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Importance of fiber in pullet diets

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Introduction

Fiber sources have been traditionally associated in non-ruminant and human nutrition, with negative attributes, including a reduction in palatability and voluntary feed intake (FI), and a decrease in digestibility of most nutrients (Hamberg et al., 1989; Mateos et al., 2002; de Vries et al., 2012). However, most of these studies showing negative effects of fiber inclusion were conducted using high levels of fiber. Recent research conducted with broilers (Gonzalez-Alvarado et al., 2008; Amerah et al., 2009; Jimenez-Moreno et al., 2009a,c, 2013a), pullets (Guzmán et al, 2013a,b), turkeys (Sklan et al., 2003), and laying hens (Hetland et al., 2001, 2005) have shown that moderate amounts of selected fiber sources might benefit the development and function of the digestive tract and improve nutrient digestibility and performance. Moreover, the inclusion of extra fiber in the diets of pullets, laying hens, and broiler breeders reduces the incidence of cannibalism and mortality (Hartini et al., 2002; Mateos et al., 2012a, b; van Krimpen et al., 2009), improves animal welfare (Aerni et al., 2000; Hocking et al., 2004), and ameliorates the structure and consistency of the feces, with a lower incidence of dirty eggs (Pottguter, 2008; Amerah et al., 2009). Also, dietary fiber (DF) reduces ammonia emissions from laying hen manure (Roberts et al., 2006, 2007) and limits infective episodes by pathogens such as *Clostridium spp* (Kalmendal et al., 2011) and *Salmonella enteritidis* (McReynolds et al., 2006). Consequently, DF should be considered as a "nutrient" rather than as an "anti-nutritional factor" in animal feeding.

Role of dietary fiber in poultry diets

The fibrous fraction of the feeds encompasses a group of heterogeneous compounds differing in chemical composition and physical properties (Graham and Aman, 1991; Bach Knudsen, 2001). Dietary fiber is the most used term to define the fiber fraction of ingredients and feeds, and includes cell walls, stored non-starch polysaccharides (NSP), and lignin (Bach Knudsen, 2001). Based on their physico-chemical properties, DF can be divided into soluble and insoluble fractions with distinct effects on digestive physiology and animal metabolism. Consequently, the benefits of fiber inclusion in poultry diets will vary depending on factors such as characteristics of the fiber source, type of bird, and digestive health status.

Broilers, especially at young ages, require diets with high levels of crude protein, starch, and fat to meet needs for growth. However, the amount of fiber of these diets is limited and often, the physiological needs of the birds for structural components are not satisfied. When low crude fiber diets (< 3% CF) are fed, the development of the gastrointestinal tract (GIT), including the gizzard, is hindered resulting in reduced nutrient digestibility and poor feed efficiency (Mateos et al., 2012a, 2013; Verdal et al., 2013). Under these circumstances, the inclusion of moderate amounts of fiber might benefit GIT and gizzard development (Gonzalez-Alvarado et al., 2007; Sacranie et al., 2012). Also, the production of HCl, bile acids, and endogenous enzyme secretions is enhanced when low fiber diets are supplemented with adequate sources and amount of fiber (Rogel et al., 1987; Svihus, 2011; Sacranie et al., 2012). In addition to changes in the pH of the different digestive organs, DF also modifies the rate of feed passage of the digesta along the GIT, which might affect microbial growth and profile (Amerah et al., 2009; Perez et al., 2011; Rochell et al., 2012).

Data from different research centers indicate that the gizzard is the pace-maker of the GIT and acts as "the Director of the Orchestra" controlling the physiology, motility, and smooth work and functioning of the digestive tract. When the birds from a given flock show gizzards uniform in size and well developed, less digestive disturbances will occur, resulting in improved health status, productive performance, and wellbeing of the birds. Under practical conditions, feeding coarsely ground diets or including whole wheat or additional

fiber to the diet will improve gizzard development. The mechanisms underlying the positive effects of these feeding practices on bird performance are not fully understood, although it is believed that they stimulate gizzard activity and digestive secretions (Hetland et al., 2003). A well developed gizzard improves gut motility and may facilitate the release of cholecystokinin which in turn stimulates de secretion of pancreatic enzymes and gastro-duodenal refluxes (Duke, 1992; Amerah et al., 2009).

Dietary fiber and poultry performance

Numerous factors affect the response of avian species to the inclusion of fiber in the diet (Montagne et al., 2003; Mateos et al., 2002, 2012a). Some of these factors are related to the bird itself (i.e., species, age), some of them to the ingredient composition and nutritive value of the diet (i.e., nutrient density, fiber content, feed form), some to type (i.e. soluble vs. insoluble; lignin content; particle size) and level of fiber used, and some to the management of the birds and environmental conditions during rearing (i.e., hygiene of the barn, disease challenge, health status). Moreover, the numerous potential interactions (positives and negatives) among these factors modify the final effects as compared with effects when each of them is present alone. For example, a high level of fiber in the diet might be less adequate in healthy animals with high rate of growth than in animals challenged by the presence of microorganisms responsible for digestive disturbances (Berrocoso et al., 2013). Consequently, it is not easy to predict and give accurate recommendations on the level of "fiber" to be used in poultry diets. Factors with greater impact and interest are: a) type, particle size, and level of the fiber in the diet, b) target species and age of the bird, and c) management, hygiene conditions, and health status of the chickens.

a. Fiber type, particle size, and level of inclusion

Fiber sources differ widely on their metabolic and physiological effects in humans and animals. Depending on their properties, DF will show different fates and fermentation patterns along the GIT. The water soluble fraction of the fiber is viscous in nature and depending on the structure, porosity, and lignin content, will be fermented at different extent by the microbial population present in the crop and the large intestine. Therefore, soluble

fiber sources, such as sugar beet pulp (SBP) might show a) increased digesta viscosity which may impair nutrient digestibility, b) higher water holding capacity (because of the high pectin contents and porosity), c) bulkier digesta which may reduce FI (Guillon and Champ, 2000), and d) increased fermentation processes with production of volatile fatty acid (VFA). These VFA can be used as a direct source of energy by the mucosa cells. Also, VFA will reduce pH in the hindgut, helping in the control of microbial growth. On the other hand, insoluble fiber sources, such as oat hulls (OH) will show a) higher retention time in the gizzard (depending on particle size) which might affect FI, b) increased rate of passage in the distal part of the GIT where it will be hardly fermented, and c) abrasion of the wall of the GIT which might affect mucine and mucosa integrity and increase endogenous nutrient losses. However, DF might also improve the tonicity of the walls, defending the digestive mucosa against the adherence of potential pathogens present in the digesta. Interestingly, Jimenez-Moreno et al. (2009b) reported an improvement in ileal digestibility of most nutrients in broilers when 30 g OH/kg were added to the diet whereas no positive effects were observed for SBP. Also, Gonzalez-Alvarado et al. (2010) reported higher FI in broilers fed 30 g OH/kg than in broilers fed 30 g SBP/kg. The authors suggested that the reduction in FI in birds fed SBP was due to higher water retention that increased the bulkiness of the digesta content and the feeling of satiety of the birds. Recently, Jimenez-Moreno et al. (unpublished data) studied the effects of including 2.5% or 5.0% of 3 insoluble fiber sources (rice hulls, SFH, and OH) in broilers feeds presented as mash or pellets at expense (wt:wt) of the control diet on performance from 0 to 21 d of age. The data showed that the inclusion of insoluble fiber in a low fiber diet improved ADG and F:G of the chicks but no differences among insoluble fiber sources were detected (Table 1).

Particle size influences the fate of different events occurring in the digestive tract of broilers such as transit time, fermentation capability, and adherence to bile, microorganisms, and certain digestive and dietary components. Fiber structure and size also plays an important role on the physiology and development of the gizzard and the GIT. Particle size (and available surface) of the fiber sources depend on the nature, the grinding process (e.g. roller mills vs. hammer mills), and feed form (mash vs. pellets). The mean particle size and particle uniformity of different fiber sources will vary even when ground using a common mill with the same screen size. For example, OH particles are fusiform and quite flexible

whereas SBP are round and break easily when friction and pressure are applied (Mateos et al., 2012a). Therefore, the mean particle size will be higher for OH than for SBP because a higher percentage of OH particles will pass unchanged through the screen. Consequently, OH will be coarser than SBP, in spite of both fibers being ground under similar conditions. In addition, particle size of the fiber sources varies during the transit in the GIT, as a result of gizzard mechanical action and bacterial degradation in the large intestine. Therefore, the mean particle size of a fiber source before ingestion is not necessarily relevant to assess its effects on GIT physiology.

Table 1. Main effect[1] of inclusion (wt:wt) of insoluble fiber sources on growth performance of broilers fed mash or pellet diets from 0 to 21 d of age (Jimenez-Moreno et al., unpublished data)

Type	Level (%)	ADG (g)	ADFI (g)	F:G (g/g)
Control	-	33.0	41.9	1.272
Oat hulls	2.5	34.1	42.9	1.259
Oat hulls	5.0	33.8	42.2	1.255
Rice hulls	2.5	34.2	42.8	1.255
Rice hulls	5.0	34.7	43.9	1.265
SF hulls	2.5	33.8	42.1	1.248
SF hulls	5.0	33.7	42.2	1.254
SEM (n = 14)		0.48	0.58	0.006
		Probability		
Control vs. all fiber diets		*	NS	*
Fiber source		NS	NS	NS
Fiber level		NS	NS	NS

[1]Average of feeds presented as mash or pellets.

Jimenez-Moreno et al. (2010) compared the effects of including 30 g/kg of diet of microcrystalline cellulose, OH, or SBP. Cellulose inclusion had little effect on gizzard development or broiler growth, probably because this fiber source lacks of physical structure and does not affect the anatomy or the physiology of the GIT. The inclusion of SBP improved energy retention but had no effects on ADG, probably because of the decrease in FI observed. In contrast, OH an insoluble NSP source, improved energy digestibility and ADG of the birds. The results of these trials highlight the different response of growing birds to fiber sources differing in physico-chemical characteristics.

Level of inclusion affects the response of poultry to DF with more positive effects in diets with low fiber content and more debatable effects at moderate or high levels of fiber inclusion. An increase in DF, especially of the soluble fraction, results in bulkier digesta that can induce satiety and reduce FI. Modern broilers are characterized by their voracity and extremely high rates of growth, which requires fine tuning in the design and manufacturing of the feed. In fact, poultry diets are formulated not only to meet nutrient requirements but also to favor voluntary FI of the birds. When fiber is added to the diet, energy intake might decrease in growing birds because of poor palatability or limited capacity of the GIT at this age. Jimenez-Moreno et al. (2011, 2013a, b) studied in 3 experiments the effects of including 25, 50, and 75 g/kg of pea hulls, OH, or SBP at expenses of the control diet (wt:wt) on digestive organ size, nutrient digestibility, and broiler performance from 1 to 21 d of age. The results of these trials confirmed the positive effects of including pea hulls or OH in the diet at levels of up to 50 g/kg on most of the variable studied, including gizzard weight, gizzard pH, and growth rate. However, a further increase to 75 g/kg had no further effects on organ development and gizzard pH and in fact, reduced nutrient digestibility and growth performance of the birds. The inclusion of 25 g/kg SBP in this control diet also improved the traits studied but the effects were less pronounced that when OH or pea hulls were used. Moreover, SBP inclusion at levels of 50 g/kg, already affected FI and growth of the birds. SBP is rich in pectin, a soluble fraction of DF that increases bulkiness and viscosity of the digesta and hinder the diffusion and absorption of nutrients in the small intestine. Consequently, an excess of dietary pectin might reduce FI and nutrient utilization at a higher extent that and excess of insoluble fiber. All these data confirm that broilers fed low fiber

diets benefits from additional fiber but that an excess might not be recommended. The level of fiber to be used in poultry diets will depend on the age of the bird as well as on the type of fiber used.

b. Target species and age of the bird

Numerous studies have demonstrated the effects of DF on different aspects of broiler and laying hen production, but the information available in pullets is scarce and more elusive. In fact, few papers have been published in recent years on the potential benefits of DF on pullet performance and subsequent egg production during the laying cycle. Guzmán et al. (2013b) included 2 or 4% straw, SFM, or SBP at the expense (wt:wt) of the control diet in pullets from 0 to 5 wk of age and reported higher ADG but similar F:G ratios when the insoluble fiber sources were added (Table 2). However, pullets fed SBP tended to grow less than pullets fed SFH, although the differences did not reach significance. In a recent research (Kimiaeitalab et al., unpublished) we compared the pH in the different organs of the GIT of broilers and pullets from 1 to 21 d of age fed commercial diets with or without an additional fiber source. The treatments were organized as a 2 x 2 x 2 factorial with 2 type of birds (broiler vs. pullets), 2 type of diets (commercial starter diet for broilers vs. commercial starter diet for pullets), and 2 levels of SFH in the diets [0 vs. 30 g/kg in substitution (wt:wt) of the control diet]. Results obtained for selected digestive organs and main effects at 21 d of age are shown in Table 3. Pullets had higher crop pH (4.71 vs. 4.51; $P < 0.001$) and lower gizzard (2.20 vs. 2.43; $P < 0.05$), ileum (6.59 vs. 6.79; $P < 0.10$), and cecum (5.72 vs. 6.12; $P < 0.001$) pH than broilers (Table 3). Also, the inclusion of SFH to the diet reduced gizzard pH (2.20 vs. 2.43; $P < 0.05$) but had little effect on the pH of the other organs.

Table 2. Influence of fiber source and level of fiber inclusion in the diet on growth performance of pullets from hatching to 5 week of age (Guzmán et al., 2013a)

Fiber inclusion		ADFI	ADG	F:G
Source	Level (%)	(g)	(g)	(g/g)
Control	0	19.8	8.2	2.41
Cereal straw	2	20.2	8.4	2.40
	4	20.5	8.5	2.41
Sunflower hulls	2	20.5	8.7	2.36
	4	20.5	8.5	2.41
Sugar beet pulp	2	20.3	8.4	2.41
	4	20.4	8.3	2.47
Fiber inclusion				
Control		19.8	8.2	2.41
Fiber diets (average)		20.4	8.5	2.41
Fiber source				
Cereal straw		20.4	8.5	2.41
Sunflower hulls		20.5	8.6	2.38
Sugar beet pulp		20.4	8.4	2.44
Inclusion level (%)				
2		20.3	8.5	2.39
4		20.5	8.4	2.43
Sd[1]		0.72	0.32	0.088
Probability				
Control vs. fiber diets[2]		*	*	NS
Fiber source		NS	†	NS
Inclusion level		NS	NS	†
Fiber source x inclusion level		NS	NS	NS

[a-b-c]Within a column, means without a common superscript differ ($P < 0.05$).

[1]Standard deviation (n=10 replicates per treatment).

[2]Control (no additional fiber) vs. the average of the 6 fiber containing diets.

Table 3.- Influence of inclusion of 30 g sunflower meal (SFH)/kg diet on pH of the crop, gizzard, ileum, and ceca of pullets and broilers at 21 d of age[1] (Unpublished data)

	Crop	Gizzard	Ileum	Ceca
Type of bird				
Broiler	4.51	2.43	6.79	6.12
Pullet	4.71	2.20	6.59	5.72
P	**	*	†	***
Fiber inclusion				
Control	4.61	2.43	6.61	5.87
SFH	4.78	2.20	6.78	5.97
P	†	*	†	NS

[1]Only selected main effects are presented.

It has been postulated that pullets will benefit from high fiber diets during the last part of the growing period because of better development of the GIT which will favor voluntary FI at the onset of the laying period. An increase in FI will improve BW of the young hens, resulting in better egg production and an increase in egg weight. Recent studies conducted in our laboratory (Guzmán et al., 2013a, b) have shown that an increase in the fiber content of the diet increases gizzard weight, reduces gizzard pH, and improve GIT development at 5, 10, and 17 wk of age. However, the effects of fiber inclusion on increasing the capacity of the GIT is not long-lasting and disappeared quickly with time, once the bird received a diet low in fiber. In fact, when the hens received a common commercial layer diet, productive performance was similar for all hens, irrespective of the level of fiber used during the rearing phase (Bouali et al., 2013). These results are consistent with data of Harzalli et al. (2013) showing that pullets adapt their GIT quickly to changes in feed form of the diet during the rearing phase.

The data available suggest that pullets are less susceptible to changes in DF content of the diet than broilers of the same age. It seems that, pullets will show a less positive response to additional fiber when fed low fiber diets than broilers but also a less negative response to additional fiber when fed high fiber diets. Broilers might respond better to the

inclusion of moderate amounts of fiber than pullets and laying hens because diets for broilers are low in fiber and broilers are more voracious and have higher desire for feed than pullets or adult birds. In fact, modern broilers have a high capacity for feed consumption and when fed high-energy pelleted diets might over consume feed, resulting in poor F:G and excess of carcass fat (Svihus, 2011). DF might reduce slightly energy intake and such a reduction might result in better feed efficiency and less carcass fat. However, a reduction in FI in pullets or laying hens because of an excess in DF, might hinder productive performance.

c. Management, hygiene conditions, and health status

Dietary fiber has a protective effect against a range of metabolic problems and diseases in humans including obesity, constipation, colon cancer, and cardiovascular diseases. Also, DF might protect the mucosa layer of the GIT again external agents reducing the incidence of non-specific colitis and suppressing at a certain extent the growth of different pathogens (Mateos et al., 2012a). The inclusion of fiber in the diet affects in different ways the mechanisms of defense of the host. Some effects (e.g., increased digesta viscosity, effects on mucosa integrity) might have negative connotations on growth but others (e.g., reduced pH, changes in rate of passage, increased digestive wall tonicity, improved reverse peristaltism) might reduce the adherence of pathogens to the digestive mucosa preventing potentially harmful bacteria from entering the intestinal tract. In fact, the response to DF of susceptible animals to microbial infection, such as weaning pigs and young birds might depend not only on the type of diet used but also on the physiological and health status of the animals, with more noticeable effects under poor management practices (Berrocoso et al., 2013; Mateos et al., 2013).

Opposite to general believes, the inclusion of certain insoluble fiber to the diet, such as OH and SFH, do not necessarily increase moisture content of the excreta. Moreover, a poor consistency of the excreta is more evident when poultry are fed diets low in fiber than when fed diets containing moderate amounts of fiber. In a recent experiment (Jimenez Moreno et al., unpublished) we determined water intake, water to feed intake ratio, and moisture content of the excreta in broilers fed mash or crumble diets with or without the inclusion of 2.5 or 5% of 3 insoluble fiber sources (rice hulls, SFM, and OH) from 6 to 9 d and from 18 to 20 d of age. Water intake increased with pellet feeding at all ages, and also

from 18 to 20 d of age when the fiber sources were included in the diet. However, fiber inclusion did not affect water to feed intake ratio at any age. Moreover, DM content of the excreta was not affected by feed form or by the inclusion of any of the insoluble fiber sources in the diet at any age. Coarse fiber particles might held large amount of water and improve the consistency and structure of the droppings which in turn will facilitate management and improve the hygiene conditions of the barn (Amerah et al., 2009; Mateos et al., 2012a). Thus, excreta score is usually improved when a source of insoluble fiber is included in the diet of broilers, pullets, and laying hens. Also, the incidence of stereotypies, cannibalism, and mortality in pullets, commercial layers, and broiler breeder hens, benefits from an increase in DF, especially in non-debeaked flocks, reared at high density on parks or in flocks subjected to an aggressive light program under intensive production systems (Bearse et al., 1940).

Summary

Dietary fiber has been considered for long as a diluent of poultry diets with negative effects on nutrient digestibility and growth of broilers, pullets, and turkeys, and on productive performance of egg-laying hens. This traditional thinking is probably correct when high levels of soluble fiber, such as SBP, are included in mash diets for young birds. However, it might not be the case when moderate amounts of certain insoluble fiber sources, such as OH are included in high energy diets with less than 30 g CF/kg. Under these circumstances, the inclusion of additional insoluble fiber improves digestive organ development with increases in HCl production and endogenous enzyme secretions which favor solubility of the mineral sources and pepsin activation, and increases crude protein and starch digestibility. Most of these effects are a consequence of improved gizzard size and functioning with an increase in gastro-duodenal refluxes that allows for a better mixing of nutrients and digestive enzymes. The information available indicates that poultry requires a minimum amount of fiber in the diet for optimal GIT function and performance. However, current knowledge on fiber properties and physiological and metabolic changes within the bird induced by the fiber source, does not allow any clear recommendation on levels of use. Probably, diets for broilers with less than 30 g CF/kg will benefit from the inclusion of 20 to 30 g/kg of an

insoluble fiber source. However, an excess of DF will hinder nutrient digestibility, energy intake, and growth performance, with more negative effects in young broilers than in pullets or egg-laying hens. Moreover, pullets and adult birds might benefit of moderate to high levels of fiber (up to 60 to 70 g CF/kg) because of reducing stereotypies and stress with lower incidence of cannibalism and mortality, indicative of improved animal welfare.

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The Crop: Role in Poultry Performance and Health

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Introduction

Predicting the future of broiler nutrition and defining required research to overcome barriers can be difficult because of the ever changing nature of the birds as well as the socio-economic changes that affect the way birds are raised and fed. As it should, prediction relies heavily on previous research and industrial experience. However, often application of existing knowledge or examining nutritional issues in a more holistic fashion is also useful in predicting future best practice and research requirements.

The digestive tract of vertebrate animals is extremely complex and has evolved to serve its primary function of supplying nutrients to the host animal, while at the same time serving as a barrier to infection and harm from compounds within the digestive tract. As can be expected, functions of gastrointestinal tract (gut) segments are interdependent to provide for efficient digestion and other gut functions. Evolution of the gut took place in a natural environment, where omnivorous chickens accessed a wide range of foods and were not always able to find the level of nutrients necessary for maximum growth and reproduction. The importance for gut segments would also change to match the nature of the feedstuffs being consumed. For example, the gizzard, and caeca and caecal fermentation would increase in importance in the presence of fibrous, poorly digestible materials that might be found in winter. It is well understood today that gut segments change to reflect diet changes and that communication mechanisms are in place between gut segments to optimize nutrient retention.

With increasing use of high quality ingredients in animal feeding, the importance of all segments of the gut and their interrelationships has often been forgotten or neglected with emphasis instead being directed to delivery of digestible nutrients to the broiler. However,

more recently the importance of segments such as the proventriculus/gizzard and caeca has received renewed attention. A well developed gizzard has been demonstrated to have relevance in digestion and gut health and the caeca play a critical role in determining gut health and colonization by zoonotic organisms. For reviews of these gut segments see Svihus (2011) and Svihus et al. (2013). The crop has also been the subject of a wide range of research, but its importance in broiler feeding remains either poorly understood or neglected in research and poultry feeding. Based on the interdependence of gut segments and the relatively neglected crop, the objectives of this paper are to examine the digestive process that occurs in the crop, define its role in improving broiler nutrition and health, and then speculate on how it can be manipulated to the benefit of broiler production.

Crop anatomy

The crop is a thin walled diverticulum of the esophagus and retains the basic structural pattern of the digestive tract (Hodges, 1974). Extending from the lumen are non-keratinized stratified squamous epithelium of the mucous membrane, lamina propria (connective tissue), muscularis mucosae, sub-mucosa (a layer of loose connective tissue), muscularis externa and the serosa. The structure of the crop is well innervated and vascularised implying potential for interaction with other gut segments and possibly a variety of roles related to digestion and gut health. Mucous glands are found in the oesophagus and also at the interface between the oesophagus and crop, but are not found in the crop diverticulum (Fuller and Booker, 1977). Similarly, structural differences have been noted between the oesophageal area of the crop (slightly convoluted surface with a high density of bacteria) and the apex of the diverticulum (flatter surface, numerous sloughing epithelial cells, fewer bacteria) (Bayer et al., 1975). The non-secretary nature of the crop suggests a limited capacity for digestion, but moisture and enzymes derived from saliva, feed and bacteria play a role in initiating the digestive process. Demonstrated bacterial adhesion to starch granules is just one example of how digestion can occur in the crop (Bayer et al., 1975). The importance of the crop in diet digestion will presumably be much affected by the proportion of the feed that enters the crop and the amount of time it spends there. The nature of the crop epithelium indicates that it is not an important area for absorption. However, some have suggested that absorption by diffusion of organic acids such as lactic acid and DL-2-hydroxy-4(methylthio) butanoic acid

may be possible (Cutler et al., 2005; Richards et al., 2005). Again, residency time of these compounds in the crop would likely impact this potential.

Feed residency in the crop

The degree to which feed enters and how long feed is resident in the crop is variable and highly dependent on the nature of bird feeding behaviour, feed presentation (e.g., meal vs. ad libitum) and bird management (e.g., periods of darkness). Estimates of crop retention time in birds given ad libitum access to feed and 24 hours of light per day are 7.4 and 24.6 minutes for broiler and White Leghorn chicks, respectively (Shires et al., 1987). In this setting, it appears that feed is primarily directed to and accumulates in the gastric stomach and small intestine. Therefore, not only will crop residency be short, but it is also likely that most feed will not enter the crop (Savory, 1985). An extreme contrast to this situation is broiler breeders, during the rearing period and fed on an every-other-day feeding schedule, where the crop does not empty until 20 hours after feeding (Deep and Classen, University of Saskatchewan unpublished). Similarly, the time feed spends in the crop can be affected by lighting programs with extended periods of darkness. In turkeys, digesta remained in the crop for 9 hours after the end of the photophase in turkeys given a 14L:10D lighting program (Cutler et al., 2005). Turkeys and chickens are nocturnal and eat during the day if not limited by day length or other environmental factors such as high temperature. Feeding behaviour has a diurnal pattern in birds given a dark period with the nature of the pattern dependent on the length of the dark period (Schwean-Lardner et al., 2013). Feed intake may or may not be high immediately after lights come on, and increases again prior to lights going off (Buyse et al., 1993; Schwean-Lardner et al., 2013). Early morning feeding can be rationalized by hunger associated with the extended period of time during darkness, but when not seen may be attributed to the capacity of the crop to store feed for an extended period of time and therefore a lack of hunger (Scanes et al., 1987). The late day increase in feed intake is anticipatory and takes some time for birds to learn after first being exposed to a dark period. The rationale is that birds increase feed intake so that their nutritional needs are met for at least a portion of the dark period. Buyse et al. (1993) found that the crop contained only small quantities of ingesta during the photoperiod in a 14L:10D lighting program, in apparent agreement with Shires et al. (1987). However, the ingesta content of the crop

increased dramatically (10.5-fold) at the beginning of the scotophase as a result of late day feeding. The crop content decreased gradually during the scotoperiod and of note feed transit time was longer during the night; this was also found by others (Cutler et al., 2005; Duve et al., 2011; Scanes et al., 1987). These authors speculated that the storage of feed in the crop, its gradual release and the increased food transit time at night resulted in the majority of the bird's nocturnal energy needs being met. Anticipatory feeding appears to be affected by the length of the scotoperiod. In a comparison of 16L:8D and 13L:4D:3L:4D lighting programs, Duve et al. (2011) found that birds on the latter lighting program failed to display anticipatory feeding before either of the dark periods and suggested that its absence was caused by a lack of need to feed rather than not predicting the dark period. This research plus other work (Schwean-Larder et al., 2013) suggest that dark periods of greater than four hours are necessary to induce anticipatory feeding behaviour. In conclusion, use of the crop as a feed storage device in minimal when highly nutritious feed is readily available and there are no constraints on feeding behaviour (like a commercial broiler barn). However, birds utilize crop storage in response to hunger (e.g. food deprivation) or regular periods of darkness (Savory, 1985).

The control of gut emptying is complex, but the proventriculus/gizzard play a central role (Chaplin et al., 1992; Jackson and Duke, 1995). Signalling includes neurological (vagus nerve; Denbow, 1989) and hormonal (e.g. ghrelin; Kaiya et al., 2009; glucagon-like peptide-1, Tachibana et al., 2003) mechanisms.

Bacterial population

Lactobacilli dominate the bacterial community of the crop, but Coliforms, Streptococci and Bifidobacteria are also present (Fuller, 1973; Fuller and Booker, 1974; Fuller and Turvey, 1971; Hilmi et al., 2007; Peinado et al., 2013; Petr and Rada, 2001). At least some Lactobacilli are capable of binding to the crop epithelium to form biofilm layers that are relatively uniquely found on non-secretory stratified squamous epithelium (Edelman et al., 2002; Fuller and Turvey, 1971; Lebeer et al., 2011). Adherence and colonization are vital to prevent removal with passing digesta, and also to permit attached Lactobacilli to seed the "new" digesta contents as epithelial cells are sloughed. The exact nature of the crop

microflora continues to increase in clarity as newer techniques of identification become available (Cousin et al., 2012; Hammons et al., 2010).

Bacterial colonization of the crop is initiated either just prior to or after hatch (Barnes et al., 1980). Colonization is variable in young chicks immediately after hatch, and the speed and nature of crop bacterial colonization is influenced by a variety of factors in the diet. This is a relatively important part of the bird's life as they also are most susceptible to colonization by non-desirable pathogenic and zoonotic organisms at that time (Gast and Beard, 1989; Smith and Tucker, 1980). Factors affecting speed of Lactobacilli colonization include probiotics, prebiotics, organic acids, medication (Rada and Marounek,1996), and the feedstuffs themselves (Rubio et al., 1998). Lactobacilli can be eliminated from the crop by anti-bacterial agents such as penicillin and monensin, and when this occurs, the number of Coliforms increases (Rada and Marounek, 1996). Of note, Escherichia coli (E. coli) are capable of binding to crop enterocytes. Their colonization can be inhibited by adhering strains of Lactobacilli (ST1 Lactobacillus crispatus) because of shared adhesion sites, but not by Lactobacilli that are weakly adhesive (Lactobacillus crispatus strain 134mi) (Edelman et al., 2003). Similarly, Lactobacilli are capable of inhibiting Salmonella colonization of the gut (Gusils et al., 1999). The ability of Lactobacilli to prevent colonization can be attributed to a number of mechanisms including competing adherence sites, stimulation of the immune system, antibacterial agents and low pH as a result of fermentation. These studies (and many others) support the concept that a stable and dominant position of adhering Lactobacilli in the crop is essential for gut health and the development and maintenance of a balanced crop microflora (Fuller 1973, 1977).

The absence of feed, such as during feed withdrawal prior to slaughter or during moulting procedures, results in a shift in the bacterial community and predisposition of the crop to Salmonella and Campylobacter colonization. Feed withdrawal increases the potential for pathogen presence as a result of decreased Lactobacilli colonization. Among other changes decreased Lactobacilli numbers result in decreased production of lactic acid and other short chain fatty acids (SCFA), and increased pH (Durant et al., 1999; Hinton et al., 2000a). The presence of Salmonella and Campylobacter in the crop of broilers at slaughter represents a human disease risk because of the higher potential of carcass contamination at slaughter from crop than caecal rupture (Corrier et al., 1999; Hargis et al., 1995; van Gerwe et al.,

2010). This emphasizes the need to reduce Salmonella colonization during grow-out and maintaining this status during the feed withdrawal period. Inclusion of organic acids or fermentable substrates in water can reduce the Salmonella and Campylobacter contamination of crops and broiler carcasses (Byrd et al., 2001 (lactic acid); Chaveerach et al., 2004 (acidified drinking water); Hinton et al., 2002b (sucrose, glucose))

Feed withdrawal in laying hens also markedly increases the survival of Salmonella in the crop (Humphrey et al., 1993) and may provide an environment that increased the expression of genes necessary for intestinal invasion (Durant et al., 1999).

Role in gut health and pathogen colonization

The presence of pathogens harboured in the crop and additional organisms that pass through the crop to the lower gut suggest the need for expression of both adaptive and innate immune responses in the crop. Research has confirmed this logic with the demonstration of well-developed lymph nodules in the upper alimentary tract in the form of oesophageal tonsils (Arai et al., 1988). A further demonstration of immune competency in the crop is the development of lymphoid aggregates in crop walls following challenge with Salmonella enteritidis (Seo et al., 2003; Vaughn et al., 2008a, 2008b) and the presence of secretory immunoglobulins As (IgA) that specifically bind to Salmonella enteritidis antigens (Seo et al., 2002, 2003). The high expression of β-defensin gallinacin-6 (Gal-6) in the oesophagus and crop, and the demonstration of its antimicrobial activity against food-borne pathogens, demonstrates that the crop can play a role in chicken innate host defense (van Dijk et al., 2007). Taken together, these findings suggest an influence of the crop in local and total digestive tract health and pathogen colonization in addition to its conventionally described role in feed storage.

The crop is a part of the acidic barrier formed by the crop and gizzard that reduces passage of bacteria including pathogenic (Clostridium) and zoonotic (Salmonella, Campylobacter) genera to the distal gut with the gizzard being the more potent component of this barrier. Sekelja et al. (2012) investigated the relationship between bacterial phylogroups in the excreta and gastrointestinal tract of broiler chickens fed fine or course Brewer's spent grain. When Brewer's spent grains were fed in the fine form, they found that two Clostridial phylogroups were related to the bacterial populations in the caecum/colon and the small

intestine, and a Lactobacillus phylogroup related to the microbiota in the crop and gizzard. When the same diet was fed with course Brewer's spent grain, gizzard development was stimulated, and excreta phylogroups showed less relationship to the bacteria present in the proximal gastrointestinal tract, supporting the importance of gastric barrier function. The impact of crop microbiota on caecal flora was demonstrated by research using dietary butyric acid in unprotected and partially protected form to study Salmonella Enteriditis colonization in the crop and caeca (Fernández-Rubio et al. 2009). Both sources of butyric acid reduced crop and caecal colonization, but the unprotected butyric acid was more effective in the crop and less effective in the caeca as expected. However, the still important effect of the unprotected butyric acid in the caeca suggests that controlling colonization in the crop benefits the entire digestive tract.

Crop pH and fermentation

The pH of the chicken crop can vary with values ranging from below 5 to greater than 6 (Bowen and Waldroup, 1969; Hinton et al., 2000a; Józefiak et al., 2006; Rynsburger, 2009). The pH varies with the degree of crop fermentation primarily by Lactobacilli and the production of lactic acid and other SCFA (Cutler et al. 2005; Józefiak et al., 2006). In turn, fermentation is influenced by factors such as the presence of substrate (feed or prebiotic) and again colonization by Lactobacilli (Barnes et al., 1980; Fonseca et al., 2010). The pH of the crop can also be impacted by other non-fermented components of the diet. Feed ingredients have been found to have differing acid binding capacity that can affect crop pH with mineral ingredients having the highest capacity followed by protein ingredients and then energy sources (Lawlor et al., 2005).

As noted above, extended dark periods result in anticipatory eating and crop storage (Cutler et al., 2005). In turkeys provided with a 14:10D lighting program, feed gradually emptied from the crop for 9 hours after the beginning of darkness and during that time pH decreased from 5.9 to 5.0; at the same time levels of lactic acid and SCFA were increasing. This demonstrates that the presence of feed in the crop for an extended time enhances fermentation. In a second experiment, Cutler et al (2005) found that the number of Salmonella typhimurium in crop digesta decreased from 7.1 to 4.9 (log10)/gram of crop

digesta over an 8 hour period starting after the end of the photophase. This research confirms the beneficial effect of fermentation on crop pH and subsequently bacterial colonization.
The low pH has direct inhibition effects against a variety of pathogenic and zoonotic organisms and also enhances the impact of butyric acid when it is used in water or broiler feed. The effectiveness against these organisms increases at lower pH because butyric acid in its dissociated form can cross bacterial membranes and acidify the bacterial cell cytoplasm. Therefore, unprotected free sodium butyrate should be more effective against bacteria at the acidic pH of the upper portion of the digestive tract (Van Immerseel et al., 2006).

Pendulous crop

Loss of tone in crop muscle can result in pendulous crop, a condition more prevalent in turkeys than broiler chickens. Despite the low incidence in broilers, research on pendulous crop may provide clues on management and nutrition required to produce a "healthy" crop. The etiology of pendulous crop is not clear, but the incidence of pendulous crop has been shown to have genetic, environmental and nutritional influences (Asmundson and Hinshaw, 1937; Wheeler et al., 1960). In a comparison of feeding glucose monohydrate or starch to turkeys, a high incidence of pendulous crop was found for the birds fed glucose monohydrate and none were found for birds fed starch. In addition, abnormal fermentation was found as a result of yeast and fungi colonization of the pendulous crop birds. Preliminary evidence from turkey lighting research (Vermette, Schwean-Lardner and Classen, University of Saskatchewan unpublished) has found a higher incidence of pendulous crop in birds given 23 in contrast to those receiving 14, 17 and 20 hours per day. It may be that crop feed storage is required to maintain a scheduled flow of feed through the crop and maintenance of a desired Lactobacilli biofilm on crop epithelium, thereby preventing abnormal colonization by other microbiota.

Beneficial effects on bird performance

Exposing birds to darkness and meal feeding both promote crop storage of feed, and also improve feed efficiency (Schwean-Lardner et al., 2012; Su et al., 1999; Svihus et al., 2010). The improvement in feed efficiency in these cases has been attributed to factors such as

altered metabolism during darkness (Apeldoorn et al., 1999) and a more concave growth curve (Buyse et al., 1996), and improved nutrient retention (Buyse et al., 1996). An argument can be made that increased food softening, the initiation of the digestive process due to endogenous and exogenous enzymes, and slower digesta transit (at least during darkness) as a result of feed presence in the crop may result in improved nutrient digestibility and feed efficiency. Of interest, in a comparison of graded levels daylength (14, 17, 20, 23 hours), gizzard size increased and jejunum and ileum weights decreased with shorter days (Classen, Schwean-Lardner and Fancher, University of Saskatchewan unpublished).

In a crop with lower pH as a result of fermentation, conditions are optimized for some enzymes, both endogenous and exogenous. In particular it is well established that phytase hydrolysis in the crop can be extensive (Onyango et al,. 2005; Lan et al., 2010). Of interest is the finding that β-glucanase supplementation of broiler diets containing oats or barley increased the lactic acid concentration and lowered the pH in the crop (Józefiak et al., 2006). In contrast, the presence of β-glucanase decreased butyrate levels suggesting a shift in the crop bacterial population and stimulation of Lactobacilli growth. This demonstrates that exogenous enzymes are active in the crop and this influences conditions in the lower gut that beneficially affect production characteristics. Enzyme use improved performance criteria, but it is not possible to relate this improvement to effects in the crop. Unfortunately, the lighting program used was not identified so it is also not possible to know whether increased crop storage would enhance the β-glucanase effect. These are just two examples of positive nutritional effects occurring in the crop.

Developing and maintaining a healthy crop

The crop, though often relegated to being just a storage organ, can play a role in broiler health and nutrient retention, as well as colonization by zoonotic organisms. Positive effects appear to relate to regular utilization of the crop for feed storage. It can be expected that a well utilized crop will have a well developed Lactobacilli biofilm throughout the entire crop surface and not just near the oesophagus. In turn this will result in active fermentation and the production of lactic acid and SCFA, and lower crop pH. These plus other benefits of Lactobacilli colonization will promote a healthier gut with less colonization by pathogenic or

zoonotic organisms, and provide an environment that increases feed digestibility. Because of the susceptibility to colonization by undesirable organisms in newly hatched birds, early promotion of crop Lactobacilli colonization is highly desirable.

Providing birds with darkness is an easy method of stimulating crop utilization. However, the use of continuous or near-continuous light is predominant in the early part of a broiler's life with introduction of more substantial periods of darkness no earlier than 4 days of age and most often 7 or more days of age. The degree of crop utilization during this period is not known, but it is common to judge crop fill on the day after placement, with a recommendation that ~90% of birds will have feed in their crop. This degree of fill may be related to it being the result of the first meal, and as feeding behaviour acclimatizes to continuous light the degree of crop storage may be decreased. It is possible that earlier provision of darkness would increase crop storage of feed. This has been recommended to synchronize flocks and also provide young chicks with rest (Malleau et al., 2007), a practice that was found to have no adverse effects on early performance or carcass composition. Early introduction of darkness is also now recommended for laying hen pullets (Lohmann LSL Management Guide; http://www.hylinena.com/UserDocs/products/LSL-LITE_Commercials_North_American_Edition_2012.pdf) with birds receiving a dark period upon arrival at the farm and then provided with an intermittent lighting program (4 x 4L:2D) until 7 to 10 days of age when they are switched to a regular lighting program. The management guide lists flock synchronization, stimulation of weak chicks, easier assessment of the flock and lower mortality as advantages of this lighting program. Although the exact nature of the intermittent lighting program has not been thoroughly investigated for this age, possibly a program of this nature would encourage crop fill and the suggested benefits. It is of interest that in natural brooding the presence of the broody hen synchronizes chick behaviour and stimulates long inactive and active periods (Riber et al., 2007). Is this related to enhanced crop use and can it be mimicked using intermittent lighting?

In broilers, a longer dark period could be initiated after the intermittent phase to encourage anticipatory feeding prior to the scotophase. A longer dark phase would appear to be appropriate because of the evidence that 4 hours of darkness is required to develop anticipatory feeding behaviour. Alternately, a longer dark period could be combined with intermittent lighting or meal feeding during the "photophase". Simulating dawn and dusk

would be recommended for both intermittent and diurnal phases of the broiler life cycle to provide a stronger signal of the upcoming dark period.

Near broiler market age, it is not uncommon for broilers to be returned to near continuous light to increase ease of bird handling and preparation for feed withdrawal. This may reduce crop Lactobacilli numbers and make the crop more susceptible to Salmonella and Campylobacter colonization, a situation that will worsen during feed withdrawal. Maintaining a longer dark period until marketing and then planned feeding prior to broiler harvesting may reduce this problem by maintaining a healthy crop and then providing feed longer in the crop (with fermentation and low pH) during withdrawal. Is feed in the crop at feed withdrawal a bad thing?

Enhancing the maturation of the crop microbiota after hatch may provide a less susceptible environment to pathogenic load and appropriate probiotics (Beasley et al., 2004; Hilmi et al., 2007) and prebiotics may be useful to accomplish that goal (Lebeer et al., 2010). Although in ovo application of probiotics would seem appropriate to ensure earliest possible bacterial colonization (Edens et al., 1997), the author is unaware of successful application.

Conclusions

The crop is often not considered in making broiler nutrition or management decisions. However, there is evidence that a functional crop can play a role in bird performance and health and the safety of broiler meat. For this to happen, the early establishment of Lactobacilli in the crop and providing substrate for fermentation by ensuring regular crop feed storage are essential. It seems logical that combinations of nutritional and management techniques are required to achieve a functional crop, including use of probiotics, probiotics, organic acids, exogenous enzymes, meal feeding and lighting programs. This paper has focused exclusively on the crop, but in the larger picture all segments of the gut should be considered when planning for successful broiler production. Therefore, in addition to decisions on the crop, nutrition that stimulates gizzard size and activity, diets that provide ingredients digested at rates that provide for efficient production and maintenance of the small intestine, and dietary constituents that fuel healthy caecal fermentation may be an important method of improving broiler performance and health in an antibiotic free era.

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Nutritional Imprinting: Early Dietary Manipulation

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Definitions

Most traits of economic importance in animal production undergo continuous phenotypic variations due multi gene changes and environmental factors. Numerous quantitative trait loci that impact agronomic traits have been identified, but generally the specific set of genes responsible, are not defined. Studies where genome-wide associations have been looked at have shown that the variability of complex traits is only partially explained by genetic variation (Manolio et al., 2009). Studies in humans and animals have shown that it is not only the DNA sequence that affects the phenotype but that epigenetic changes that can be transmitted from one generation to the next are involved (Jablonka and Raz, 2009).

But what is epigenetic? In the literature one can find numerous definitions for epigenetics and numerous terms applied to the same apparent mechanism. There are many discussions in the literature on what the term "epigenetics" refers to and this leads to numerous definitions (Holliday, 2006; Bird, 2007; Jablonka and Raz, 2009; Ho and Burggren, 2010; Hanley et al., 2010; Fresard et al., 2013). Some definitions limit the epigenetic effect only to phenotype modifications without defining or needing to show gene expression changes (Ho and Burggren, 2010) others are broader and include gene expression changes (Holiday, 2006; Bird, 2007; Jablonka and Raz, 2009). Epigenetic changes can be mediated by DNA methylation, chromatin folding and its attachment to the nuclear matrix,

packaging of DNA around nucleosomes, non coding RNA and covalent modifications of histone tails. For the purpose of this review the definition of epigenetic changes will involve both phenotypic as well as changes in gene expression that persist through the life of the animal and/or subsequent generations (mainly F1) and that are changes that are greater than the normal adaptations as for example those seen to nutrient deficiency states.

Background on Epigenetic Regulation

One of the fundamental assumptions of Mendelian genetics is that a specific allele behaves in the same way no matter from where (which parent) it originates. But as with many assumptions in science this rule does not always hold true. Initial observations in mice looking for the effects of paternal or maternal impacts using genomic translocations indicated that identical chromosomal regions were not equivalent and acted differently in embryo development between maternal and paternal sources (Cattanach & Kirk, 1985). Although the terminology surrounding this phenomenon has been referred to as gametic imprinting the interpretation is described as "an allele-specific reversible epigenetic modification dependent on the parent of origin allele" (Ruvinski, 1999). It was later hypothesized that the reason for gametic imprinting in viviparous species was to ease the conflict during pregnancy between the maternally and paternally inherited genes. Imprinting in mammals is thought to have evolved as a result of the fetus being directly nourished by maternal tissues. The paternal genes stimulating growth of the fetus while the maternal genes restricting growth so that the mother may successfully deliver the offspring. Imprinting can therefore be thought of as a compromise between the maternal and paternal alleles or a compromise between the mother and the fetus. CpG islands are short, dispersed regions of unmethylated DNA with a high frequency of CpG dinucleotides relative to the bulk genome. Other mechanisms including histone modification and chromatin structural modifications can also be responsible for epigenetic regulation of gene expression (Razin, 1998).

The growing incidence of metabolic diseases in humans, such as obesity, diabetes, and cardio-vascular disease has sparked interest and research efforts into both their genetic and environmental (nutritional among others) basis. Fetal programming as it was initially referred to in humans (Baker, 2004), encompasses the role of developmental plasticity in

response to environmental signals, including nutrition, during early life and its potential adverse consequences (risk of cardiovascular, metabolic and behavioral diseases) in later life. The first studies in this field highlighted an association between poor fetal growth and chronic adult diseases. However, environmental signals during early life may lead to adverse long-term effects independently of obvious effects on fetal growth. Adverse long-term effects reflect a mismatch between early (fetal and neonatal) environ- mental conditions and the conditions that the individual will confront later in life. The mechanisms underlying this risk remain unclear. However, experimental data in rodents and recent observations in humans suggest that epigenetic changes in regulatory genes and growth-related genes play a significant role in fetal programming.

The maternal diet, and therefore the nutrient supply to the developing embryo is one of the principal environmental factors influencing growth and development of the offspring. A reliable and balanced supply of amino acids, lipids and carbohydrates and micronutrients such as vitamins and minerals 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) in the case of nutrients 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 (Fresard et al., 2013). 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 was not elucidated then. More recently studies have shown the gene expression changes occur as a result of a heat stimulus in the neonatal period (Yossifoff et al., 2008; Kisliouk et al., 2009, 2010, 2011). These researchers reported that the expression of brain-derived neurotrophic factor, an important regulator of thermotolerance acquisition in the hypothalamus of the chick, was different between birds grown under normal temperature conditions and thiose subjected to high temperatures within the first 3 days of life. These changes were associated with increased methylation of CpG sites in the promoter of the brain-derived neurotrophic factor gene. Guo et al., 2012 reported that epigenetic changes were part of the immune changes associated with susceptibility of chicks to Salmonella. Luo et al 2012a and b, reported that various histone profiles and gene promoters were differentially methylated in Mareck disease virus sensitive and -resistant strains of chicken.

This suggests that epigenetic changes may participate in the modulation of resistance to specific diseases (Fresard et al., 2013).

Although little is known about the underlying molecular mechanisms, there is evidence that feed stress may alter gene expression in part through epigenetic epigenetic changes. For example, Xu et al., 2012 found that when 3 day old chicks were subjected to a 24-hour fasting, there were methylation changes in histone H3 in the anterior hypothalamus, the area of key control of body temperature and food intake. Feed stressors such as feeding calcium (Ca) and phosphorus (P) deficient diets in the starter (Yan et al., 2005) or during the first 90 hours post placement (Ashwell and Angel, 2010) affect the ability of broilers to handle successfully or not deficient ca and P diets latter in life beyond the normal compensatory adaptation that occurs when nutrient deficiencies are fed. These changes were partially mediated through increased methylation of the NaP co-transporter (Ashwell and Angel, 2010).

There are other examples of potential epigenetic effects mediated by changes in the embryonic or neonatal period of poultry development. For example, exposure of the embryo to green monochromatic LED light appears to improve growth in broilers and turkeys (Rozenboim et al., 2004; Halevy et al., 2006; Zhang et al., 2012). This can be partially explained by the enhancement that was seen in the differentiation and proliferation of myoblasts (Halevy et al., 2006).

In birds, the effects of environmental challenges over a single generation on behavioral traits and the impact seen on gene expression and DNA methylation in offspring has resulted in interesting results from an epigenetic perspective. Spatial learning was shown to be affected in chickens subjected to unpredictable light rhythms as compared to that in birds that were exposed to predictable light rhythms (Lindqvist et al., 2007). These researchers showed that these effects were seen only white leghorn chickens but not red jungle fowl and more importantly, that these effects were transmitted to the F1generation reared under normal conditions. In another study, exposure of chickens to an unpredictable light schedule also triggered transmission of adaptive behavior to the F1 generation and interestingly, female offspring were more affected than males (Natt et al., 2009). Transcription differences in the parents, acquired in response to environmental challenges were partially passed on to the offspring. This was shown in several studies with poultry

associated with stress related genes (Goerlich et al, 2010), immune genes (Natt et al 2009), and brain-derived neurotrophic factor gene (Lindqvist et al., 2007). Heritable differences in DNA methylation between red jungle fowl and white leghorn chickens associated with spatial learning were found showing that DNA methylation was involved in the multigenerational epigenetic effect (Natt et al, 2012).

Nutrient Regulation of Gene Expression

The mechanism by which nutrients specifically regulate the expression of genes in vertebrates in general is poorly understood. Surprisingly, the basis of the fundamental understanding of how gene expression is regulated resulted from studying how bacteria respond to nutritional changes. The induction of proteins that transport and hydrolyze lactose in response to adding lactose to growth media was the first examination of gene expression (functional genomics) of any kind (Jacob and Monod, 1961). Research targeting the nutritional regulation of gene expression in eukaryotic cells has progressed considerably slower due to the complexity of mechanisms controlling gene expression and the difficulty in identifying specific metabolites to which the intestinal epithelium (IE) cells respond (Traber, 1997; Sanderson, 1998). The IE cell must respond to the contents of the digesta through specific interactions and react to this "sensing" mechanism by "signal transduction" pathways to alter gene expression (Yeh & Holt, 1986).

Several genes have been identified as being regulated by nutrient levels in the diet (reviewed by Kelley and Sanderson, 1999). One of the earliest studied nutrient regulators of gene expression has been the micronutrient zinc (Blanchard and Cousins, 1996). Zinc deficiency has been well characterized clinically and is associated with changes in the expression of many genes including cholecystokinin, uroguanylin, and ubiquinone oxidoreductase (Blanchard and Cousins, 2000). Most of our knowledge on the effects of nutrients on gene expression has been acquired in animal models, many examples of which can be found in a recent overview of the mechanisms by which nutrients interact with their molecular targets to modify gene expression (Muller and Kersten, 2003).

Effect of 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 and Ferket, 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.

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. In ovo feeding "jump-starts" or stimulates the adaptation to external feeding to begin earlier than would otherwise occur after the birds hatch. In addition to the increased body weights typically observed at hatch, the positive effects of in ovo feeding may include increased hatchability (Uni et al., 2005); advanced morphometic development of the intestinal tract (Tako et al., 2004) and mucin barrier; enhanced expression of genes for brush boarder 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 et al., 2005; Tako et al., 2004; Foye et al., 2006); enhanced feed intake initiation behavior (deOliveira, 2007); and increased breast muscle size at hatch (Uni et al., 2005; Foye et al., 2006). 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.

Early Dietary Adaptation

Adaptation to low nutrient diets has been long recognized. Animals respond to nutrient restriction by increasing digestive capacity either by increasing digestion per se or absorption rates and utilization efficiency. The ability of humans to adapt to a diet low in Ca was recognized in the1950s. At that time, the Food and Nutrition Board (1948) recommended an adult daily Ca allowance of 800 mg/d. However, Hegsted et al. (1952) found that adult Peruvian males, who had lived on low Ca diets through several generations, only required 100 to 200 mg Ca/d to maintain balance. It is obvious that these Peruvians, who grew up under Ca restriction and came from parent who had also grown up under low Ca diets, were able to better utilize Ca.

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 47Ca when diets low in Ca (0.8 g/kg) were fed for eight days prior to intestinal sampling regardless of dietary P concentration, or when low P (2.5 g/kg) diets were fed regardless of dietary Ca concentration. Chickens receiving a diet with normal P (6.5 g/kg) and normal Ca (12 g/kg) absorbed less than half of the 47Ca. Duodenal P absorption in 15 to 20 d-old chicks that had been fed a low Ca or a low P diet for eight days, 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 weeks 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-(OH)2-D3 (Hunziker et al. 1982; Blahos et al., 1987) and duodenal calbindin content (Morrisey 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.

However, 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. Therefore we proceeded to investigate if birds had the capacity to adapt similarly. The application of the adaptation principle in poultry may allow for decreasing both fed and excreted P and Ca without sacrificing performance and provide an additional low cost tool to decrease P and Ca in poultry litter. Understanding the changes associated with the increase in absorptive capacity in birds that have adapted to low P and Ca diets will be the first step in determining the viability of this method. 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 days of age) on performance, bone characteristics, and nutrients absorption of broilers the grower phase (19 to 32 days of age) (Yan et al, 2005).

Broilers fed a diet moderately deficient in P and Ca from hatch to 18 days of age demonstrated the ability to adapt to the deficiency, which is shown in the increased ileal absorption of P and Ca, the increased PP disappearance, compensatory growth, and compensatory improvement in bone parameters including tibia ash, tibia and shank bone mineral density and bone mineral content in a later growth phase (18 to 32 days of age) in broilers fed the low diets early on as compared to those fed diets that met requirements (Yan et al, 2005). However, practical application of this adaptation requires more studies to further fine tune the degree, timing, and length of the restriction with the aim of either no change or improved performance, and minimal changes in bone, since bone characteristics are one of the primary determinant of downgrades of poultry products in the processing plants.

Early P restriction effects on performance

To determine the effects of diet P on performance and expression of the chicken intestinal sodium phosphate co-transporter (NaPcoT), experimental diets were formulated to be deficient in total P (Angel and Ashwell, 2008). Ross 308 male chicks were fed either a control diet (C) consisting of 11.1 g/kg Ca and 5.0 g/kg available P which met

or exceeded NRC (1994) recommendations or a restricted diet (L) containing 5.9 g/kg Ca and 2.5 g/kg non phytate P (nPP) from hatch to 90 hours. All birds were then fed a control diet (C) that meet or exceeded NRC (1994) recommendations and was based on average USA industry use in 2006, of Ca and P until d 22. From day 22 to 38 of age, broilers were either maintained on a C diet containing 7.0 g/kg Ca and 3.0 g/kg nPP or a low diet (L) consisting of 4.0 g/kg Ca and 1.2 g/kg P. The three dietary treatments, C- C-C, C-C-L, and L-C-L met or exceeded all other NRC (1994) nutrient recommendations and were formulated to meet average USA industry use concentrations. Performance data were collected for each dietary phase including weight gain, feed conversion, bone ash, and specific nutrient digestibility. These data are presented in Table 1.

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 days of age) than those fed a control diet in the first 90 hr. Not only were the broilers fed the L diet early, heavier at 38 days of age, but they were more efficient in converting feed to gain, had higher tibia ash and higher P digestibility than those fed the C diet in the first 90 hr of life. Despite this improved ability to handle deviancies in Ca and P in latter growth phases, these "conditioned" birds were not able to catch up with those fed the control diets throughout all growth phases. Of note is that the increase in apparent P digestibility seen in the "conditioned" birds at 38 days of age was much greater than the increased seen in the "non-conditioned" birds as compared to that of the controls. Thus, the expected increase in digestibility when deficient diets are fed was seen in the "non-conditioned" (C-C-L) birds but there was an increase over that that can potentially be the response to the low diet being fed in the first 90 h (L-C-L). This clearly establishes that there was a "conditioning or imprinting" in these birds meaning that modifications are occurring in these birds that are long term and that allow for improved P utilization when P deficient diets are fed in the grower/finisher phases.

Table 1. Impact of early dietary deficiencies of phosphorus (P) and calcium (Ca) on performance and apparent digestibility (AD) of P (Angel and Ashwell, 2008)

Treatment [1]	C-C-C	C-C-L	L-C-L	SEM	P values
Hatch to 90 hour					
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, g/kg	133.7		111.7	0.57	<0.001
90 hour AD P, g/kg Ca in diet	576.2		663.9	6.67	<0.001
Apparent P absorption, gr/90 h	0.147		0.096	0.004	<0.001
91 hr to 8 days of age					
Body weight, g (8 days)	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
9 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
22 day AD P, g/kg	514.6		529.4	16.12	0.513
23 to 38 days of age[4]					
38 days Body weight	2345.9[a]	2235.4[c]	2285.6[b]	20.158	0.001
Gain, g	1450.9[a]	1333.0[c]	1390.6[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, g/kg	126.2[a]	105.3[c]	118.4[b]	1.71	<0.001
38 day AD P, g/kg Ca in diet	453.9[c]	515.4[b]	601.1[a]	1.562	<0.001

[1]Treatments are: Control (C) – C –C (fed in all phases) diets that met or exceeded NRC (1994) nutrient recommendations (including those for Ca and P); C-C- Low (C-C-L) fed the C diets from hatch to 22 d of age and then the 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 met or exceeded all NRC (1994) nutrient recommendations except for those of Ca and P. From hatch to 90 hr the L diet contained 5.9 g/kg Ca and 2.5 g/kg non phytate P (nPP) while the C diet contained 11.1 g/kg Ca and 5.0 g/kg nPP. From 22 to 38 d of age the L diet contained 4.0 g/kg Ca and 1.1 g/kg nPP, while the C diet contained 7.0 g/kg and 3.0 g/kg nPP.

[2] Up to 22 days of age, treatment C was replicated 16 times and treatment L eight times. Between 22 and 38 days of age all treatments were replicated 8 times.

[3] Means within row with different superscript letter differ (as specified in the P value column). Where there are only 2 means in a row the P value column shows significance.

These data indicate that, in broilers, during the period immediately post hatch there is a phenomenon occurring that permanently alters the bird's response to its environment. But is this an epigenetic change involving methylation? Gene expression measurements for the chicken sodium phosphate co transporter (NaPcoT) were determined using real-time quantitative PCR. The differences in CT (expression) values for gene expression were determined to be different using a student's t-test and the relative difference in expression caused by the dietary treatment was determined by correcting for the efficiency of the PCR as calculated by the standard curves of dilutions of pooled cDNA across the experiment. Expression levels and N-fold expression changes (changes relative to the control) are reported in Table 2. Feeding the L diet in from hatch to 90 hours had a significant effect on the expression of the NaPcoT as seen by the average n-fold increase of 2.8 in the mRNA levels in the small intestine. Nearly identical stimulatory effects were seen across all segments of the intestine with relative expression decreasing from duodenum to ileum. This pattern is similar to that in trout where a 2 fold induction of the NaPcoT was observed as a result of a 40% reduction of diet P (Sugiura et al., 2003). This is also in the range of the induction of expression of the NaPcoT as a result of feeding a low P diet in mice (Segawa et al., 2004). In this experiment with mice, the effect of reducing P from 5.0 g/kg to a2.50 g/kg induced NaPcoT expression an average of 2.3 N-fold and this change was shown to not be involved in the vitamin D signaling pathway since knockout mice showed similar response to that of wild type mice. The influence of vitamin D on the absorption of Pi (inorganic P) has long been known but further evidence has shown that 25-hydroxyvitamin-D3-1α-hydroxylase which leads to an increase in the level of 1,25-dihydroxy-vitamin D3 does not influence the expression of the NaPcoT in the intestine in mice (Capuano et al., 2005). Therefore the regulation of expression of the NaPcoT by dietary P must function through a novel pathway that is not influenced by vitamin D or its metabolites.

When methylation determinations were done on the samples obtained from birds in the 90 hour Ca and P trial apparent methylation was observed at 29 positions within the 52 predicted CpGs in the PCR products produced in the control samples (Ashwell and Angel, 2008). As a comparison only 19 CpGs were methylated in the DNAs extracted from the neonatally programmed duodenums.

This 43% reduction in methylation of cytosines in this region may be involved in the increased gene expression observed in the programmed birds relative to controls. Further characterization of the differential methylation patterns is needed both post programming as well as later in life to verify these observations.

Table 2. Impact of early dietary deficiencies of phosphorus (P) and calcium (Ca) on sodium phosphate co-transporter (NaPcoT) gene expression (Ashwell & Angel, 2008)

Dietary treatment	Tissue	Gene expression[1]		n-fold[2]
		18s rRNA	NaPcoT mRNA	
90 hr				
	Jejunum	16.1±0.3[a]	24.4±0.3[a]	1.0
	Ileum	16.2±0.3[a]	25.3±0.4[a]	1.0
Low P	Duodenum	16.0±0.3[a]	24.5±0.3[b]	3.1
	Jejunum	16.2±0.3[a]	26.1±0.4[b]	2.9
	Ileum	16.2±0.4[a]	26.9±0.2[b]	2.5
day 38				
	Jejunum	16.2±0.3[a]	23.7±0.4[a]	1.0
	Ileum	15.8±0.4[a]	24.9±0.4[a]	1.0
Low P	Duodenum	16.6±0.4[a]	25.4±0.4[b]	2.5
	Jejunum	16.1±0.5[a]	25.3±0.6[b]	2.7
	Ileum	16.8±0.5[a]	26.7±0.4[b]	1.8

[1]Expression values are presented as the average ± the Std Dev (n=8).
[2]n-fold change in gene expression was calculated by the 11 11 Ct method of Pfaffl (2001) including the amplification efficiency for both genes. 18s amplified with 94% and Na/Pi IIb amplified with 91% efficiencies respectively as determined by the standard curves of diluted cDNA. Expression levels for the control diet were set as 1.0 for fold change effects within a tissue and within an age.
[ab]Means within a column and age with common superscript letter do not differ ($P<0.05$).

These data along with the other preliminary data demonstrating long term effects on performance and gene expression as a result of nutritional neonatal programming in the chick are strong evidence for the role of epigenetics in the regulation of these phenomena.

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. A study by Rao et al. (2009) where the effect of feeding a low protein diet (100 g/kg) versus a normal breeder diet (150 g/kg) to females of an inbred indigenous Chinese breed (Langshan) decreased egg weight and laying rate as well as hatch weight of the off springs. But key here was that these researchers reported that at 28 days of age both body weight and pectoralis major muscle weight were greater in chicks hatched from eggs laid by the low protein fed hens than that for birds hatched from eggs laid by hens fed the normal protein diet.

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 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 well be sending an epigenetic message to their progeny that they will live in a world of limited nutrient resources.

Researchers have recently recognized that breeder nutrition and management can have significant effects on progeny performance beyond what had been thought. Ross broiler breeders fed different amounts of cumulative nutrients by feed restriction to control body weight can affect progeny performance; and this effect on progeny differs among growth-restricted hens or males (Romero- Sanchez et al., 2007). Cumulative nutrient intake during rearing of Ross 308 female broiler breeders was positively correlated with the growth rate of male broiler progeny, especially among young broiler breeder flocks. In another experiment, Brake and Romero- Sanchez (2008) reared Ross 344 males on two cumulative feeding programs providing either 29,580 kcal ME and 1,470 g CP or 33,500 kcal ME and 1,730 g CP to produce either a low or high body weight at

22 weeks of age, respectively. In contrast to what was observed in the weight-restricted hens, the low body weight males had progeny that were heavier at 42 days of age than those that of males with higher body weights. Based on these studies, epigenetic effects are more likely associated with breeder hen management and the resources she passes onto her progeny via the egg.

Conclusions

By helping to understand the interaction between nutrients and molecules in an organism through its life span and to subsequent generations, will greatly affect our ability to change the way animals grow. Although the complexity of this proposed integration is exceeding the current bioinformatics tools and capacities, its implications for nutritional research can be enormous. Unlike biomedical interventions (drug therapy), nutrition is chronic, constantly varying, and composed of a very large amount of known and unknown bioactive compounds. Furthermore, nutrition touches the core of metabolism by supplying the vast majority of nutrients (both macro- and micronutrients) for maintaining metabolic homeostasis. This homeostasis stretches from gene expression to lipid metabolism and from signaling molecules to enzyme cofactors. Thus, nutrition by its nature *needs* to be studied in an integrated way. So far, most of the tools for this integration were lacking, thus maintaining an unbridgeable gap between classical nutrition (studying physiology with a focus on biochemical pathways) and biomedical sciences (determination of disease-related molecular mechanisms). In applying Systems Biology to nutritional sciences, these paradoxical extremes are bridged and the complexity of the relationship between nutrition and health can be met in an integrated approach. Many hurdles need to be overcome, before this research area reaches maturity.

The data included in this review along with other preliminary data demonstrating long term effects on performance and gene expression as a result of nutritional manipulation in the chick are strong evidence for the role of epigenetics in the regulation of these phenomena. Further work must be conducted to elucidate the specifics of neonatal programming in the chicken and the extent of its effects on DNA methylation. Demonstrating these epigenetic effects in the

chicken will provide both a significant contribution the understanding of the regulation of genomes, particularly in oviparous species but also provide a potential mechanism for improving performance and the economics of poultry production beyond incremental improvements. Nutrition research in the future will increasingly focus on the ways in which genes are affected by what we feed breeders, how these breeders are managed, how eggs are incubated and neonatal changes in environment. If these effects can be better understood and harnessed then dietary conditioning offers the opportunities for nutrition to impact both the animal and its offspring are almost limitless.

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The 'Omic' Revolution - Implications for Animal Production

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"OMICS", is a term that refers to the recently developed high throughput technologies that include genomics, functional genomics (transcriptomics), proteomics, and metabolomics. These state-of-the-art technologies can be applied to the measurement of metabolism on an organism, tissue, or cell at a molecular level. Genomics is the study of an organism's entire genome and especially the analysis of the relationship between genetics and the phenotype (the measurable traits of the organism). The whole genome sequence is now available for most livestock species and this has allowed researchers to discover and assess the unique genomic features and the complexity and structure of the genomes of livestock. This provides the molecular basis to link genomic information to economically important traits. For example, these tools can be used to "genotype" individual animals and begin to predict their breeding value or performance for traits such as feed efficiency, product quality or even susceptibility to disease. Transcriptomics (measuring abundance of messenger RNA) studies when and how the genes in the genome can be turned on and off, which allows new molecular phenotypes to be identified or new insights into the processes underlying metabolism; for example under different nutritional management or across different stages of production. Furthermore, proteomics and metabolomics can determine the entire set of proteins and metabolites in a biological system and how they can directly determine the biological process relating to traits of interest. The integration of "omics" technologies generates information that links variation in the genome with metabolism and nutritional physiology of livestock species. In turn, producers can use this information to apply a systematic approach to improve the breeding and management of their animals: improving performance potential, health, or the animal's response to different nutrition or management measures. Application of livestock omics has great potential for the future development of breeding and nutritional strategies to the improve efficiency and sustainability of animal production.

Issues facing the New Zealand Poultry Industry and Legislation Updates

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Novel Strategies to Manage the Impact of an Unnecessary Feed Induced Immune Responses

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Introduction

Enzymes are the catalytic cornerstone of metabolism and as such they are the focus of intense research worldwide. Feed grade microbial enzymes continue to a fast growing field in commercial animal production. The global market of animal nutrition enzymes is currently close to 700 million USD and is expected to double this estimate by 2017.

Exogenous enzymes have been used commercially for over three decades to improve nutrient digestibility in various grains including wheat, barley and more recently in corn/sorghum/soy based diets to supplement the bird´s developing endogenous enzymes.

Energy sparing enzymes

Additional benefits of other NSP enzymes such as β-mannanase on sparing energy effects by hydrolyzing feed borne inmunogens are largely unrecognized in the industry. A critical role in feed induced immune responses is played by immunogens such as β-manans present in soybean meal, sunflower, copra, guar meals and other leguminous feedstuffs.

Since the early 1990s, the usage of β-mannanase in diets for monogastric animals as a nutritional aid has become widespread, due to the ubiquitous use of soybean meal or other leguminous plants as protein sources. The mode of action of β-mannanase is monogastric animal is complex and provides an energy sparing effect by reducing unnecessary innate immune responses by eliminating immunogens in feed ingredients and other ingredients. A key group of immunogens are galacto mannans present in significant quantities in soybean products, guar meal, copra meal, and sunflower meal.

The active ingredient of B-mannanase, endo-1, 4-β-mannanase, is commonly known as β-mannanase. β-mannan fibers of various types are found in all vegetable feed ingredients, and soybean meal is the main source of β-galactomannan (simply called β-mannan below) in most poultry and swine feeds.

STRUCTURE OF β-MANNAN AND THE β-MANNANASE REACTION

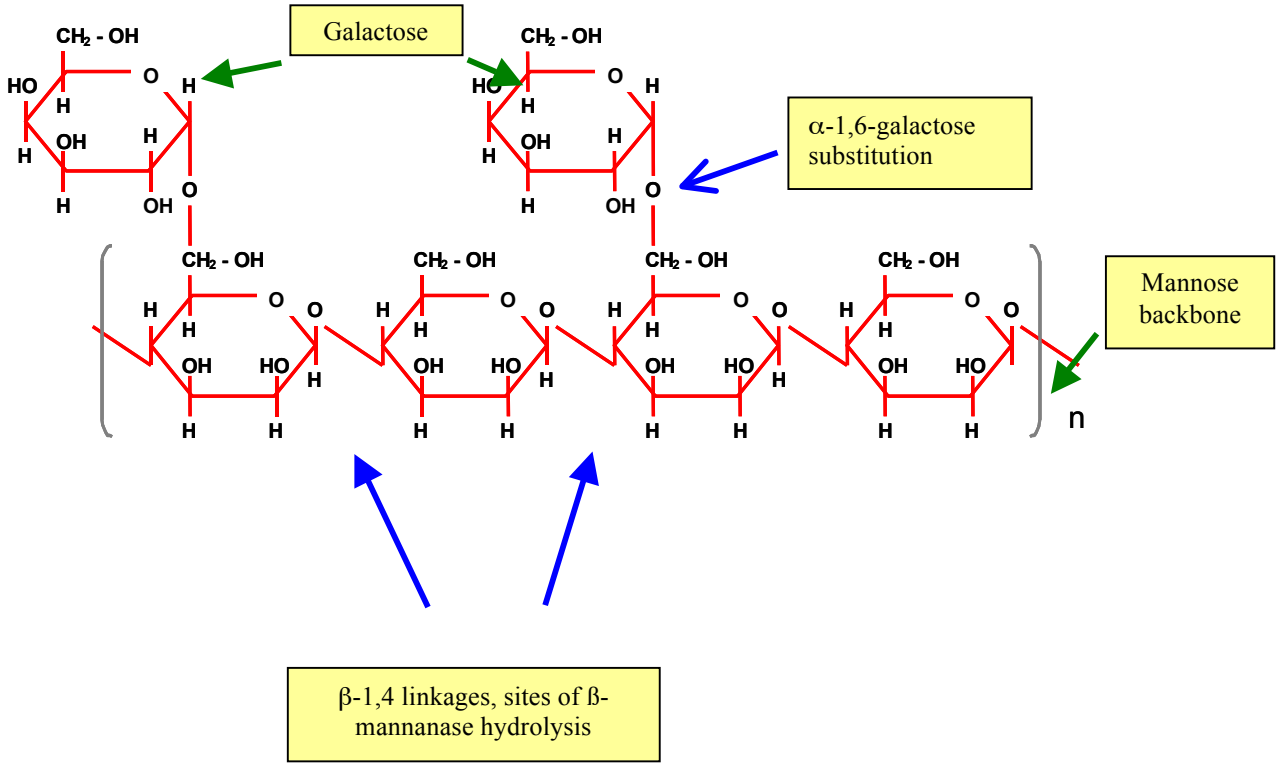
β-mannan (ß-galactomannan, ß-glucomannan, and β-galacto glucomannan) are non-starch polysaccharides (NSP). The derivatives are basically a β-mannan backbone where some of the mannose units in the backbone are modified with an added galactose side chain and in the case of glucomannan; some mannose backbone is replaced by glucose. The structure of β-galactomannan similar to that found in soybean meal is shown in figure 1.

The adverse effects from β-mannans become much more pronounced with increasing solubility. The water solubility of β-mannan increases as the number of galactose side chains on the mannan backbone increases, and a galactose/mannose ratio of close to 1: 2 indicates a very high solubility. Soluble β-mannan is often referred to as β-galactomannan. ß-mannans are heat resistant compounds that survive all feed processing procedures.

β-mannans or β-galactomannans as they often are referred to in their soluble forms, are powerful anti-nutritional factors for monogastric animals. β-galactomannans have been shown to reduce the absorption of glucose, the secretion of insulin, inhibit the production of IGF-1 (Insulin-like Growth Factor-1), decrease the retention of nitrogen, reduce the absorption of water, increase fecal moisture content, reduce the levels of intestinal enzymes, and reduce the metabolizable energy content of feed.

ß-D-Mannanase EC 3.2.1.78, endo-1, 4-ß-D-mannanase) is an endohydrolaze enzyme, which cleaves the β-1,4 linkages of β-mannan, β-glucomannan, β-galactomannan, and β-galacto glucomannan, thereby reducing them to a complex mixture of small mannose oligosaccharides that typically have less than 10 sugars per molecule after a complete digestion.

Figure 1. Structure of β-galactomannan and sites of β-mannanase hydrolysis



In glucomannan, some backbone β-1,4-mannose residues are replaced with glucose

Feed induced immune response (FIIR) - β-galactomannan is recognized by the innate immune system

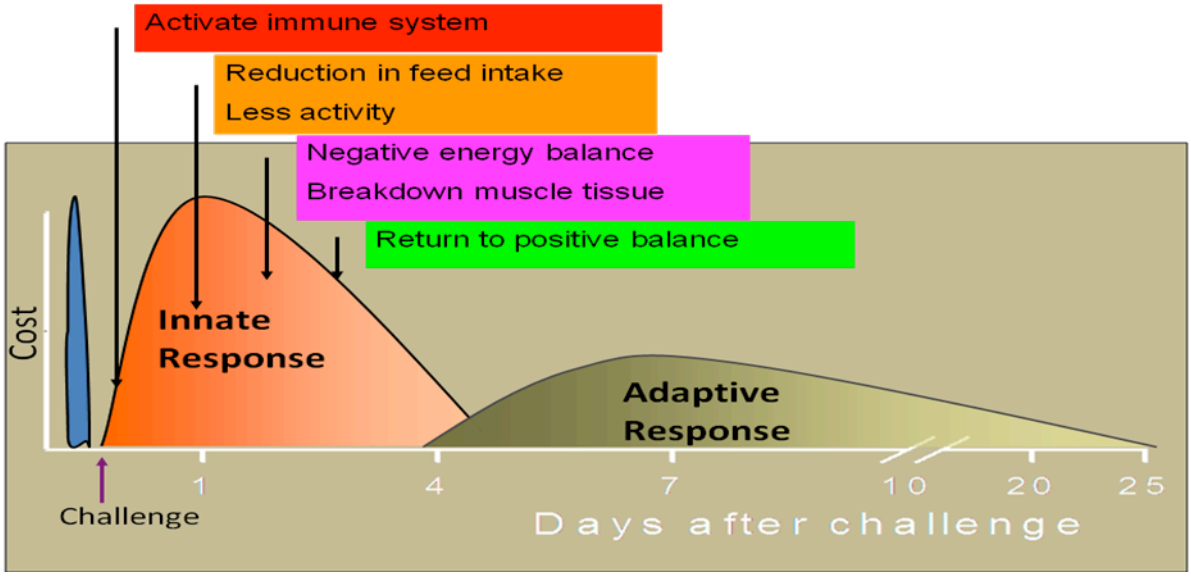
The innate immune system the organism's first line of defense and has been highly conserved through evolution with analogous elements found from insects, to plants to humans (Anderson et al., 2001). The innate immune system with its powerful and rapid response is essential for survival due to its role to fight infections prior to the development of adaptive immunity in animals (Anderson et al., 2001; Klasing, 2007). A cascade of immune activity to block the threat is triggered whenever a Pathogen Associated Molecular Pattern (PAMP) is recognized. However, this benefit can compromise the overall host immune status consistent with the challenge of the undesired autoimmune diseases occasionally triggered by faulty regulation of the adaptive immune system, research has recently shown that the innate immune system reacts in a counterproductive way against non-pathogen food components that happen to be analogues of PAMPs (Anderson, 2001) and lead to Feed Induced Immune Response (FiiR). A case in point, the innate immune system mistakenly recognizes large β-mannan molecules as a pathogen. The mistake occurs because a) mannose sugars, as found in large β-mannan fibers, also exist in different molecular structures on the cell walls of many pathogenic microorganisms; and b) the innate immune response is based on recognition of pathogen associated molecular patterns (PAMP). This recognition is unspecific in the sense that the response is triggered whether the molecular pattern is part of a pathogenic microorganism or, in this case, of an otherwise benign fiber. The importance of mannose recognition likely evolved because many pathogenic microorganisms have mannose molecules organized in their cell structure. Even low levels of 0.2-0.25% β-galactomannan can provoke a significant immune response, and our research indicates that the negative effects may be aggravated by simultaneous enteric infection or challenge. The metabolic response to an immune challenge is one where nutrients are repartitioned to immune related processes that increasingly take priority over growth related processes as the level of challenge or stress becomes more severe. This repartitioning effect can, even in the presence of subclinical challenges, result in significant loss of performance.

β-mannanase efficiently hydrolyses soluble β-mannan fibers into small mannose oligosaccharides that no longer can provoke an immune response (Barnes et al., 2002; Didierlaurent, A., Simonet, M. and Sirard, J-C. 2005; Klasing, 2007).. The high solubility of the mannanase reaction products enables potential binding directly to any mannose binding receptors on pathogen surfaces or epithelial cells of the GI tract to potentially inhibit binding interactions, the first step for infection.

B-mannanase improves the performance of monogastric animals by hydrolyzing β-mannan that reduces or eliminates a wasteful immune activation. This reduces the negative feedback on the anabolic processes, which allows the feed digestion, nutrient absorption, and tissue accretion to occur more effectively. Hence, it reduces the waste of nutrients by counterproductive immunological processes.

The timing of resource use for the innate system and the adaptive system are very different (Figure 2). The innate reaction is not based on antibodies (adaptive immunity), instead it is based on immune cells and binding proteins that continually screen for distinct pathogen associated molecular patterns (PAMP) that are not typically found in mammalian cells. Through this strategy, cells of the innate system are able to recognize and detect invading pathogens (Anderson et al., 2001). The innate immune system is activated whenever a pathogen associated molecular pattern is recognized, and the response is the same whether the PAMP is pathogen associated or not. Examples of PAMP include bacterial cell wall fragments, viral nucleic acids, endotoxins, β-1,3-glucan, α-mannans and β-mannans and many other molecular patterns commonly associated with pathogens.

Figure 2. Innate vs. Adaptive Immune Responses (Klasing, 2007)



Acute phase proteins

Acute phase proteins (APPs) are a large and varied group of glycoproteins. They are synthesized by liver parenchymal cells and released into the bloodstream as part of the initial response to a variety of stressors such as local inflammation, bacterial infection, endotoxin injection, abnormal cell proliferation, and thermal or mechanical injury (Barnes et al., 2002; Johnson et al., 1999). The synthesis of APPs is thought to be stimulated by monokines such as I1-1, I1-6 and tumor necrosis factor (TNF). Although the response pattern of individual APPs to various stressors or disease may differ, changes in their plasma concentrations are generally regarded as being sensitive, although non-specific, indicators of inflammation.

The biological functions of APPs are also highly variable with some functioning as proteinase inhibitors, enzymes, transport proteins, coagulation proteins, and modulators of the immune response.

All APPs appear to play a role in the restoration of homeostasis after injury, tissue necrosis, or infection. Some Apps such as C-reactive protein (CRP) and serum amyloid A (SAA) in humans, haptoglobulin in ruminants, and alpha 1-acid glucoprotein (AGP) in poultry, are considered "major" acute phase proteins and have been used to detect and monitor infection, inflammatory disease, and cancer (Barnes et al., 2002).

In broiler experiments, measuring the level of the APP has been used to test for innate immune system stimulation (Johnson et al., 1999; Murata et al., 2004) α1-acid glycoprotein (AGP) as it correlates with innate immune response (Holt P.S. and R. K. Gast. 2002; Takahashi et al., 1994; Hulten et al., 2003).. A 42 day broiler pen trial showed significant improvement in feed conversion, and also significant reduction in AGP (Anderson and Hsiao, 2006). Results are shown in Table 1. β-mannanase was essentially able to provide the same effect as the addition of the growth promoting antibiotic BMD into the diet, although the mechanism is clearly different as there are no antibiotics in β-mannanase.

Table 1. Broiler Trial, 0-42 Days

	0-21d	*21-35 d*	*35-42d*
ME	2960	3020	3080
CP	22.0	19.4	17.5
Lys	1.2 0	1.03	0.90

Treatment	WAFC Stat.	AGP Stat.
Control	1.820 a	304 a
BMD	1.772 b	246 b
Mannanase	1.756 b	225 b

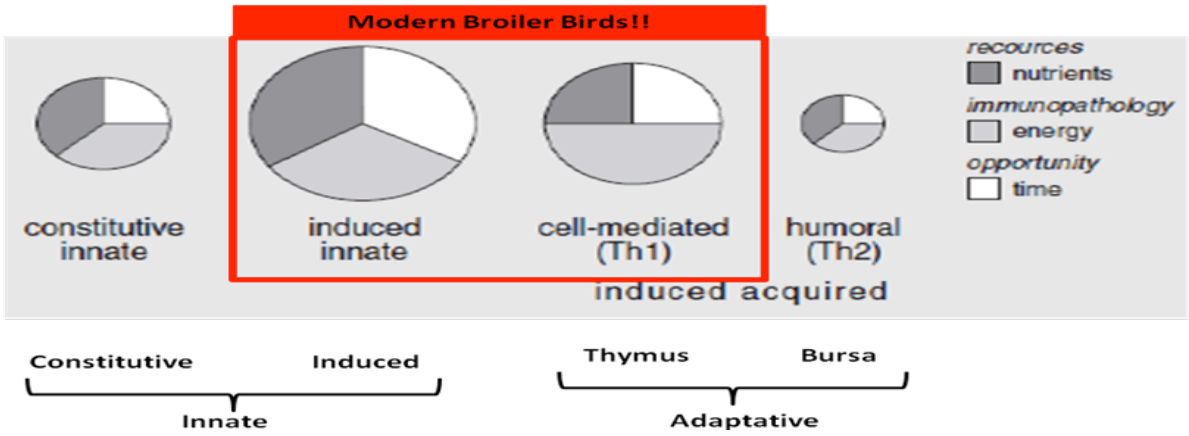
Cobb X Cobb broilers were used with 50 birds/pen; 10 cages per treatment; 0.93 sq. ft./bird; 50 g/ton BMD (when used): 60 g/ton Salinomycin; water and feed *ad libitum* , 42 days trial at SPR. The energy and crude protein for each period are shown above. The treatments were (a) control diet (without BMD antibiotic or β-mannanase), (b) BMD added but no β-mannanase, and (c) β-mannanase added (without BMD). Chicken AGP was determined by Radial Immunodiffusion test plates for chicken α-1-acid glycoproteins were obtained from Cardiotech Services, Inc., 2149 Emerson Avenue, Louisville, KY. Chicken blood was collected into anticoagulant-coated tubes, and plasma was prepared through centrifugation to remove cells. Plasma samples were frozen for shipment and refrigerated until use. Kit directions were followed, except plates were incubated at room temperature for 2-3 days before reading, and the polynomial curve fit function of Microsoft Excel was used to make an equation

to calculate unknown values from diameters. Generally 30 birds were analyzed per experimental treatment. The serum level APPs (like AGP) can be influenced by several factors including physical injury, thus outliers can be expected, typically 3-6% in a treatment group. Values greater than two standard deviations above the mean value are removed for analysis. AGP measurements less than two standard deviations below the mean were never observed.

Immune responses of modern broilers

Cheema et al (2003) recently concluded Genetic selection for improved broiler performance has resulted in a decrease in the adaptive arm of the immune response (adaptive responses) but an increase in the cell-mediated and inflammatory responses (Innate responses. The primary implication is that modern commercial broilers tend to use a more expensive immune response when their immune system is activated (Figure 3).

Figure 3. Immune response of a modern commercial broilers



Innate and adaptive immunity are essential for host survival and health. The innate response is generalized to broad classes of foreign (nonself) immunological challenges, including pathogens. The innate response has systemic effects on host physiology, and activation of this system can divert substantial amounts of nutrients away from growth (Barnes et al., 2002). Acquired immunity is targeted at specific antigens, and response to a particular pathogen involves a very limited subset of lymphocytes. The acquired response is exquisitely specific and targeted; activation of this type of immune response requires very little in the way of nutrients and causes very little change in metabolism of the bird (Humphrey and Klasing, 2004; Klasing and Calvert, 1999). For the host to be fully protected, these 2 aspects of the immune system must work in concert. Therefore, despite the

negative effect of an innate immune response on growth and productivity of poultry, attempts to improve performance through manipulation of innate immune function must be done with this in mind. The most effective means of reducing the effect of the immune system on bird performance is to reduce exposure to foreign antigens and food borne immunogens .

Implications of using energy sparing feed grade enzymes in poultry feed formulations

A trial conducted with 2,400 day-old male Ross 308 broiler chicks, randomly distributed in 24 pens and assigned to two treatments: 1) Control and 2) B-mannanase = Control + 110 mL/MT B-mannanase (Tables 2 & 3). The birds were bedded on clean wood shavings over once-used litter. Starter feed was fed from days 0-20 and grower feed from days 21-41. All diets were prepared as mash without antibiotic or anticoccidial and offered ad libitum.

Table 2. Summary Composition of the Basal Diets (E Esteve, IRTA 2005)

	Starter, Day 0-20	Grower, Day 21-41
Corn	54.43	59.08
Soybean meal, 48% CP	23.45	15.60
Full fat soybeans, extruded	14.45	18.28
Lard	3.00	3.00
Guar gum meal	0.50	0.50
ME, kcal/kg	3,050	3,150
Crude protein, %	21.0	19.0
Total lysine, %	1.25	1.08

Table 3. Results, Days 0-41 (E Esteve, IRTA 2005)

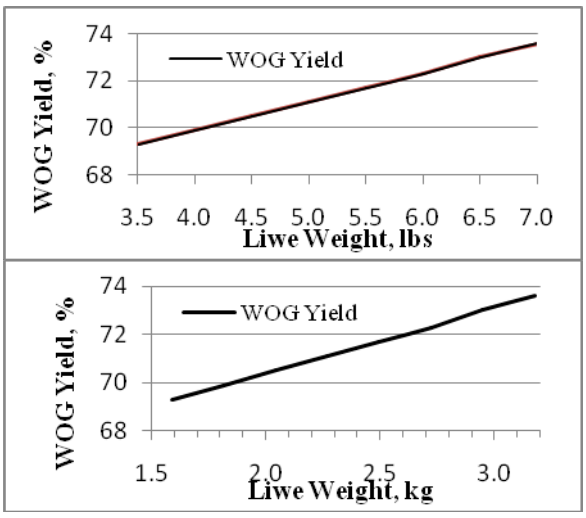
	Control	B-mannanase	P value
Final Weight, kg	2.085	2.166	P<0.05
Daily Gain, g/day	49.8	51.7	P<0.05
Daily Feed Intake, g/day	90.0	90.5	NS
Feed/gain	1.810	1.751	P<0.01
Pasted Vents, Day 10, %	15.8 ± 3.52	9.2 ± 1.23	P<0.001
Litter Score, Day 35, (0 = very dry, 4 = very wet)	3.5 ± 0.85	2.8 ± 0.62	P<0.01
Body Weight Homogeneity, CV %	4.7	4.0	NS
Skin Pigmentation, Axilla (wing pit)1 L	54.3	55.3	P<0.001
a	9.5	9.7	NS
b	14.6	13.4	P<0.001

In this study, similar mortality was observed, when corrected for mortality before 7 days of age. β-mannanase improved daily gain by 3.8% ($P<0.05$) and feed conversion by 3.3% ($P<0.01$). Frequency of pasted vents was reduced by 42% and β-mannanase improved skin pigmentation, resulting in a richer color ($P<0.001$), Table 3.

Influence of flock uniformity on processing yields in broilers

The yield of the whole broiler without giblets or WOG yield is a very important measure of sellable meat. WOG = Without Giblets (heart, liver, and gizzard) and WOG yield refers to the weight of the carcass as it leaves the chiller in percent of live weight. Live weight and uniformity are among the many factors that affect WOG Yield. The correlation between WOG yield and eviscerated body weight (calculated on dry yield basis prior to the chiller with no water uptake) for a widely used broiler breed is shown in Figure 4.

Figure 4. Calculated WOG Yield for a major broiler breed



A conservative estimate based on the above trials therefore suggests that β-mannanase has the capability to improve the WOG yield by 0.4 percentage points. The value of this improvement will vary depending on the market and products manufactured by each plant. β-mannanase improves uniformity by reducing the number of underperforming birds The value of uniformity is often undervalued by feed ingredient decision makers who primarily focus on cost of production or feed cost.

Uniformity affects the carcass value of broiler at processing, and is mainly important for plants that specialize in further processing, as opposed to whole birds. It stands to reason that any improvement in live weight uniformity in broiler grow-out operations will improve the consistency of the final products. It is crucial to focus on the impact of uniformity as it generally leads to higher WOG yield and this reduces processing loss from because the machinery is better matched to the size of the birds. Recent data has shown that it also leads to more birds meeting stringent customer specifications that command premium prices. β-mannanase also can potentially reduce the number of birds that are downgraded into lower value price categories. More uniform flock as also benefit

personnel at the processing plant because it leads to higher line speed / efficiency at the processing line so there is Less rework due to contamination at evisceration, short hocks (feet cut above the hock joint – equivalent to the human ankle), broken wings, torn skin.

At the barn level more uniform flocks lead to fewer culls, more birds within optimal weight range, shorter production cycles, and healthier birds

Conclusions

The value of β-mannanase doesn't come from energy releasing but from preventing a Feed Induced Immune Response (FiiR) associated primarily with β-mannans and other immunogens in feed. The degree of immune stress by mannan is also influenced by the degree of intestinal leakage. β-mannans do stimulate the immune system as observed by increased serum AGP production. The inclusion of an energy sparing enzyme such as β-mannanase significantly reduces serum AGP levels when animals are exposed to β-mannans, therefore, supporting the energy sparing mechanism. Values from feeding B-mannanase are much more appreciated in the field conditions such as improvement in mortality, culls, uniformity (process yield) and litter quality.

Future research should focus on establishing the impact of adding energy sparing and energy releasing enzymes on host innate immune function, intestinal integrity, the commensal flora and their interactions to maximize poultry health and profitability. There are clear indications that the flora is beneficial but also has the potential to be harmful, and increasing knowledge of how the flora interacts with the immune system should further our knowledge on how to maximize intestinal, integrity, optimize innate immune function and maximize net energy for growth and productivity.

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Dietary modulation for optimum eggshell quality

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Introduction

Eggshell is an architectural marvel and is easily prone to breakage with any mechanical stress. The problem is more severe when the shell quality is poor. The reduced shell quality increases the risk of eggshell breakage and may contaminate the surrounding eggs. The shelf life of the contaminated eggs is reduced, besides lowering consumer acceptability. According to Roland (1977), 7.77% of eggs collected are shell less, thin shelled or ultra-thin shelled, the incidence is much more compounded by higher environmental temperature. The incidence of eggshell defects and cracked eggs increased up to 21% of total eggs produced in hot tropical environment (Njoku and Nwazota, 1989). On average, the estimated total cracked eggs or lost from the point of lay till reach their final destination ranged from 13 to 20%. The approximate loss incurred by the layer industry in India due to poor shell quality is calculated at US $ 99.8 million per annum.

Common egg shell abnormalities

The most common eggshell abnormalities found in India are thin shelled, shell less, corrugated, misshapen and shell less eggs.

Thin shelled / shell-less eggs

Imbalance in dietary levels of calcium (Ca), phosphorus (P), cholecalciferol (CC), magnesium (Mg), manganese (Mn), copper (Cu), fluorine (F) or feed contaminated with pesticides, fungicides, fungal toxins, environmental stress, disease outbreaks, improper lighting schedules, old age, hard water, etc. may cause thin / shell less eggs depending on the severity of cause.

Corrugated eggs

Corrugated eggs are produced, when the layer suffers from infectious bronchitis, lathyrogen toxicity, consume pesticide contaminated feed and Cu deficiency. In the latter two cases eggs are usually larger than normal size due to excess water accumulation in the albumen.

The article reviews the nutritional causes of eggshell defects and dietary modulations being followed in India to ameliorate eggshell defects and improve the shell quality.

Pre-lay nutrient requirement

Shell gland derives Ca from both dietary source and medullary bone particularly during peak phase of eggshell formation. Dietary Ca alone is not adequate for shell formation and bird needs to mobilize Ca from medullary bones to ensure optimum quality of eggshell. Therefore proper deposition of Ca in medullary bone during pre-laying phase is essential to sustain optimum shell quality in the laying phase. In most commercial practices, about 2% Ca is provided in diets from 17th week of age to 5% average egg production of the flock, at which time a shift is made to regular layer diet. Supplementation of diet with higher levels of Ca during the pre-laying period was assumed to increase in medullary bone Ca storage (Hurwitz, 1976).

Calcium, phosphorus and cholecalciferol

Sound egg-shell formation depends on the availability of Ca and carbonate ions (CO_3) during shell formation in the uterus. The modern layer, producing about 320 eggs with an average egg size of 55 g requires about 700 to 750 g Ca which is equivalent to more than 33 times that found in the hen's body.

Ca requirement

Calcium metabolism is highly dynamic in laying hen. At sub-optimal level of dietary Ca, medullary bone will supply about 40% Ca required for shell calcification (Johnson, 1986). Optimum level of dietary Ca reduce the need for skeletal Ca, thus

reducing the need for dietary P. Shell quality was reported to reduce within 24 hours of feeding low-Ca diet (1.5%) and restored to normal within 24 h of feeding on the high Ca (5.5%) diet (Keshavarz, 1986). Sub-optimal levels of dietary Ca (3%) increases feed intake and this in-turn increases egg weight (Keshavarz *et al.,* 1993).

NRC (1994) has recommended 3.25 g Ca and 0.25 g non-phytin phosphorus/hen/day. During the past several years, a number of authors suggested that the shell quality can be improved with Ca intake above the NRC suggested requirements (Keshavarz and Nakajima, 1993, Rama Rao *et al*., 2003).

Particle size and source of Ca were reported to influence requirement of Ca in diet. Peterson and Hoej (1980) reported lower levels of Ca (about 3.0%) in diets based on oyster shell grit and higher levels (3.5%) in diet based on powdered limestone. For optimum shell quality and egg production, WL laying hens require a minimum of 3.5 to 4.0 g Ca/bird/day (El-Boushy and Papadopoulos, 1979; Keshavarz *et al.,* 1993; Leeson *et al.,* 1993; Karunajeeva, 1978; Pejin, 1989; Chandramoni *et al.,* 1988). Higher dietary Ca levels (4.00 to 4.25 g / hen / d) were reported for post peak production compared to young pullets (37-52 weeks) for optimum shell quality. The reasons for higher Ca requirement during post peak production is due to bigger egg size, reduced ability of bird to utilize dietary Ca and resorption from medullary bone (Petersen, 1965). Additional supplementation of Ca source (2 g/hen) for a brief period (about a week) is being followed in flocks having the problem of shell quality (Camps and Sevajares, 1996) in India.

A short term experiment (196 to 336 d of age) conducted at this laboratory (Rama Rao *et al*., 2003) suggested requirement of 35g Ca/kg diet for optimum egg production and shell quality. A long term experiment was conducted with laying hens (25 to 72 weeks of age) to study the effect of feeding 4 levels of Ca at a constant level of NPP in diet (S V Rama Rao Personal Communication, **Table 1)**. The results suggested that EP improved significantly with increase in dietary Ca form 3.5 to 3.75% and further increase in Ca concentration did not show any additional benefit on EP. Higher dietary Ca concentration ($\geq$4%) significantly reduced egg weight compared to those fed $\leq$3.75%. Shell quality parameters were not affected by variation in concentrations of Ca in diet from 3.5 to 4.25% in layer diet.

Table 1. Effect of feeding graded concentrations of Ca on performance of WL laying hens (25 to 72 weeks of age)

Ca, %	EP, %	Egg weight, g	Shell defects, %	Shell weight, %	Shell thickness, mm
3.50	87.79^{B}	58.55^{A}	2.587	9.268	400
3.75	90.60^{A}	57.98^{AB}	2.440	9.276	399
4.00	91.58^{A}	57.57^{B}	2.468	9.284	399
4.25	90.89^{A}	57.35^{B}	2.572	9.388	402

Rama Rao, unpublished data

Source of Ca

These has been a constant debate on the merits of using oyster shell grit *vis a vis* stone grit in layer diet. Contradicting results were reported about the beneficial effects of oyster shell grit over lime stone (Duke and Charles, 1983; Harte *et al*., 1923). Hart *et al.* (1923) reported that dolomite limestone reduced eggshell strength due to its high magnesium (Mg) content. Variation in Ca content and presence of fluorine in stone are the other major causes of reduced shell quality in laying hens fed stone grit. However, there is no difference between good quality (38 to 39% Ca) limestone and shell girt in maintaining eggshell quality (Roland, 1986).

Particle size of Ca source

The process of shell formation in the shell is maximum approximately 7 hours post ovulation and the secretion rate decreases 2-3 hours before oviposition (Johnson, 1986). Ionic Ca and bicarbonate should be available at the required level especially at peak hours of shell formation, which usually takes place during 2300 to 400 hrs. Residence time of Ca sources in the digestive system is an important factor for formation of eggs with optimum shell quality (Hamilton *et al.,* 1985; Roland and Harms, 1973). Pulverized lime stone gets absorbed and excreted quickly, so Ca may not be available during the period of eggshell formation. Large particle of Ca source may lodge and slowly dissolve in gizzard and thereby Ca will be available during night when the shell calcification process is in active phase. The improvement in shell quality might be due to constant "metering" of Ca from the gizzard in to the blood stream of the hen during the day and night. Stronger eggshells were produced with larger particle size compared to ground Ca sources (Miller and Sunde, 1975; Kuhl *et al.,* 1977). In practical layer diet, better result can be obtained using 1/3 to 2/3 of the larger particle size of Ca source (Roland, 1986). Most desirable particle size of Ca source for laying hens is 1 to 1.5 mm, (Leibetseder, 1987; Rao *et al.,* 1992). However, at adequate levels of Ca (2.75, 3.25 and 3.75%), variation in particle size (2-4, 4-6 and

6-8 mm) of the mineral source in diet did not influence the production performance and egg shell quality (Rama Rao and Raju, 2004). In the diet of old laying hens (>45 weeks), addition of large particles of Ca source significantly improved eggshell quality (Brister *et al.,* 1981).

Phosphorus requirement

A bell shaped response curve can be reconstructed with regard to the response of eggshell quality to dietary P concentration (Harms, 1982a), with an optimal level of 0.4 to 0.5% of total phosphorus in a corn - soy diet (Edwards and Suso, 1981).

A decrease in dietary total P from 0.8, 0.6 or 0.47 % resulted in improvement in specific gravity and shell thickness (Holder, 1981). Non phytate phosphorus (NPP) can be reduced from 0.4 to 0.2% in layer diet without affecting the shell quality (Summers, 1995). Whereas, increasing the dietary NPP from 0.25 to 0.45% reduced eggshell thickness (Hoscain and Rezende, 1996). Egg production and eggshell quality did not alter due to variation in NPP (**Table 2**) between 0.2 to 0.35 % (Rama Rao *et al*., 1999). Another long term experiment was conducted with WL laying hens (22 to 72 weeks of age) by feeding 8 graded concentrations of NPP (0.15 to 0.325%) with an increment of 0.025% in the authors laboratory (**Table 3**, unpublished data). Results suggested that optimum egg production and feed efficiency was observed at 0.275% NPP, but the eggshell quality parameters were not affected by variation in dietary NPP in the range of levels tested.

Table 2. Performance of WL laying hens (266 to 350d of age) fed diets with graded concentrations of NPP (g/kg)

Parameter	NPP, %			
	0.2	0.25	0.3	0.35
Egg production, %	94.6	92.2	92.4	91.1
Egg weight, g	59.8	59.8	59.4	57.3
Shell weight, %	9.73	10.00	9.62	9.69
Shell thickness, mm	0.379	0.390	0.380	0.378

Rama Rao *et al*. (1999).

Table 3. Performance of WL laying hens (22 to 72 weeks of age) fed diets with graded concentrations of NPP (%)

NPP %	Egg production, %	Feed/egg	Egg shell defects, % per month	Shell wt., g	Shell Thickness, mm
0.150	87.99^{bc}	117.4^{ab}	0.229	5.22	0.400
0.175	87.83^{bc}	119.3^{a}	0.274	5.28	0.406

0.200	88.39^{abc}	115.3^{bcd}	0.234	5.19	0.401
0.225	88.23^{abc}	114.1^{bcd}	0.220	5.22	0.398
0.250	89.78^{ab}	113.3^{cd}	0.239	5.18	0.398
0.275	90.13^{a}	112.8^{d}	0.220	5.21	0.402
0.300	87.43^{c}	116.2^{abcd}	0.243	5.19	0.397
0.325	88.35^{abc}	116.6^{abc}	0.229	5.22	0.400

Rama Rao (unpublished data)

Cholecalciferol requirement

Cholecalciferol (CC) plays a vital role in eggshell formation. This vitamin is involved in synthesis of Ca binding protein, which facilitates Ca absorption / transportation in intestine and shell gland. A level of 300 ICU D_3/kg diet is the optimum level for WL laying hens (NRC, 1994). The movement of Ca across the shell gland occurs through the association with the CC dependent Ca binding protein (CaBP). Metabolites of CC are reported to have higher bio-efficacy than the parent compound. At 0.57 µg/kg, 1∝-OH cholecalciferol was 10.9 times as active as CC for shell thickness and 6.7 times for shell strength. 25 hydroxy CC was 2.8 times as actives as CC for both shell thickness and strength (Kaetzel *et al.,*1978).

Incorporation of CC metabolites (1, 25 $(OH)_2$ CC or 25-OH CC) proved more efficient in improving the eggshell quality compared to its parent compound (Polin and Ringer, 1977; Tsang *et al.,*1990). In young pullets, supplementation of 1.1 mg / kg 25-hydroxy CC had no significant effect on egg specific gravity (Roland and Harms, 1976). However, Mcloughlin and Soares, 1976) reported that 25-hydroxy CC (600 IUC) in combination with oyster shell improved eggshell quality especially in older hens in their second year of production. Increasing the supplemental CC from 500 to 2000 icu/kg layer diet significantly improved eggshell strength (Supic and Pejin, 1988). However, additional supplementation of CC (300 to 2400 icu/kg) in old laying hens (72 to 88 weeks of age) fed adequate levels of Ca and total P (3.81 and 0.71%, respectively) did not show any beneficial effect on egg production and egg shell thickness (Panda *et al*., 2006).

As a general practice, CC is being added in layer diets whenever there is an issue of egg shell quality. In layer diets during post peak egg production phase where

the egg size is increased and utilization of minerals reduced, CC is supplemented at additional levels of up to 3600 icu/kg.

Feeding time

The shell strength of eggs laid in the morning is not as good as that of those laid in the afternoon (Roland *et al.,* 1973; Choi *et al.,* 1981). Usually shell calcification takes place during late hours of night. Therefore, availability of key nutrients essential for shell calcification at the time of shell formation is a paramount key factor that determines the shell strength. The normal practice of feeding the laying hens during the early hours of the day makes the nutrients unavailable to the birds during eggshell formation (early hours of fore noon). Calcium source should be available in the digestive tract for absorption during phase of eggshell formation, which helps in proper eggshell formation. The thermogenic effect of feeding coincides with the period of increased environmental temperature (summer season), which further aggravates the heat stress. The thermogenic effect of feeding will last up to 8 to 10 hours at higher environmental temperature (32°C) compared to at 20°C (only 2 hours) (Van Kampen, 1977). Feeding during late evening would ensure minimal stress and also availability of ionic Ca for eggshell formation.

Bird usually consumes maximum feed during forenoon and relatively small quantity during the afternoon. Therefore, the feed intake is usually negligible in the night and blood Ca will be exhausted early and less Ca will be available for shell formation (Scott *et al.,* 1971). Though some quantum of shell grit is lodged in gizzard, its absorption from the digestive tract may not be effective when other nutrients like CC, P and ammo acids, etc. are lacking in the digestive system (Farmer and Roland, 1986). Under normal feeding practices, little Ca is available to the hen after mid night when midnight feeding will make the Ca available during eggshell formation. In this practice, the lights are switched on for a period of half an hour at midnight and the hens are allowed to eat. This provides a source of Ca during the critical phase of shell formation (Harms *et al.,* 1996).

Mycotoxins

The shell strength of eggs produced by hens fed aflatoxin contaminated feeding is uncertain. Several investigators have reported that shell percent or shell thickness of

eggs produced during aflatoxin feeding are normal (Hamilton and Garlich, 1971; Huff *et al.,* 1975). Increase in the percent shell of hens receiving aflatoxin was reported (Hamilton and Garlich, 1972; Washburn *et al.,* 1985), which was attributed to reduced egg weight compared to the reduction in shell deposition.

Mycotoxins also disturb the utilization of Ca and P in chicken by damaging several vital organs like liver and kidney. Aflatoxin and ochratoxin causes pathological changes in liver and kidney, respectively which will impact the conversion of CC into its active metabolites (25 and 1,25 DH CC). Therefore, the requirement of CC increases in diets contaminated with fungal toxins. However, zearalenone (10 to 800 PPM) or citrinin (50 and 250 PPM) did not influence the eggshell quality (Ames *et al.,* 1976; Allen *et al.,* 1981).

Fungicides and chemotherapeutic agents

Fungicides are being used as seed / grain protectants during storage. Further, utilization of certain chemotheraputic agents like antibiotics, sulfonamides, coccidiostats, etc. became necessary to control the certain diseases in poultry. Water supplementation of sulphanilamide (125 or 150 mg/lit) reduced the intestinal Ca absorption (16 to 22%) and eggshell thickness (22 to 29%) and increased uterine contractions. Further, sulphanilamide was reported to inhibit shell calcification by reducing the secretary ability of shell gland by blocking activity of carbonic anhydrase (Dalgaard *et al.,* 1952). However other sulfa compounds like sulphaguanidine, sulphadimidine, sulphaquanidine sulphaquinazaline, sulphaquanaxaline will not affect shell quality (Scott *et al.,* 1944). Organo phosphates (malathion, coumaphos) and carbomates (carboryl) are less toxic to poultry compared to chlorinated hydrocarbons (DDT, BHC). White Leghorn laying hens fed 10 mg/kg DDT in diet depressed the eggshell thickness (Smith *et al.,* 1970). There were changes in the physical characteristics of the eggshells including reduced porosity. Deleterious effects DDT (10 or 50 PPM) on eggshell quality were more pronounced on hens in their second year of lay compared to those on young pullets (Cecil *et al.,*1973).

Egg production drops severely from 95 to less than 5% within 4-5 days in laying hens fed thiram contaminated feed. The eggs produced by hens fed thiram contaminated feed are mostly defective in shape, shell less eggs and watery albumen. Eggshell defects were attributed to reduced absorption of Ca by thiram in the

gastrointestinal tract (Edwards, 1987) thereby decreased serum Ca levels (Weldig *et al.,* 1968). Thiram stimulates contractions of oviduct muscles and resulting in expulsion of non-calcified eggs or eggs with defective shape and corrugated surface. Toxic effects of thiram in chicks can be counteracted with copper sulphate supplementation at 200 mg/kg in diet (Weidong *et al.,* 1990), but no such benefits were observed in WL laying hens (Nageswara *et al.,*1996).

Methyl mercury chloride at 10 mg/kg mercury content resulted in thin shelled and 'Sandpaper' eggs and truncation of the shell with corrugations (Scott *et al.,* 1975).

Common practices to minimize the losses due to pesticide toxicity include force molting, starvation, feeding high protein, high Ca diets, hepato-protective agents, etc. These practices showed some improvement in performance under practical conditions.

Trace minerals

Certain trace minerals (copper and manganese) have a significant role in formation of egg shell organic matrix.

Copper (Cu) is required in the cross linking of lysine in shell membrane organic matrix. Copper is co-factor for amine oxidase (Kim and Hill, 1966) and deficiency of Cu leads to reduced formation of lysine-derived cross-links i.e. formation of desmosine and isodesmosine. In Cu deficiency, the cross linking may not take place (Baumgarter *et al.,* 1978). Eggs produced on Cu deficient diet are abnormal in shape, size and narrow end is less distinct. Eggshell surface is wrinkled. Eggs acquire more water during shell formation, therefore, egg size will be bigger having higher proportion of thin albumin. The subsequent mineralization over these defective membranes results in wrinkled and misshapen eggs. Increasing dietary concentration of zinc to 50 PPM improves shell quality (Tanatarov, 1985).

Manganese (Mn) plays a role in synthesis of protein polysaccharides of shell matrix (Leach *et al.,* 1969). Deficiency of Mn in layer diet affects both the quality and

quantity of shell matrix (Longstaff and Hill, 1972). Supplementation of Mn up to 200 mg/kg in layer diet improved the eggshell quality and decreased the number of damaged eggs (Tanatarov, 1985; Whisenhunt and Maurice, 1985; Ochrimenko *et al.,* 1993). The percentage of broken eggs reduced and shell thickness and shell weight were increased by use of 500 mg/kg Mn in layer diet (Sus, 1975). Manganese deficiency results in fewer, but larger cones probably due to the fusion of several mammellary cores during shell formation. The shells are also thinner and have translucent areas (Leach and Gross, 1983). Hossain and Rezende (1996) did not find change in eggshell quality with varying levels of Mn in the diets (25, 50 or 75 mg/kg) of commercial laying hens. The inconsistent results could be due to wide variation in Mn content of feed ingredients from different geographic regions (Shyam Sunder, 1998).

Organic forms of trace minerals are reported to be better available and improve egg shell quality. The bio-availability of TM from inorganic form was reported to be less available compared to organic form particularly in diets containing higher concentrations of Ca (Basauri, 1998).

Salinity

Underground water usually contains higher concentrations of dissolved salts. Ground water increased the incidence of eggshell defects up to 34% in laying hens (Balnave, 1990). Supplementation of drinking water with 0.2 to 2 g NaCl / liter significantly increased the incidence of eggshell defects (Balnave and Scott, 1986; Balnave *et al.,* 1989). Increasing salt intake through the drinking water (500, 1000 and 2000 mg/lit.) or feed (1000 and 2000 mg/kg) reduced shell thickness (Pourreza *et al.,* 1994). The sensitivity of hens to saline drinking water appears to increase with age, presumably associated with increased egg size. Shell quality was returned to normal by providing town water (3 mg Na and < I mg Cl (Yoselewitz and Balnave, 1989a) in pullets but not older birds.

The deleterious effects of saline drinking water can be reduced through supplementation of the diet with organic Zn (Moreng *et al., 1*992). Zinc methionine improved the shell quality through increased activity of carbonic anhydrase in the uterus (Yoselewitz and Balnave, 1989b). The problem of poor shell quality cannot be alleviated by reducing or eliminating the NaCl from the diet. Increasing Ca level up to

2.8 or 3.3% can alleviate these ill effects of chlorine (0.25, 0.86%). Increasing dietary sodium content relative to chlorine increased blood bicarbonate level, eggshell strength and eggshell strength (Austic and Keshavarz, 1988).

Sodium chloride up to 1.0% in layer diet (40-80 weeks) improved eggshell quality and hens could tolerate 2.0% NaCl without adverse effects on eggshell quality (Hartal, 1991). Variation in results obtained might be due to variation in the concentration of salt in drinking water, Ca, Na and Zn in diet. The concentration of Ca binding protein and specific activity of carbonic anhydrase was significantly lower in hens receiving saline drinking water (Yoselewitz and Balnave, 1989b). This response may be related to the reported inhibiting effect of the Cl ion on carbonic anhydrase activity (Waygood, 1955). The reduced egg shell quality with saline drinking water could also be due to decreased uptake of Ca by the shell gland (Pourreza *et al.,* 1994).

Incidence of eggshell defects observed with saline drinking water can be reduced significantly by supplementing ammonium bicarbonate (Yoselewitz *et al.,* 1990) or ascorbic acid (Balnave *et al.,* 1991; Balnave and Zhang, 1992) in water or feed.

Higher environmental temperature

The ambient temperature during most part of the year in India is above the ideal temperature zone (55 to 75^{o} F) for layer. Higher environmental temperature is detrimental to the eggshell quality in several ways. Panting to expel metabolic heat produced in summer causes metabolic alkalosis, which limit ion exchange in the uterus, by lowering carbonic anhydrase activity in the shell gland and kidney (Goto *et al.,* 1979). Heat stress minimized blood flow (30- 40%) to ovarian follicles and shell gland due to peripheral vasodilatation (Wolfenson *et al.,* 1981). Reduced feed intake due to heat stress also limits several nutrients, leads to bone desorption and hyper phosphataemia which inhibits the formation of $CaCO_3$ (Mongin, 1968) in shell gland. Heat stress also reduces conversion of CC to its active metabolites (Scott, 1966). High environmental temperature also increases water intake which has an adverse effect on Ca solubility.

The effects of heat stress can be reduced by several ways

During heat stress, feed intake drops and utilization of Ca and CC reduces, and therefore, it is logical to increase Ca concentration in diet during summer. Supplementation of bicarbonate (Patience *et al.,* 1987; Balnave and Muheereza, 1997), or potassium (Teeter and Smith, 1986; Smith and Teeter, 1993) proved to improve the shell quality during heat stress. However, care need to be taken to avoid excess Cl in the diet.

Carbonated drinking water can improve the shell quality during heat stress (Smith and Teefer, 1993). Eggshell quality and performance of laying hens fed ascorbic acid (100 mg/kg) improved at elevated environmental temperature (34°C).

Prostaglandins may be responsible for premature expulsion of soft shelled or shell less eggs. Acetylsalicylic acid is an inhibitor of prostaglandin synthesis. Supplementation of acetylsalicylic acid (0.05%) in diets of laying hens producing soft or shell less eggs increased the shell thickness (Blog and Hester, 1991).

Sodium Bicarbonate

Supplementation of sodium bicarbonate could improve eggshell quality by neutralizing the metabolic acidosis associated with shell formation (Mongin, 1968; Cohen and Hurwitz, 1974; Wideman and Buss, 1985). Some workers reported that addition of sodium bicarbonate in diet (1.2 to 10 g or 50 g / kg) or water (2.5 to 10 g / L) improved eggshell thickness (Frank and Burger, 1965; Mongin, 1968; Makled and Charles, 1987). A reduction of dietary chloride to the minimum requirement may improve shell quality by increasing bicarbonate resorption by kidney. It was reported that a level of 0.2 % sodium chloride will provide adequate chloride for egg production (El-Boushy and Raterink, 1985). The use of sodium bicarbonate in layer diets during summer is not uncommon to improve eggshell quality. But the benefit of sodium bicarbonate use depends on the concentration of NPP in diet. Inclusion of sodium bicarbonate in the layer diet increases the shell quality for hens given low-phosphorus diets (0.3%), but not for those given high phosphorus (1.21%) diets (Britton and Zumbado, 1984).

Ascorbic acid

Ascorbic acid synthesis is impaired during the period of high environmental stress (Perek and Kendler, 1963). Ascorbic acid has been suggested to promote mineral mobilization from skeleton (Thornton, 1970). Ascorbic acid is involved in calcium

absorption or resorption from skeleton (Orban *et al.,* 1993) and synergism exists between ascorbic acid and vitamin D_3 (Weiser *et al.,* 1988). Ascorbic acid is also essential for synthesis of steroid hormones, shell organic matrix and bile acid formation (Kolb, 1985). Work done at the authors laboratory (Panda *et al*., 2008, **Table 4**) showed significant improvement in shell breaking strength, shell thickness and shell weight in WL laying hens fed diet supplemented with 200 or 400 mg ascorbic acid/kg diet under tropical summer (30.2 to 43.4^{o}C with 34 to 72% relative humidity). Supplementation of ascorbic acid is more beneficial when used under environmental stress or nutritional stress. Increasing the levels of ascorbic acid (100, 250 and 500 PPM) resulted in increased in egg specific gravity and shell percent (Zapata and Gernat, 1995), which was attributed to increased conversion of CC to $1,25(OH)_2D_3$ (Weiser *et al.,*1988).

Ascorbic acid alleviates the ill effect of heat stress by reducing the concentration of corticosterones in the bird (Schmeling and Nockles, 1978).

Table 4. Effect of supplementing ascorbic acid on eggshell quality in WL laying hens

Ascorbic acid, ppm	Specific gravity	Egg breaking strength, N	Shell thickness, mm	Shell weight, %
0	1.072	20.89b	0.356b	9.01b
200	1.073	25.03a	0.376a	9.22a
400	1.070	25.25a	0.375a	9.23a

Panda *et al*. (2008).

Organic acids

Supplementation of short chain fatty acids is reported to increase the intestinal morphology, digestion and absorption of nutrients. An experiment was conducted on WL laying hens (38 to 54 weeks of age) by feeding sodium (500g/Ton) and calcium butyrate (370g/Ton). Supplementation of both forms of butyric acid significantly improved eggshell thickness and egg density (Rama Rao Personal communication, **Table 5**).

Table 6. Effect of feeding two forms of butyric acid on egg shell quality in WL laying hens (38 to 54 weeks of age)

Treatment	Eggshell wt., %	Shell thickness, mm	Density	Breaking strength, N
Control	8.984	0.379	1.047	13.317
Na butyrate	8.985	0.385	1.052	13.501
Ca butyrate	9.039	0.384	1.052	11.750

Rama Rao, unpublished data

Protein and amino acids

The poor shell quality associated with larger egg size during later part of egg production can be improved by decreasing the dietary concentration of protein, methionine, linoleic acid, etc. Shell quality was significantly improved by reducing the dietary methionine (from 0.383 to 0.233) and NPP (from 0.65 to 0.25%) and by improving the Ca content (3.0 to 4.5%) in WL layer diet (Jackson *et al.,* 1987).

Probiotics

Feeding of certain probiotic preparations (*Lactobacillus sporogenes*) was also found to enhance the egg shell quality (Panda *et al*., 2007, **Table 6**). This beneficial effect of feeding the probiotic may be attributed to a favorable environment in the intestinal tract, created by the beneficial bacteria, which might have helped to assimilate more Ca (Mohan *et al*., 1995; Panda *et al*., 2003), which was evident by increased concentration of Ca in serum with probiotic supplementation.

Table 6. Effect of feeding *L sporogenes* on eggshell quality in WL laying hens

Trait	*L sporogenes*, 6 x 10^8 CFU/g		
	0	100	150
Specific gravity	1.067	1.067	1.065
Shell strength, N	36.56^{b}	38.64^{ab}	40.58^{a}
Shell weight, %	9.33^{b}	9.48^{ab}	9.78^{a}
Shell thickness,	0.35^{b}	0.38^{a}	0.38^{a}

mm			

Panda *et al*. (2008).

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Gut Health in Poultry – A Nutritionist's Perspective

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Introduction

The integrity of the gut and its functionality are central to achieving efficient performance in both meat and egg-producing poultry (Choct, 2009). For the last 50 years poultry producers have had access to antibiotics to modify the gut flora to improve feed utilization and maintain a healthy gut in their birds. However, this situation is changing with the association of the use of antibiotics in animals with the emergence of antibiotic-resistant bacteria that affect human health (Collignon et al, 2009). The poultry industry is now faced with finding ways of promoting and maintaining gut health by cost-effective means without using antibiotics.

The purpose of this article is to discuss some of the particular techniques used in poultry to successfully maintain a high level of gut health and performance based on experience in the field rather than controlled experimental science but building on science that has already been done.

Feed Particle Size

Feed particle size has been shown to affect performance in both broilers and layers due in part to its involvement in gizzard development and in feed passage rate (Amerah et al 2007). Feeding whole wheat has been shown to be beneficial to gut health (Hetland et al, 2002) but may have some practical problems. We have encountered problems in the field with free range broilers avoiding whole wheat to get at the pelleted feed (Figure 1). Although the gizzards of these birds were well developed some birds appeared to have wheat compacted in them (Figure 2). It was speculated that this was because the wheat was very hard and dry and the birds were not able to effectively grind it up. In this case cracking the wheat to achieve a coarse particle of 1-3 mm and including it in the pelleted feed resulted in less feed rejection and better growth.

Figure 1. Whole wheat left in feed pan.



Figure 2. Whole wheat in gizzard contents

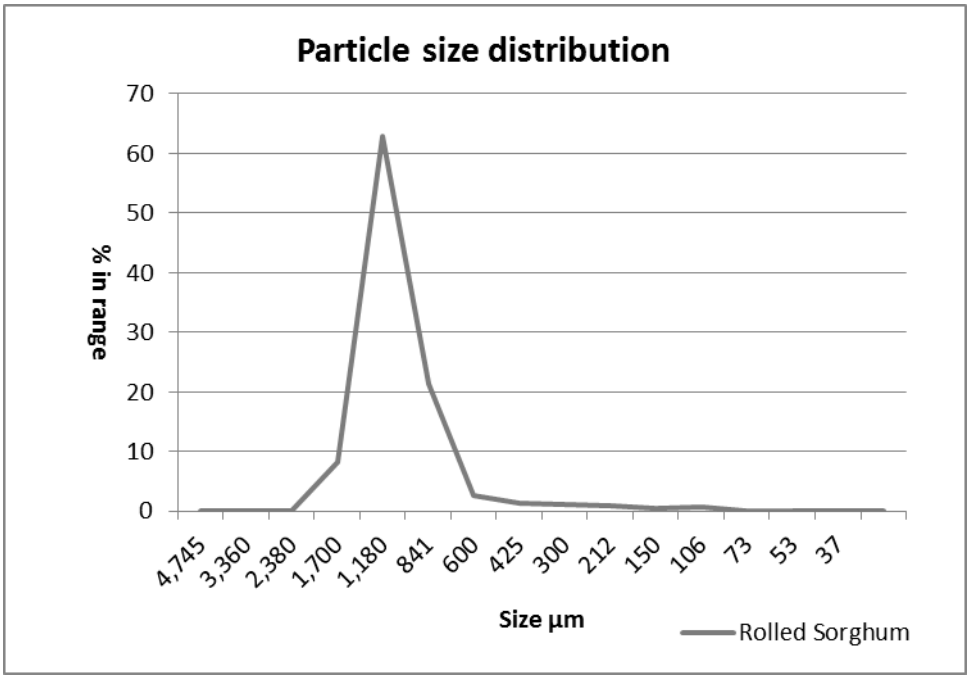


Feeding a more evenly distributed particle size is beneficial to both broilers and layers with regard to gizzard development and wet droppings. In layers fed mash diets providing a coarser but even particle size is less of a problem but the commercial broiler industries in most countries favour pelleted feeds for reasons of increasing feed intake and therefore growth. An interesting compromise has been reached in Japan and Korea where the bulk of the broiler industry feeds a crumbled Broiler Starter but feeds mash in the Grower and Finisher stages. This is has the advantage of getting the birds off to a good start but promotes gizzard development and gut health later in the program.

Most layers in Australia are fed mash but a considerable proportion is fed crumbles or short pellets. In mash diets particle sizes vary according to how the grains are processed. Roller mills set to produce coarser particle sizes (>1 mm) tend to yield more even particle distributions than hammer mills. Further, roller mills tend to produce a sharp edged particle with less dust whilst hammer mills produce a more rounded particle, which results in considerably more dust (McKinney 2006). The latter is probably an advantage in producing durable pellets but the shaper edged particles produced by roller mills may provide more physical stimulation of the gut lining and therefore lead to better gut health. Some livestock suppliers give recommendations for particle size whilst some are fairly vague. The Isa layer production manual (Isa, 2010) suggests that 85% of the feed should be between 0.5 and 3.2 mm. Hy-Line, in its latest manual (Hy-Line 2014), recommends that 70% of particles should be between 1-3 mm. With modern roller mills fairly precise control of grain particle sizes is possible and a typical analysis of grain particle distribution from

a triple roller mill is shown in Figure 3. With a target size range of 1.5-2mm and a peak of 1.8mm more than 80% of particles are between 1 and 2mm in diameter.

Figure 3. Particle size distribution of rolled sorghum



The advantages of having an even particle size are less feed separation in the feed conveyors, less chance for the birds to selectively feed and better consumption of micro-ingredients that are of fine particle size.

Fibre Content of the Diet

The role of fibre in poultry diets for both broiler and layers is receiving increasing attention as producers look for ways to improve gut health and productivity (Mateos et al, 2013; Pottgüter, 2013). Further, it is lignified, insoluble fibre that seems to have the positive effects on gut health. Wood shavings increased the size of the gizzard in broilers and layers and increased starch digestion in the latter, whilst oat hulls increased starch digestion in broilers (Hetland et al, 2003). In practical terms increasing the level of fibre in the diet has been seen as necessitating a reduction in nutrient density which, with modern genetics and the high price of ingredients, is not necessarily economic. Adding conventional fibre sources, such as wheat offal, requires an inclusion of >10% for a 1% increase in crude fibre. Feed fibre products

derived from trees have provided a source of fibre which is very concentrated (>60%) meaning that significant increases in feed fibre can be gained at relatively low inclusion rates. These sources of fibre have proved particularly successful in formulations for free range layers where high nutrient density is required in the early lay period. Although seemingly expensive concentrated fibre products have proved economic on some farms because of the reductions in mortality that appear to have resulted from their use.

In data from a free range farm inclusion of tree fibre, which was initially used at 0.4%, has been increased progressively to 0.83% at the request of the farm managers who have noticed that feather loss has been substantially reduced. Mortality in successive flocks on this farm has also fallen since concentrated insoluble fibre was included in the diet (Figure 4).

Figure 4. Mortality in successive batches of free range layers.

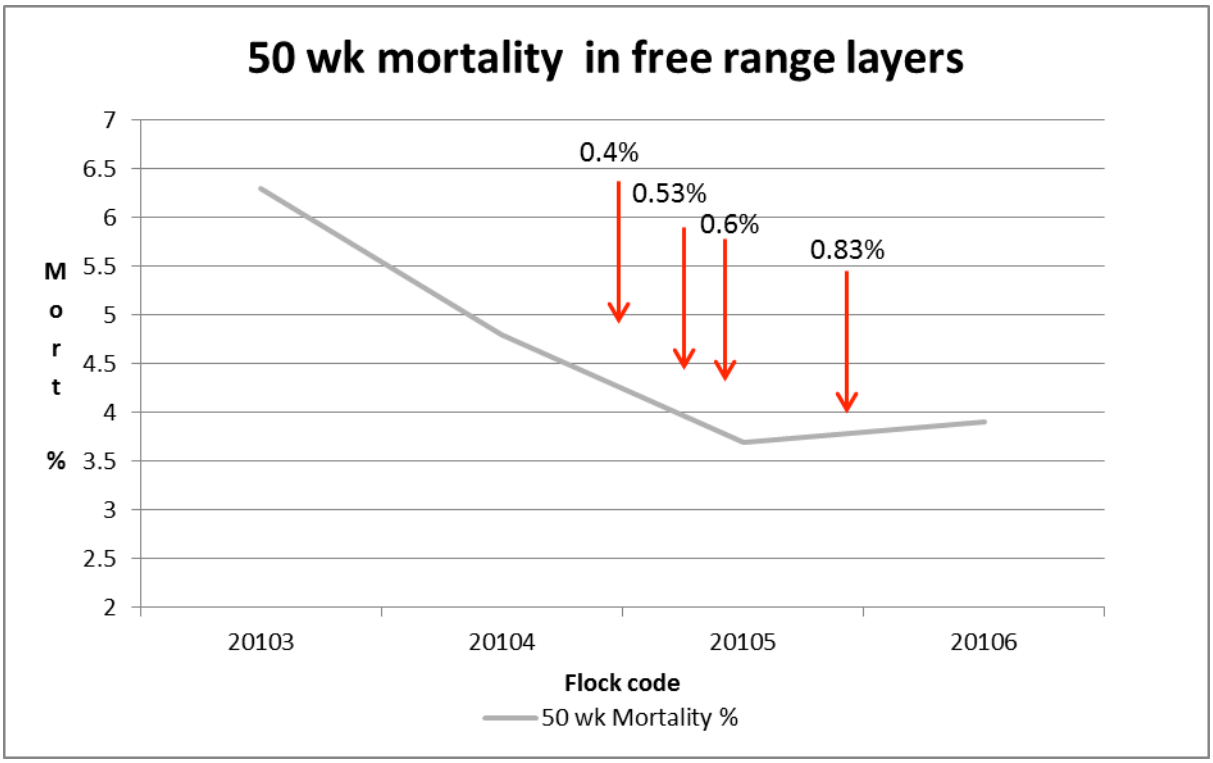


Figure 5 shows successive flocks of Hy-Line Brown layers in the same house and range, both at 39 wk of age. The 2012 flock was fed a diet with 0.53% fibre product and was already showing obvious signs of feather damage at 39 wk. The 2013 flock which was fed a diet containing 0.83% concentrated fibre product had almost no feather damage, particularly at the tail or neck.

Figure 5a Feather cover in Hy-Line hens 2012 **Figure 5b** Feather cover in Hy-Line hens 2013





Non-antibiotic feed additives

In recent years there has been an explosion in the number of non-antibiotic feed additives available for poultry. Some, like organic acids, the MOS derivatives, probiotics and exogenous enzymes are well established and supported by trial work. Another class of compounds, the so called phtytogenic compounds, are also prolific; some having very little data to support the claims of the manufacturers. However, a number do have activity in monogastric animals and some of their effects have been recently reviewed (Windisch at al, 2008). However, while most of these additives have been shown to have some effects on performance parameters very few have demonstrated any effect on important problems for poultry such as coccidiosis.

One such additive has appeared with claims for helping prevent coccidiosis. Its actives are not defined precisely but described as being a mixture of predominantly aldehydes and terpenes. Its proposed modes of action are three-fold: disruption of the cell membrane and mitochondrial membranes (aldehydes), blocking of ATP production (aldehydes) and disruption at two points in the TCA cycle (terpenes).

Because it looked somewhat promising it was tested in a free range laying flock that had been diagnosed with inflamed small intestines, undigested feed in the

small intestines and wet droppings. Post mortem examination of several birds revealed coccidiosis (Isaac, 2013). The producer was also concerned about poor egg shell quality and dirty eggs in these flocks. Faecal samples were collected from two houses and sent to a parasite diagnostic centre for analysis. Faecal and Caecal samples showed the presence of coccidial oocysts. The phytogenic additive was included in the feed at 1 kg/t with a concentrated fibre product added at 10 kg/t. Faecal samples were taken from the same flocks at four regularly spaced intervals and checked for oocysts and the results showed a gradual reduction in oocysts (Table 1).

Table 1. Oocyst counts per g faeces or caecal contents.

	2/11/2012		3/12/2012		21/12/2012		25/01/2013	
	Pooled	Caecal	Pooled	Caecal	Pooled	Caecal	Pooled	Caecal
Shed A	0	0	500	240	120	NA	0	0
Shed B	0	1140	360	20	40	NA	-*	-*

* Flock depleted

Faecal and caecal oocyst counts decreased over time following the addition of the phytogenic additive to the feed. A follow up visit to the farm in 2013 indicated that shell quality had improved and dirty eggs were markedly reduced. Since then the additive has been used successfully in preventing coccidiosis in pullets on organic feed where no other additives are allowed. It was suggested the addition of concentrated fibre also contributed to drying up the droppings and reducing the frequency of dirty eggs.

Conclusions

Increasingly, as changes to poultry production methods become driven by forces other than economics the requirement to look for alternative methods of maintaining production will increase. Although many alternative additives and feeding strategies exist, not all will work and some will work in some instances and not in others. However, it is now clear that with methodical on-farm investigation, drawing on the available science, there are ways to successfully raise poultry without using antibiotics in the feed.

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