

NUTRITIONAL IMPRINTING: EARLY DIETARY MANUPULATIONS

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Introduction

Land application of poultry litter is of increasing concern in areas of intensive poultry production in the United States (Sharpley, 1999). Poultry litter and or excreta are in general relatively high in phosphorus (P) due to the poor utilization of phytin P (PP) (Nelson, 1976) by poultry. The Environmental Protection Agency (EPA) passed federal regulations that limits the amount of poultry litter that can be applied to soils, based mainly on litter P content (EPA, 2003). To address the P issue, poultry nutritionists have developed several nutritional strategies, that include feeding diets with P concentrations closer to requirements (Yan et al., 2001; Dhandu and Angel, 2003), dietary supplementation with feed additives such as microbial phytase (Simons et al., 1990; Denbow et al., 1995; Yi et al., 1997; Angel et al, 2005) and vitamin D3 metabolites (Edward, 1993; Biehl and Baker, 1997; Edward, 2002; Angel et al, 2005), and use of genetically modified feed ingredients with lower concentrations of PP (Cromwell et al., 1998; Waldroup et al., 2000; Li et al., 2000).

Another environmental concern in poultry production and one that state and federal environmental agencies are currently trying to limit is nitrogen (N) emissions. It is generally thought that the main compound emitted from poultry operations is ammonia (NH₃). The control of NH₃ emissions in poultry houses is a major concern for the poultry industry. Not only does NH₃ impact bird health and performance (Quarles and Kling, 1974; Miles et al., 2004), but there is also a concern of its negative impact on public health and the environment. Once NH₃ is emitted into the atmosphere it can contribute to the formation of fine particulate matter (PM_{2.5}) which may cause respiratory illness in humans (Fierro, 2000) and increase the development of atmospheric haze and acid rain (NRC, 2003). Pending revisions of the Clean Air Act will set a PM_{2.5} limit which may indirectly regulate NH₃ emissions and require the implementation of control measures on poultry house facilities. One of the most efficient and possibly the most economical method of controlling NH₃ production from poultry facilities is to reduce the amount of N excreted from the birds. In general, N excretion is directly related to the animal's N (protein) intake, and growth rate that is related to the amount of N retained by the body. Numerous nutritional studies have demonstrated that dietary manipulation in monogastric animals can be a useful tool to reduce NH₃ emissions from poultry and livestock facilities (Reece et al., 1979; Ferguson et al., 1998). Several nutritional strategies have been developed to reduce N concentrations in poultry excreta and consequently reduce atmospheric NH₃ emissions. Some of these strategies include reducing dietary protein concentrations while providing a

adequate essential and some non-essential amino acids at the correct balance in the diet by supplementation with synthetic amino acids (Ferguson et al., 1998), minimizing feed and water waste (Ferket et al., 2002), separate sex and phase feeding, enzyme supplementation (Zanella et al., 1999), and minimizing feed nutrient variability (Van Kempin, 2001).

Most of the strategies mentioned above partially address environmental concerns as well as production cost challenges related to diet P and protein. Most of these strategies have proven affective to varying degrees but they have the potential to increase cost and increase risk by increasing the possibilities of lower productivity in the field and at processing. Alternative strategies need to be studied. One of these alternatives is epigenetic regulation of gene expression by early nutritional imprinting that result in changes in genes f significant economic importance to the poultry industry. These genes modifications brought about by an early-life dietary change need to be long lasting such that once the early nutritional modification is replaced with adequate or deficient feeds, the ability of the animal to better utilize specific nutrients is maintained.

Background on Epigenetic Regulation

The growing incidence of metabolic diseases in humans, such as obesity, diabetes, and cardiovascular disease has sparked interest and research efforts into both their genetic and environmental (nutritional) basis. The maternal diet, and therefore the nutrient supply to the developing oocyte, embryo or fetus, is one of the principal environmental factors influencing the development of the offspring. A reliable and balanced supply of amino acids, lipids and carbohydrates is required to support the high rates of cell proliferation and the key developmental processes that take place during fetal development. Eukaryotic cells have evolved a complex series of nutrient sensors that are able to regulate gene expression in response to imbalances in the supply of nutrients. In adults these systems serve two purposes; first to protect the cell from damage caused by acute deficiencies and second to optimize homeostatic control to deal with a prolonged excess or deficiency of a particular nutrient. This second process may have a critical impact on the long term health of the offspring. It has been proposed that adverse nutritional conditions during fetal development lead to adaptive changes in metabolism that lead to a 'thrifty phenotype' in the offspring (Hales and Barker 1992). Poor nutrition in early life produces permanent changes in glucose-insulin metabolism, including a reduced capacity for insulin secretion and insulin resistance (Hales and Barker 2001). However, if this 'programming' of metabolism during embryonic and fetal development is inappropriate for the long term nutritional environment where the animal will live in it may lead to adverse long term consequences (Sayer et al. 2004, Yajnik 2004, Barker 2004). The initiating factor(s) for fetal programming may be nutrient(s) interacting directly with genes and their regulatory elements at the cellular level, altering patterns of growth and gene expression.

It is becoming apparent that embryonic and fetal cells have a complex system to integrate nutritional signals from their environment and adapt their development accordingly to ensure survival. Human diets are comprised of complex mixtures of protein, fats, carbohydrate and vitamins. The full impact of inappropriate programming of metabolic regulation is only just beginning to be appreciated. The available evidence suggests that nutrient sensing regulatory systems are present in many critical tissues during early development. It remains to be seen whether they play an important part in establishing homeostatic control mechanisms early in life.

Similar observations to those found in placental organisms have been made in the chicken where conditioning in early-life imparts long-term effects. The first report of this type of response was to temperature or thermal stress. The basis for these studies was to identify a mechanism to impart

tolerance to acute heat stress in chickens produced in sub-tropical climates. It was found that excessive thermal input during the first week of life modulated the response to thermal stress later in life (Yahav and McMurtry, 2001). By simply increasing the brooding temperature from 30°C to 37.5°C for 24 hours within the first 5 days post-hatch birds are able to tolerate 6 hr of exposure to 35°C at 42 days of age, while “unconditioned” birds are unable to acclimate. The mechanism for this conditioned response is unknown. Several studies speculate that it is during the period immediately post-hatch (neonatal) that the chick is developing the connections in the brain for the detection and regulation of body temperature (Katz and Meiri, 2006; Labunsky and Meiri, 2006).

Early dietary adaptation

Adaptation to low nutrient diets has been long recognized. Animals respond to nutrient restriction in general by increasing absorption rates and utilization efficiency, which decreases excretion of the restricted nutrients. The ability of humans to adapt to a diet low in Ca was recognized in the 1950s. At that time, the Food and Nutrition Board (1948) recommended an adult daily Ca allowance of 800 mg per day based on studies done with US adult males. However, Hegsted et al. (1952) found that adult men in Peru, who had lived on low Ca diets for long periods, only required 100 to 200 mg Ca per day to maintain Ca balance. It is obvious that these Peruvians, who grew up under Ca restriction, were able to better utilize Ca.

Although several reports demonstrate the ability to program chicks through early dietary manipulation to improve Ca and P utilization later on in life, there has been little work reported in the literature applying this concept with dietary protein utilization. The lysine requirement for broiler chickens has been studied extensively, and recommendations have been made for different growth phases (Garcia et al, 2006). Lysine is often considered first co-limiting amino acid with methionine in broiler diets, and is added in synthetic form to meet the bird's requirement. The interaction between protein and lysine is considered an important factor which affects broiler performance and carcass quality.

Adaptation to P and Ca restricted diets has also been previously reported in chickens. In an in-vitro trial, using ligated duodenal loops, Morrissey and Wasserman (1971) observed that broiler chicks absorbed a higher percent of a labeled ^{47}Ca (ranging from 70 to 90%) when diets low in Ca (0.08%) were fed for eight days prior to intestinal sampling regardless of dietary P levels, or when low P (0.25%) diets were fed regardless of dietary Ca levels. Chickens receiving a diet with normal P (0.65%) and normal Ca (1.20%) absorbed less than 50% of ^{47}Ca . Duodenal P absorption in 15 to 20 d-old chicks that had been fed a low Ca or a low P diet for eight d, as measured by ligated duodenal loop technique in vivo, increased by 49 and 87%, respectively (Fox et al., 1981). Blahos et al. (1987) reported an increase in duodenal and ileal P absorption in broiler chickens fed a low Ca diet for two wk and a smaller, but still significant increase in duodenal but not ileal P absorption in chicks fed a low P diet. The adaptation to P or Ca restriction was believed to be a result of an increased level of circulating $1,25\text{-(OH)}_2\text{-D}_3$ (Hunziker et al. 1982; Blahos et al., 1987) and duodenal calbindin content (Morrissey and Wasserman, 1971; Montecuccoli et al. 1977). By comparing the duodenal calbindin concentration and its changing pattern with age for 1991 and 2001 strains of broilers, Bar et al. (2003) concluded that modern broilers exhibit higher capacity of adaptation to P or Ca deficiency and this capacity remains high for the whole growth period.

Focus on Early Nutrient Imprinting on improving the utilization of Phosphorus and Protein by broilers

No literature could be found on work conducted to evaluate the long-term effects of early P or Ca restriction on growth performance, bone mineralization, and P absorption in poultry. Thus, we proceeded to investigate if birds had the capacity to adapt to low P diets. The application of the adaptation principle in poultry may allow for decreasing both diet and excreted P without sacrificing performance and provide an additional low cost tool to decrease P in poultry litter. The goal of this work was to determine if adaptation occurred in broilers and then to try to identify the mechanisms of this adaptation. We evaluated the ability of the chicken to adapt to a moderate early life deficiency in P and Ca and characterized this adaptation changes by examining the impact of the previous P and Ca status (starter phase, hatch to 18 d) on performance, bone characteristics, and nutrients absorption of broilers the grower phase (19 to 32 d) (Yan et al, 2005).

In summary, broilers fed a diet moderately deficient in P and Ca from hatch to 18 d demonstrated the ability to adapt to the deficiency. This was shown in the increased total P and Ca ileal absorption (Table 1), the increased PP disappearance, improved growth, and improvement in bone measures including tibia ash, tibia and shank bone mineral density and bone mineral content in a later growth phase (18 to 32 d).. These published data indicate that in birds during the period immediately post hatch there is a phenomenon occurring that permanently alters the bird's response to its environment. This adaptation or conditioning, which-ever term you choose to use, is a real observable fact for which no underlying mechanism has been previously proposed.

A second experiment was done to determine the effects of diet P on performance and expression of the chicken intestinal NaPcoT, experimental diets were formulated to be deficient in total P. Ross 308 chicks were fed either a control diet (C) consisting of 1.11% Ca and 0.50% available P (NRC levels) or a restricted diet (L) containing 0.59% Ca and 0.25% available P from hatch to 4 d of age (90hr). All birds were then fed a control diet (C) consisting of NRC recommended levels of Ca and P until d 22. From day 22 to d 38 the birds were either maintained on a C diet at NRC levels of Ca and P at 0.7% and 0.3% respectively or a restricted diet (L) consisting of 0.4% Ca and 0.12% P. The three dietary treatments, C-C-C, C-C-L, and L-C-L met all other NRC (1994) nutrient recommendations. Performance data were collected for each dietary phase including weight gain, feed conversion, bone ash, and specific nutrient retention. These data are presented in Table 2.

Broilers fed the moderately deficient diet (L) to 90 hr were better able to handle a deficiency in P in the grower/finisher phase (22 to 38 d of age) than those fed a control diet in the first 90 hr. Not only were the broilers fed the L diet early on heavier at 38 days of age, but they were more efficient in converting feed to gain, had high tibia ash and higher P retention than those fed the C diet in the first 90 hr of life. This clearly establishes that "imprinting" or modifications are occurring in the animal that are long term and that allow for improved P utilization when P deficient diets are fed in the grower/finisher phases.

TABLE 1. Total P apparent absorption, phytate P disappearance, and calcium apparent absorption up to distal ileum of 18, 21, 23 and 32 d old broilers as affected dietary treatments ^{1,2} (from Yan et al, 2005)

	Control-Control	Control-Low	Low-Low	SEM ³	P-value
 %				
Total phosphorus apparent absorption					
d 18		49.5 ^b	56.0 ^a	1.3	0.0034
d 21	44.1 ^b	43.5 ^b	54.7 ^a	1.0	<0.0001
d 23	43.5 ^b	42.8 ^b	57.1 ^a	2.2	0.0006
d 32	41.7 ^b	44.7 ^{ab}	47.2 ^a	1.4	0.0445
Phytate P disappearance					
d 18		12.3 ^b	37.2 ^a	2.1	<0.0001
d 21	7.3 ^c	21.1 ^b	35.3 ^a	2.3	<0.0002
d 23	6.8 ^c	18.5 ^b	40.0 ^a	3.2	<0.0001
d 32	6.0	12.6	15.5	4.5	0.1836
Calcium apparent absorption					
d 18		60.9 ^b	71.1 ^a	2.0	0.0033
d 21	55.5 ^b	58.5 ^b	70.7 ^a	0.9	<0.0001
d 23	57.7 ^b	59.2 ^b	72.0 ^a	2.2	0.0003
d 32	48.2 ^c	57.6 ^{ab}	60.5 ^a	3.9	0.0323

¹ For d 18 and 21, data are means of 7 pens with 8 birds per pen. For d 23 and 32, data are means of 6, 4, and 7 pens with 5 birds per pen for control-control, control-low, and low-low groups, respectively.

² Determined non-phytate P levels were 0.43 % and 0.29 % for the control and low starter diets respectively, 0.40 % and 0.29 % for the control and low grower diets, respectively. The analyzed calcium levels were 0.95 % and 0.63 % for the control and low starter diets, respectively, 0.86 % and 0.65 % for the control and low grower diets respectively.

³ Standard error of mean (weighted where n was not equal).

^{abc} Means in rows with common superscripts do not differ significantly (P<0.05).

Initial work has been done looking at whether nutritional imprinting can be used to change the ability of the broiler to handle low protein diets in the finisher and withdrawal phases where the greatest proportion of the protein is fed (on amount fed per bird). Broilers fed a high lysine diet in the pre-starter phase were able to tolerate a decrease in protein in the finisher and withdrawal phases. Angel et al., (unpublished) found that body weight of 42 day old broilers fed a high lysine pre-starter and a low protein finisher and withdrawal diets was slightly, but significantly lower than the body weight of birds fed the control diet. However, feed efficiency, carcass yield and breast yield was not affected by the low protein diets. Additionally N excretion from birds fed the low protein Fn and Wd diets was 20.6% lower (P < 0.01) compared to the N excretion from birds fed the control diets. Replication in this study was limited and the goal of the work focused on reducing N excretion. The findings related to the impact of early lysine were incidental and merits further research. More information is needed to determine the impact of this feeding strategy on flock performance and breast yield of birds raised in a floor pen environment before this strategy can be fully implemented under commercial conditions.

Based on the controlled battery work done to date it appears that nutritional imprinting can be a potent management and nutritional tool that imparts on the birds the long term ability to more efficiently utilize deficient concentrations of dietary P and protein when these are fed in the finisher and withdrawal phases. More work is needed to, one determine the how well the P imprinting concept works in floor pens and under conditions more similar to those seen in industry and two to further explore the initial finding with high lysine in starter diets.

Table 2. Impact of early dietary deficiencies of phosphorus (P) and calcium (Ca) on long term utilization of P and Ca and on performance (Angel et al., unpublished)

Treatment ¹	C-C-C	C-C-L	L-C-L	SEM	P values
90 hr					
Weight gain, g	41.2 ²		36.2 ²	0.775	<0.001
Feed to gain ratio	0.83		0.88	0.016	0.028
Toe ash, %	13.37		11.17	0.057	<0.001
Apparent P absorption, %	57.6		61.3	0.667	<0.001
Apparent P absorption, gr/period	0.147		0.096	0.004	<0.001
Apparent Ca absorption, %	64.41		65.59	1.221	<0.001
Apparent Ca absorption, gr/period	0.242		0.120	0.006	<0.001
90 hr to 8 days of age					
8 days Body weight, g	150.2		141.8	0.248	<0.001
Weight gain, g	63.5		64.5	1.222	0.557
Feed to gain ratio	1.15		1.12	0.027	0.367
8 to 22 days of age					
22 days Body weight, g	904.9		884.7	6.530	0.025
Weight gain, g	754.2		742.2	6.605	0.179
Feed to gain ratio	1.40		1.40	0.010	0.967
Apparent P absorption, %	51.46		52.94	1.612	0.513
22 to 38 days of age⁴					
38 days Body weight	2345.9 ^a	2235.4 ^c	2275.6 ^b	20.158	0.001
Gain, g	1450.9 ^a	1333.0 ^c	1380.6 ^b	15.766	0.03
Feed to gain ratio	1.82 ^{ab}	1.89 ^a	1.76 ^b	0.033	0.013
38 day Toe ash, %	12.62 ^a	10.53 ^c	11.24 ^b	0.171	<0.001
38 day Apparent P absorption, %	60.11 ^a	45.39 ^c	56.54 ^b	1.562	<0.001

¹Treatments are: Control (C) – C –C (fed in all phases) diets that met National Research Council (NRC, 1994) nutrient recommendations (including those for Ca and P); C-C-L fed the C diets from hatch to 22 d of age and then the Low (L) diet from 22 to 38 d of age; L-C-L fed a L diet from hatch to 90 hr, a C diet from 90 hr to 22 d and a L diet from 22 to 38 d. The L diet that met all NRC (1994) nutrient recommendations except for those of Ca and P. From hatch to 90 hr the L diet fed contained 0.59% Ca and 0.25% available P while the C diet contained 1.11% Ca and 0.50% available P. From 22 to 38 d of age the L diet contained 0.40% Ca and 0.11% available P while the C diet contained 0.70% Ca and 0.30% available P.

²The C treatment was replicated 16 times while the L treatment was replicated eight times up to 22 d of age.

³Each treatment was replicated eight times from 22 to 38 d of age.

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