me may finally come to appreciate the theories of Jean Lamarck and the ancient Greek philosophers: inheritance is indeed based on the genetic code and how the genes are expressed. However, the science of epigenetic manipulation has just hatched...and we still have a lot to learn!

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