

# Invited Speakers

## S2.2

### The Role of Carbohydrates, Protein and Fat in Litter Quality

SR COLLETT

The University of Georgia, College of Veterinary Medicine, USA

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In modern poultry production, litter is used to enhance bird health and welfare, and in order to achieve this, litter moisture should not exceed 25%. Litter becomes wet when the rate of faecal and urinary water excretion exceeds the rate of evaporation from the litter.

An increase in faecal water (diarrhoea) or urine volume (polyuria) may be the result of normal homeostatic process or disease. Water intake, urine volume and faecal moisture are altered by dietary mineral content, anion-cation balance, feed passage time and several feed ingredient characteristics. The content and quality of protein, carbohydrate and lipid in the diet can affect water excretion rate directly, by creating an osmotic gradient across the intestinal and renal tubule lining. More subtle effects may cause diarrhoea indirectly by inducing changes in the intestinal ecology that result in an inflammatory response. Diarrhoea resulting from inflammation of the intestinal lining (enteritis) is a more serious problem. The digestion and absorption of nutrients is compromised, and changes in the composition of the intestinal flora are more difficult to correct.

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### Introduction

Litter is used routinely in meat bird production to enrich the rearing environment and enhance bird health and welfare. The litter acts as, a cushion to increase comfort, an insulator to help keep floor temperatures within comfort zone and a buffer against fluctuations in moisture content. A variety of litter materials and management practices have been employed around the world depending on the regional economics of poultry production and litter material available.

Pine-shavings, straw or oat-hulls are the most commonly used litter materials, while alternatives such as hard wood shavings, paper or paper products, bark and pelleted products are less frequently used. In most parts of the world, litter is removed and replaced after each grow-out cycle, but in most US operations and some Central and South America operations, litter is reused for several cycles before being replaced. Both the type of litter material and the litter management practices affect its ability to enhance bird health and welfare.

The staggering improvements in genetic potential for rate of gain in meat type birds have altered the dynamics of managing litter quality and in particular litter moisture content. Intensification of farming practices, increased feed and water intake, the removal of in-feed antibiotics, and changes in the diet have together increased the importance of the litter in buffering the lag between water addition and removal.

Recent advances in molecular study techniques have highlighted the importance of the gut microbiota (microbial community) in maintaining gut integrity and homeostatic function. Equally important has been the realization that the microbiota that inhabit the litter originate from the hindgut (faecal and caecal excretion) and act as a source of challenge to the upper gut (coprophagia and retro-peristalsis). The survival of these organisms in the litter is influenced by the complex ecology of the litter microenvironment but is primarily dependant on the litter moisture content.

Economy of scale has driven the poultry industry to intensify while consumer pressure has provided sales incentive to ensure that welfare is a significant part of the economic equation; no sale no profit. Maintaining litter quality is undoubtedly integral to balancing profitability with welfare.

### Litter Quality

Litter quality is a rather nebulous term use to express the ability of the bedding material to satisfy purpose. Although there are several litter characteristics used to describe litter quality, moisture content is the most important. Birds appear to be most comfortable when litter moisture is around 25%. *Poultry litter becomes wet when the rate of water addition (urine/faeces/spillage) exceeds the rate of removal (evaporation) and the moisture content rises above 25%* (Collett, 2007).

Poultry litter supports a very diverse microbial community that derives sustenance from both the litter material itself and the bird's excreta. In addition to these raw ingredients, the richness in microbial diversity is increased by the cascade of nutrient networks that are established over time. The microenvironment within the litter bed is what directs the microbial community evolution and shift. When the litter moisture drops too low, bacterial activity declines and when litter moisture rises, microbial activity increases.

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With healthy flocks the rate of water addition to the litter is, for the major part, stocking density dependent. House design in terms of floor space, feed trough space, nipple drinkers per bird, heating, cooling and ventilation capacity is based on metabolic mass. To optimize metabolic efficiency and hence performance, the birds have to be kept within their thermoneutral zone, their metabolic engines have to be kept stoked with feed and their metabolic waste has to be removed. To optimize economic efficiency, stocking density is pushed to match house design capacity. As a flock approaches depletion date and the metabolic mass of the flock approaches house design capacity, the margin for error in house environmental management narrows.

Improvements in bird growth rate, and house design, have permitted the use of higher stocking densities at younger ages. In modern small-bird (1.72kg) production systems, birds will reach target weight as early as 34 days. Under such conditions stocking density reaches house design capacity (34kg/m<sup>2</sup>) as early as 4 weeks of age. At this age coccidiosis challenge is also peaking as indicated by oocyst count (Renya *et al.*, 1983). So just when stocking density and house ventilation capability are reaching their limits, the inflammatory response provoked by *Eimeria* parasite invasion is compromising solute transfer across the intestinal lining, to the point where nutrient assimilation decreases and faecal moisture increases. Although there is only a minor change in the efficiency of nutrient assimilation, the resultant change in the nutrient base for the downstream microbiota is sufficient to cause shifts in community composition. The increase in intestinal mucus and undigested nutrients (protein in particular) promotes the growth of, amongst others, Clostridial organisms (Collier *et al.*, 2008). Microbiota diversity declines and pathogens such as *Clostridium perfringens* dominate. The metabolites (toxins) of these unfavourable members of the intestinal microbial community cause further inflammation (Wages and Opengart, 2003). Shifts in composition of the microbial community of the caecae can also reduce the bird's ability for post renal concentration of urine thus increasing water loss. To make matters worse the inflammatory mediators, cell debris and mucus that pass down the intestinal tract onto the litter creates a non porous cap on the litter, thus reducing its absorptive capacity. The liquid fraction of the excreta collects on the litter surface, birds become wet and uncomfortable and performance suffers. Once the litter is saturated the surface area for evaporation is reduced, litter temperature drops and it becomes very difficult if not impossible to control litter moisture.

## Water Balance

Water is the biological transport medium for nutrient absorption and waste excretion. In living organisms, water balance is a critical part of homeostasis and is achieved by a carefully regulated process called osmoregulation. This involves a combination of active and passive solute transfer in order to balance water gain (*intake* and *synthesis*), with water loss (*excretion* and *insensible loss*).

Water gain is in reality regulated by drinking since it accounts for around 80% of total water intake. The other sources of water, feed moisture and metabolic water synthesis are diet dependant variables which are beyond the bird's control (Leeson and Summers, 2005). The urge to drink is initiated when the thirst centre is stimulated by intracellular dehydration (osmoreceptors), extracellular dehydration (mechanoreceptors) or angiotensin II secretion (rennin-angiotensin axis) (Goldstein and Skadhauge, 2000; Kanosue *et al.*, 1990; Kaufman *et al.*, 1980).

Water is lost during homeostasis, as vapour from the skin and respiratory tract, and as liquid in the faeces and urine. In healthy birds that are kept within their comfort zone (thermo-neutrality) approximately 50% of water is lost as vapour and 50% as liquid (Goldstein and Skadhauge, 2000). Liquid water loss is governed by solute and water movement across the gastrointestinal and renal tubule membranes. It is this liquid water loss (faecal and urinary) that poses the greatest risk to litter quality since it requires additional energy (heat of evaporation) and effort (air temperature/humidity control) to evaporate from the litter and remove from the house. At maximum stocking densities huge amounts of water are added to the litter on a daily basis so even a minor change in water balance can alter litter moisture. It is critical for the litter to absorb and hold this water until it is removed by evaporation.

In normal healthy birds, water molecules are in constant flux across the gastrointestinal membrane, and faecal water, although dietary dependent, usually remains fairly constant (Cronje, 2007). When membrane integrity and normal homeostatic process is compromised by gastrointestinal disease or abnormal osmotic stress, there is a net efflux of water into the gut lumen and the increase in water loss results in diarrhoea. From a diagnostic point of view it is important to differentiate whether the diarrhoea is physiological or pathological (enteritis) in origin. Distinguishing physiological from pathological diarrhoea is difficult in the avian species because urinary excretion is somewhat unique in birds. Firstly, the ureters open into the coprodeum and secondly, the urine passes retrograde up the colon to the caecae before being evacuated via the cloaca with the faeces (Goldstein and Skadhauge, 2000). Urine excretion is carefully controlled by several hormone feedback systems but the kidney's capacity to concentrate urine is limited in poultry (Clark and Mok, 1986; Goldstein and Skadhauge, 2000; Gray, 1993; Gray *et al.*, 1988; Morild *et al.*, 1985). The solute concentration of normal urine is only 2-3 times that of plasma. To compensate for this, post renal concentration of urine occurs during its passage through the coprodeum, colon and caecae (Rice and Skadhauge, 1982).

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Increased litter moisture can result from physiological diarrhoea, pathological diarrhoea (enteritis) or elevated urine output (polyuria). Water intake, urine volume and faecal moisture are altered by dietary mineral content, anion-cation balance, feed passage time and several feed ingredient characteristics. Additionally, any inflammation that results from an inappropriate immune response at the gastrointestinal and renal tubule lumen surface, can potentially compromise solute and water transfer and elevate water loss above normal limits.

## **Effect of Dietary Protein Carbohydrate and Lipid on Litter Quality**

Nutrition has evolved from its infancy of merely correcting nutrient deficiencies to the point where nutrients, in addition to their function as building blocks for growth and development, are being viewed as providing dietary signals to the host genome (Francis *et al.*, 2003). The whole new science of nutrigenomics has evolved with efforts to decipher the language of nutrient signal transcription, metabolic effects and phenotypic expression. This is a very difficult task: feed is nutritionally complex and its composition notoriously variable, dietary signals vary widely in strength, target gene function is frequently unknown and the expense of such research limiting (Muller and Kersten, 2003). The prospects are however exciting and worth pursuing.

To ensure that the bird's constantly improving genetic potential for production is converted into economic gain, the poultry nutritionist has had to ensure that nutrients are supplied in the correct *form* and *balance*, at the lowest possible *cost*. In this case "cost" has to be viewed from two different paradigms. From an *input efficiency* perspective the emphasis is on minimizing diet or ingredient cost while from an *output efficiency* perspective the emphasis is on maximizing performance. In each case the aim is to maximize return on investment or profit. Companies with high marginal to capital cost ratios will favour the former, while those with low marginal to capital cost ratios will favour the latter.

It seems that perhaps too much emphasis has been placed on formulating to the bird's experimentally determined *nutrient requirement*. This approach unfortunately takes no account of the performance implications of subtle but significant shifts in health status, for example. Under research conditions, confounding factors are eliminated through careful experimental design, and population variance is minimized by meticulous control of experimental conditions. While this approach ensures statistically reliable results, it fails to reflect the consequence that subtle subclinical disease has on performance in a field situation. Instead, mortality or death is used routinely by nutritionists to measure the health response to dietary inputs; clearly a very coarse measure of health status. In modern highly intensive production systems disease is seldom clinical in nature before it impacts bird performance. The complex interaction between, nutrient, host, environment, and disease causing agent, is critical to the outcome of disease challenge and what ultimately determines its effect on performance.

## **Protein**

The cost of energy has doubled over the last few years but protein has always been an expensive ingredient and amino acid balance has for decades been the focus of diet formulation. The potential interactions and permutations of amino acid ratios make traditional approaches to determining ideal amino acid composition of the diet daunting. The more holistic approach of stochastic modelling has helped to improve the process by extending it to include and consider more variables. Recent research indicates that it may be necessary to expand this concept to include the impact of ingredient choice on the microbiota (Ley *et al.*, 2006). The composite output of the intestinal milieu (nutrient and microbiota) has been shown to influence both the efficiency of nutrient assimilation and the process of accretion.

The intestinal microbial community of birds evolves rapidly in the first three weeks of life (Hooper and Gordon, 2001; Lu *et al.*, 2006; Lu *et al.*, 2003; Torok *et al.*, 2007). This change in composition happens to coincide with improvements in protein digestibility (Huang *et al.*, 2005). Nutritionists have aimed at maximizing protein accretion during this critical early developmental phase through careful ingredient choice. If protein digestion efficiency is partially microbiota dependant, this approach may be misdirected. Highly digestible protein sources such as fishmeal are favoured ingredients in starter diets. These highly digestible proteins are also soluble, and soluble nutrients have been shown to move through the digestive tract more rapidly than insoluble nutrients (Klipper *et al.*, 2000, 2001, 2004). Proteins of animal origin are consequently much more likely to arrive in the distal intestinal tract in an undigested state. Apart from being antigenic, these large protein molecules provide a nutrient substrate for proteolytic organisms and are thus likely to generate a microbiota dominated by Firmicutes. While a microbiota dominated by these organisms is associated with more efficient feed conversion, many pathogens including *Clostridium perfringens* are also members of this group (Bäckhed *et al.*, 2007; Ley *et al.*, 2006).

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*The dilemma of modern meat bird protein nutrition appears to be that while the broiler requires a high protein diet for rapid growth, a high level of protein nitrogen in the hind gut is a potential health hazard.* By breeding for rapid growth rate and hence appetite and protein requirement, the geneticist has narrowed the gap between too little and too much dietary protein. The nutritionist has been able to determine and formulate to the optimum level of protein, by carefully controlling experimental conditions. Besides causing relative protein deficiency for the bird, even a slight change in digestion and absorption efficiency can cause a composition shift in the microbial community. The daily stressors experienced by meat birds in an intensive farming system are adequate to alter the *status quo* at the microbe/digestive tract interface. This constant risk of intestinal pathogen overgrowth raising the level of inflammation to pathological levels, was likely the primary reasons why prophylactic in feed antibiotics became a necessity.

The perceived risk associated with feeding animal protein to poultry has in many countries forced the shift to all vegetable diets. With all vegetable diets, the nutritionist has had to deal with the increased risk of wet litter because firstly, high dietary potassium elevates urine output, and secondly, the high non-digestible carbohydrate and protein fractions increase faecal moisture. Even where the use of animal protein is still permitted, soya is still the largest source of protein in avian diets. With this much reliance on soya as a source of protein there is a high risk of incomplete heat inactivation of its naturally occurring trypsin inhibitor leading to enteritis and wet litter (Leeson and Summers, 2005).

In addition to affecting growth rate and feed conversion, inefficient protein assimilation increases the excretion of nitrogen (undigested protein) which negatively affects litter quality. This increase in nitrogen excretion occurs as a result of increased protein passage (faecal nitrogen) and nitrogen excretion consequent to body protein recycling (urinary nitrogen) (Akester *et al.*, 1967; Denbow, 2000; Duke, 1982). The elevation in urinary nitrogen has the propensity to cause osmotic diuresis, polyuria and wet litter. When litter moisture is normal there is typically enough water present for the microbes to be able to convert excreted nitrogen into ammonia. Even these low levels of ammonia emission from the litter have been shown to compromise innate immunity and depress performance. Any elevation in litter nitrogen and, or litter moisture above normal can raise ammonia levels in the house to dangerous levels.

Much of the research effort on protein nutrition has been directed at balancing amino acid requirement with supply. Requirement is unfortunately a moving target and nitrogen excretion a reality. The rate of nitrogen excretion increases as the level of dietary protein increases and the impact of this on litter quality can therefore be reduced by improving amino acid availability or reducing dietary total protein. Both of these objectives can be achieved by adding exogenous enzymes to the diet to improve amino acid availability since this will allow a reduction in total protein. Despite the fact that there is ample research to demonstrate that the use of exogenous enzymes improves digestibility, protein enzymes are not commonly used in meat bird diets (Choct and Annison, 1992; Rosen, 2000; Rosen, 2001). This is surprising, since this practice can potentially improve feed efficiency and gut health while reducing cost, nitrogen excretion and carbon footprint.

## **Carbohydrate**

Dietary water soluble non starch polysaccharides (NSP) such as: pentosans and  $\alpha$ -amylase inhibitors in wheat,  $\beta$ -glucans in rye and barley, and sucrose, stachyose and raffinose in soya, have the propensity to cause wet litter. These NSP stimulate mucus production, increase ingesta viscosity and compromise digestion and absorption (Choct and Annison, 1992; Collier *et al.*, 2003; Iji, 1999). In addition to increasing faecal moisture, the relative abundance of nutrient (non-digested fraction) in the hind gut causes unfavourable shifts in the microbial community of the hind gut, thus potentially reducing the efficiency of post renal concentration of urine. Exogenous enzyme addition to diets with high levels of these ingredients has become standard practice in order to avoid the wet litter problems associated with higher inclusion rates of these ingredients (Choct and Annison, 1992; Leeson and Summers, 2005; Rosen, 2000; Rosen, 2001).

Despite the fact that birds do not produce the enzyme ( $\alpha$ 1:6-galactosidase) necessary to digest the relatively high levels of NSP (mainly sucrose, stachyose and raffinose) present in soybean meal, routine addition of enzymes to soya based diets is uncommon. These non-digestible carbohydrates can however, potentially alter the microbial nutrient profile in the hind gut, destabilize the hind gut microbial community and increase faecal water, thus contributing to wet litter problems (Leeson and Summers, 2005). This is particularly noticeable with undercooked soya since the antinutritional factors (trypsin inhibitors, lectins and isoflavones) exacerbate the degree of intestinal inflammation, protein indigestion, and the excretion of nitrogen and water (Leeson and Summers, 2005).

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Interestingly, the negative effect of the NSP tends to dissipate as birds mature. One plausible explanation for this is that microbial contribution to the digestive process improves as the microbiota adapts to the dietary ingredients, evolves and matures. It has been demonstrated in mice for example, that the lack of gut flora results in intestinal obstruction with inspissated mucus, emphasizing that the mucolytic activity of microbial enzymes is essential for normal digestive process (Hooper and Gordon, 2001; Hooper *et al.*, 2001). An increase in NSP digesting organisms and microbial mucolytic activity of the evolving intestinal microbiota, would explain the age related waning of NSP antinutritional effects.

## Lipid

The use of lipids in meat bird diets has become more attractive, as grain derived energy cost has increased. Full-fat soya has proven to be a very high value product but thorough grinding is essential to release the cell-associated lipids and ensure efficient digestion (Leeson and Summers, 2005). As with all added fats and oils, the liberated lipid in full-fat soya is very vulnerable to oxidation. These ingredients require careful storage and special quality control. Rancid fats are less digestible, reduce the efficiency of fat soluble nutrient absorption, cause intestinal inflammation and alter the composition of the intestinal microbial community. These changes invariably cause wet litter either directly by steatorrhoea, or indirectly by affecting gut flora and gut wall integrity through oxidative damage.

Apart from their effect on digestion and absorption, the type and quality of dietary lipids can alter the nature and extent of the bird's inflammatory response. Dietary polyunsaturated fatty acids (PUFA) incorporated into the cell wall during growth provide the substrate for immune system communication molecule synthesis (Klasing, 1998; Korver and Klasing, 1995; Korver and Klasing, 1997). Immune cell phospholipid membrane composition will correlate with dietary PUFA content. The inflammatory response in birds fed ingredients that are rich in linoleic acid will be dominated by prostaglandins, leukotrienes and thromboxanes. These cytokines are synthesized from arachidonic acid which is an n-6 PUFA acid derivative. The response in birds fed fish-oil will in contrast be dominated by Interleukin-1 and prostaglandin-E since these are n-3 PUFA derivatives (Fritsche *et al.*, 1991; Korver and Klasing, 1997). The n-3 PUFA derived cytokines are released in lower quantities in response to immune stress and are less potent mediators of inflammation than the n-6 PUFA derived cytokines (Korver and Klasing, 1997). Broilers fed diets high in n-3 PUFA will consequently tend to perform better than those fed diets high in n-6 PUFA when confronted by immunological stress (Korver and Klasing, 1995; Korver and Klasing, 1997; Korver *et al.*, 1998; Korver *et al.*, 1997). Flax seed oil contains the highest levels of omega-3 fatty acids (50% linolenic acid) and thus provides the most suitable vegetable substitute for fish meal as a source of omega-3 fatty acids (Leeson and Summers, 2005).

## Conclusion

To keep pace with genetics, the science of nutrition is evolving to the molecular level. Nutrients are beginning to be viewed as dietary signals and every effort is being made to decipher the language of signal transcription, in order to discover their metabolic and phenotypic effects. The emerging science of nutrigenomics will no doubt change the landscape of nutrition in the years to come.

The realization that the *microbiota* (composite microbial community) of the intestinal tract affects more than intestinal-health, is revolutionary. Since these organisms are capable of altering host gene expression nutritional management of this microbiome (composite microbial genome) should be considered an integral part of poultry nutrition. The dietary protein, carbohydrate and lipid content, and their ratio, quality, type and source can affect feed efficiency directly or indirectly. The direct impact of nutrients on feed efficiency can be measured by carefully designed nutrient response research. The more subtle indirect effects are health related and are consequently a lot more difficult to quantify since they are the result of changes in the composition of the intestinal microbial community, the integrity of the gut lining and the bird's water balance.

The impact of intestinal homeostatic perturbation on the house environment, especially the litter moisture content, is magnified by our need to maximize return on investment. This is particularly evident in those parts of the world where large integrated companies own the grow-out facilities, since the return-on-assets-managed, is governed by meat-yield per house. Short grow-out cycles, short down-time, high stocking densities and the practice of thinning, have been implemented to improve economic efficiencies, but have at the same time, altered the intestinal ecology, and have thereby predisposed birds to intestinal disease and wet litter.

The emphasis in monitoring has shifted from *clinical* to *subclinical* and the emphasis in management has shifted from prophylaxis to strategies which promote advantageous intestinal ecological evolution. Gut health management programs need to be improved to the point where monitoring is sufficiently sensitive to detect early changes in gut health, and program design is flexible enough to allow timely corrective action to be taken.

# Invited Speakers

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