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Review

Nutrition, feeding and laying hen welfare

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Abstract. The relationship between nutrition and welfare is usually considered to be a direct result of supplying the hen with adequate quantities of feed and water. This simple notion of freedom from hunger and thirst belies the fact that nutrients play a pivotal role in the body's response to challenges whether they relate to ambient temperature, gastrointestinal health, pathogen exposure, metabolic disorders, or social and mental stress. In all instances, maintaining homeostasis and allowing for physiologic response is dependent on an adequate and balanced nutrient supply. It is accepted that most laying hens are fed a complete diet, formulated commercially to provide the required nutrients for optimal health, egg production and welfare. In other words, the laying hen, irrespective of her housing, does not experience hunger or thirst. However, despite adequate nutrient and water supply, certain senarios can significantly affect and alter the nutrient requirements of the hen. Furthermore, the chemical composition and also the physical form of feed can significantly contribute to prevent or treat welfare and health conditions and is, therefore, a highly relevant tool to ensure and maintain an adequate welfare status. Therefore, this review takes a broader perspective of nutritional welfare and considers the nutrition of hens managed in different production systems in relation to nutritional physiology, gut microbiota, stress, metabolic disorders and feeding management.

Keywords: amino acid, calcium, diet, energy, gut health, gut microbiota, immunity, inflammation, metabolic disorder, nutritional physiology, pasture, phosphorus, stress.

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Introduction

The supply of feed to laying hens is the most significant cost of egg production and diets are formulated to meet the nutrient requirements of the hen at least cost. The modern bird has been selected for high productivity (Underwood *et al*. 2021) and if this genetic potential is to be met without compromising welfare, feeding and housing must be optimal. Diets for laying hens are formulated to ensure maximum egg output. In general, nutritional recommendations for laying hens consider breed and housing conditions. However, as most husbandry situations are complex, adjustments to the recommended daily energy and nutrient intakes may need to be made to meet specific production objectives (Fig. 1). As conditions change in the hen house or on the range, including stage of production and season of the year, the amount of each nutrient required will change and, in some cases, dietary imbalances occur that impair productivity and compromise welfare.

The five freedoms are commonly used as a framework to evaluate welfare (see Webster 2001, 2016). It is obvious that freedom from hunger and thirst involves nutrition and feeding, but it is less obvious that the other four freedoms, including freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour, and freedom from discomfort and stress have nutritional components that also must be satisfied. In this regard, feed provides the nutrients and substrates essential for the maintenance of homeostasis. Moreover, there is increasing evidence that diet and the gastrointestinal microbiota combine to play a central role not only in physical but also mental wellbeing, with obvious implications for bird health and welfare (see Villageliu and Lyte 2017; Kraimi *et al*. 2019).

Concerns of laying hen welfare have focussed on housing systems, environmental enrichment and other management strategies. The major variable for the laying hen is the management system (housing) under which it is maintained (Edwards and Hemsworth 2021). Understandably, significant amounts of money have been invested by egg producers to upgrade caged-hen facilities and to develop colony-caged, barn, and free-range systems to improve hen welfare and meet consumer expectations. The aim of egg production is to achieve the best bird performance and feed utilisation, while providing adequate hen welfare. Therefore, for some husbandry conditions and specific production objectives, adjustments of the recommended daily energy and nutrient intakes need to be made to meet these demands and manage associated challenges. However, there is limited information available on the nutritional welfare of laying hens. Within this context, the

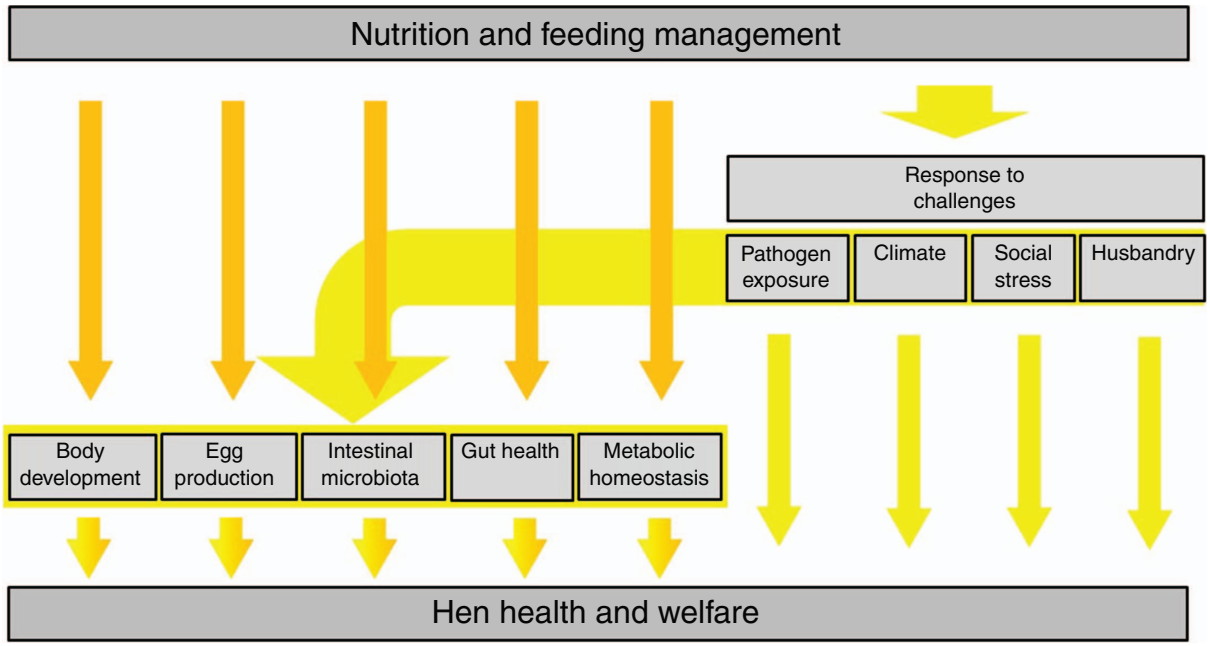

Fig. 1. Direct and indirect effects of nutrition and feeding management on hen welfare. Feed and nutrients have a direct impact (orange arrows) on body development, egg production, intestinal microbiota, overall gastrointestinal health and metabolic homeostasis. Indirectly (yellow arrows), feed provides support to mitigate challenges such as pathogen exposure, adverse climate conditions, social stress and husbandry procedures, including vaccination, beak trimming and relocation. Together, the direct and indirect effects of nutrition and feed management are central to laying hen welfare, health and productivity.

nutrition of hens managed in caged and non-caged production systems is reviewed. Nutrient requirements are discussed in relation to nutritional physiology, stress, metabolic disorders and feeding management.

Diet composition and nutrient availability

Arguably, more is known about poultry nutrition than the nutrition of most other species and this has contributed to the success of the poultry industry. With chicken meat production, nutrition research has sought to optimise growth and development, while the aim of laying hen nutrition research has been to maximise egg production or reproductive performance. However, to achieve maximum egg output, bird health and welfare must be optimised. The role of diet composition in achieving this is discussed in this section. The growing pullet is discussed in a later section.

Energy

Laying hens require energy for maintenance, determined by metabolic body mass, and for production (Leeson and Summers 2009). The energy requirements for production are primarily for daily egg production and body mass increase between the onset of sexual maturity and the attainment of mature bodyweight (BW), including feather growth (Tauson and Svensson 1980). Genotype and housing also mediate energy requirements along with several variable factors, including hen activity, ambient temperature and plumage condition that change during the production cycle (Peguri and Coon 1991, 1993).

The housing system is a major determinant of hen behaviour and activity. Tiller (2001) suggested that the maintenance energy required for hens housed in barn and free-range conditions is higher than for caged hens. While several studies have determined that hens kept in non-cage systems, such as organic, barn or free-range production, require an additional 10–15% more energy, hens may compromise egg production so as to meet their energy demands and, therefore, the expected egg output is reduced (Tiller 2001; Aerni *et al*. 2005; Leenstra *et al*. 2012; Leinonen *et al*. 2012; MacLeod 2013).

Energy requirement adjustments are also required for changes in ambient temperature (GfE 1999). An important component of this adjustment is plumage condition, which provides insulation and allows hens to regulate energy loss. A loss of feathers due to abrasion, feather pecking or moulting has to be compensated with increased levels of dietary energy to maintain body temperature, hen health and welfare. Moreover, additional dietary energy is required for the dissipation of body heat when the ambient temperature exceeds 22°C, as frequently occurs in many areas of Australia and Asia.

Laying hens are able to adjust their feed intake, to some degree, according to their energy requirements. Hens reduce feed intake when dietary energy increases. In contrast, if dietary energy content is too low, layers are unlikely to increase their feed intake sufficiently to overcome the deficit (Leeson and Summers 2009). Throughout the production cycle, the energy requirements of laying hens remain relatively constant as the demand for body

maintenance increases with age and this is offset by a decrease in egg production. Nevertheless, hen age is positively correlated with egg weight and egg mass, resulting in reduced eggshell thickness and, subsequently, a reduced number of sellable whole eggs in older flocks (Harms *et al*. 1982).

Protein and amino acids

While protein is a critical component of a layer diet, the balance of essential amino acids is crucial when formulating diets (Leeson and Summers 2009). The ideal protein concept was developed in the 1950s, as a way to define the essential amino acid requirements of birds for protein accretion and maintenance (Scott *et al*. 1969). Although sulfur amino acids (methionine and cystine) are considered first limiting amino acids in most commercial laying hen diets, lysine is used as the reference amino acid for this concept (NRC 1994). The requirements for all other indispensable amino acids are expressed as a percentage of lysine and calculated values for caged hens, as shown in Table 1; presumably, free-range hens have a similar ideal amino acid ratio. The values reported span some 20 years and show some variation due to genetic changes in laying stock and different methods of calculation. Synthetic or crystalline amino acids are added to diets to ensure that the ideal amino acid profile is achieved, thus permitting lower levels of protein to be fed. In some countries, synthetic amino acids are banned in organic poultry farming and this could result in the feeding of imbalanced diets, especially in relation to methionine, with a negative impact on bird health and welfare (Burley *et al*. 2016; van Krimpen *et al*. 2016).

Dietary content of balanced protein affects both egg number and egg size. For each 0.05% increase in sulfur-containing amino acids above 0.23%, the egg weight of layers increases by 0.7 g and the addition of methionine results in an almost linear increase of egg weight (Calderon and Jensen 1990; Waldroup and Hellwig 1995). However, if the concentration of these amino acids is at the lower limit of the requirement, reduced egg number is observed. Hens will also try to maintain amino acid requirements by increasing their feed intake, resulting in an overall increase in energy consumption. Hens maintained in free-range conditions have higher crude protein requirements for maintenance, most likely reflecting increased feed intake to meet the greater energy expenditure (Leenstra *et al*. 2014; de Almeida Brainer *et al*. 2016).

Specific amino acids have been shown to modifiy animal behaviour in many species. In caged laying hens, increasing lysine and methionine concentrations ameliorate the negative effects of stocking density on cannibalism and mortality (Balnave and Robinson 2000). Khattak and Helmbrecht (2019) demonstrated that the tryptophan requirements of hens housed in colony cages at peak production were higher than current NRC (1994) recommendations. In barn systems, dietary supplementation with tryptophan was shown to alleviate stress in layers, and be an effective treatment for hysteria (Laycock and Ball 1990). Hens fed additional tryptophan generally show decreased stress, and when also supplemented with insoluble fibre, have a reduced occurrence

Table 1. Ideal amino acid profile for laying hens reported by different authors, with the requirement for lysine (Lys) set at 100%
Arg, arginine; Ile, isoleucine; Met, methionine; M+C, methionine + cystine; Thr, threonine; Trp, tryptophan; Val, valine

Reference	Lys	Met	M+C	Trp	Thr	Arg	Ile	Val
NRC (1994)[A]	100	43	84	23	68	101	94	101
Jais *et al*. (1995)[B]	100	44	–	16	76	82	76	64
CVB (1996)[C]	100	50	93	19	66	–	79	86
Coon and Zhang (1999)[C]	100	49	81	20	73	130	86	102
Leeson and Summers (2005)[D]	100	51	88	21	80	103	79	89
Rostagno (2005)[C]	100	50	91	23	66	100	83	90
Bregendahl *et al*. (2008)[E]	100	47	94	22	77	107	79	93
Lemme (2009)[C]	100	50	91	21	70	104	80	88
Rostagno *et al*. (2011)[C]	100	50	91	23	76	100	76	95
Dalibard *et al*. (2014)[F]	100	50	82	20	68	103	76	89
Dalibard *et al*. (2014)[G]	100	53	86	21	73	108	79	92
Soares *et al*. (2019)[H]	100	–	88	21	69	109	75	90
Soares *et al*. (2019)[I]	100	–	88	21	69	104	78	91
Mean	100	48.8	88.1	20.8	71.6	104.3	80.0	90.0
CV (%)	0	6.1	4.8	9.2	6.3	10.2	6.5	10.2

[A]Based on total amino acid requirements for 32–45-week-old laying hens.
[B]Based on nitrogen balance.
[C]Based on digestible amino acid requirements.
[D]Based on total amino acid requirements.
[E]Based on true digestible amino acid requirements for maximum egg mass between 28 and 34 weeks of age.
[F]Based on true digestible amino acid requirements of light hen: feed intake of 105 g/hen.day.
[G]Based on true digestible amino acid requirements of heavy hen: feed intake of 115 g/hen.day.
[H]Based on nitrogen utilisation.
[I]Based on amino acid deletion.

of cannibalism (Mousavi *et al*. 2018). Obviously, tryptophan availability in layer diets has important implications for hen welfare.

Generally, hens housed in cages are at a lower risk of infectious (Noormohammadi 2021) and parasitic diseases (Groves 2021) than are hens housed in free-range systems. The increased disease burden experienced by free-range hens may increase their requirement for amino acids to support an upregulation of the immune response (Klasing 2007). However, some metabolic conditions appear predominantly in caged hens. Low protein and high energy diets have been implicated in the development of the fatty liver haemorrhagic syndrome (FLHS) in caged layers, as discussed below.

Calcium and phosphorus

Calcium (Ca) and phosphorus (P) are key nutrients in layer diets. These minerals have many essential roles in metabolism, especially in bone development and eggshell formation (Etches 1987; Bar 2009). In the 25 years since the NRC (1994) prescribed the poultry requirements for these minerals, the genetics of layers have changed dramatically, housing and management have improved and, subsequently, diet requirements have changed. Within this context, it is likely that the altered requirements for Ca and P has direct implications for hen health and welfare. We have recently reviewed Ca and P metabolism in laying hens and concluded that the concentrations of both nutrients in layer diets could be substantially decreased without a negative effect on the bird (Li *et al*. 2017). Furthermore, bone quality, including mineral composition, appears not to be affected by the degree of range use when an aviary system is available in the shed (Kolakshyapati *et al*. 2019; Sibanda *et al*. 2020*b*).

Cereal grains and their by-products are the major ingredients in layer diets. Cereals have low concentrations of P predominantly bound to phytate. Diets, therefore, require supplementation with inorganic P and phytase to provide adequate available P to the hen (Li *et al*. 2016*a*). Phosphorus availability can also be impaired by elevated dietary Ca concentrations, which increase the pH of digesta and as a result, decrease P absorption and retention (Li *et al*. 2016*b*). Furthermore, the high gastrointestinal pH caused by high dietary Ca concentrations decreases pepsin activity in the proventriculus and gizzard, thus reducing digestibility of protein. In contrast, high dietary phytate concentrations bind Ca, forming non-absorbable complexes (Bryden *et al*. 2007). High plasma P concentrations also decrease gastrointestinal absorption of Ca. Therefore, an optimum dietary Ca to P ratio is essential for bone health, egg production and, ultimately, hen welfare. Vitamin D_3 regulates Ca and P absorption, storage and mobilisation, but excess availability of vitamin D_3 provides no additional advantage for egg production (Li *et al*. 2017). In general, hens can tolerate a wider range of Ca concentrations, when dietary P concentrations are high (Li *et al*. 2017).

Most of our understanding of Ca and P metabolism has been determined with broilers, and similar research is required for layers. Layers have a much higher Ca requirement and Ca metabolism is also modulated by oestrogen. Currently, total dietary Ca concentrations are used for diet formulation but, for accurate formulation, availability of different Ca sources is required. This will improve the precision of feed formulation and bird welfare. Moreover, as Neijat *et al*. (2011) concluded following their study of Ca and P dynamics of layers housed in conventional and enriched cages, nutrient utilisation, along with welfare, should be assessed when comparing different housing systems.

Vitamins, minerals and feed additives

Minimum dietary concentrations of vitamin that prevent clinical signs of deficiency may not support optimum performance and welfare (Leeson *et al*. 1995; Weber 2009). This is due to continuous genetic improvement of hens, changes in the nutrient availability of feed ingredients, and ongoing changes in hen management, which all contribute to the demand for micronutrients, especially vitamins. Intensive egg production increases metabolic, social, environmental and disease stresses, resulting in increased requirements for vitamin and trace minerals. For example, vitamin E, vitamin C, selenium and zinc are antioxidants that limit radical damage and oxidative stress and help sustain health and longevity (Attia *et al*. 2016). Improved egg quality can be achieved if supra-nutritional levels of vitamin E are added to layer diets. However, very little research has been conducted on the implications of adding high concentrations of one vitamin for the absorption and metabolism of other vitamins. Weber (2009) reviewed the requirements of laying hens for vitamins and concluded that high producing layers require 5–10 times the minimum requirements determined by the NRC (1994), so as to sustain productivity and maintain welfare. The possibility of suboptimal vitamin and trace-mineral intake can be avoided by adding sufficient quantities with approporiate safety margins, as a premix to diets.

Along with a premix, poultry diets are commonly supplemented with a variety of other feed additives to improve the nutritional characteristics of the diet, hen performance, and egg quality. These include nutritional additives (amino acids, vitamin and minerals, prebiotics, and probiotics), products to add nutritive value to the egg (so called ‘designer eggs’), and products that affect the visual characteristics of eggs by improving yolk colour. The various yolk pigments added to layer diets are to meet consumer expectations and have no relevance to hen health or welfare.

The use of feed enzymes for improving the digestibility of nutrients and dietary energy content has become common practice over the past 30 years. Dietary enzymes lower feed costs and benefit the environment. For example, exogenous feed enzymes such as phytase increase dietary P availability, thus reducing the need to add inorganic P to diets, and, subsequently, decrease the amount of P excreted. As indicated above, many factors affect phytase efficacy, including phytase source and concentration, and importantly, the dietary Ca : P ratio. Phytase is most efficacious in diets containing low available P and adequate Ca concentrations (Li *et al*. 2016*b*, 2017), whereas carbohydrases, such as xylanase and β-glucanase, increase the available energy level of the diets (Mathlouthi *et al*. 2002).

Mixtures of additives are sometimes used strategically to improve bird health and welfare. Probiotics, which are live bacterial cultures, along with prebiotics (a substrate for gut bacteria, usually fibre) fall into this category and are being used increasingly in layer diets to improve gut health. This is discussed further in the section on the gut microbiota and microbiome. Combinations of feed enzymes, organic acids and essential oils can reduce the incidence of gut impaction and increase nutrient digestibility (including non-starch polysaccharides) in free-range hens with access to abundant pasture (Iqbal *et al*. 2018, 2019*a*).

Water

Water is the most important nutrient as it has a central role in all aspects of metabolism (Leeson *et al*. 1976) and is critical for bird welfare. It is difficult to access water requirements, as water intake is modulated by age, feed intake, stage of production, ambient temperature, water temperature and quality (Leeson *et al*. 1976). The intake of water and feed are directly related; hens that drink less water will also consume less feed, and, subsequently, egg production declines. Presumably, current high producing laying hens have higher metabolic demands for water than did earlier layer strains. Water intake is a sensitive indicator of bird health and, therefore, monitoring water intake of a flock is a useful guide to changes in bird welfare (Leeson and Summers 2009). Hens must have access to water of high quality at all times. However, there are situations during transport and illness when birds may be deprived of water (Rault *et al*. 2016).

Poultry farm water in Australia can come from many sources, including surface water such as dams, lakes and rivers, but also underground water or treated town water; all vary greatly in quality (DAFF 2009). Water quality should be tested, and if concentrations of nitrites, chloride (Cl) and other minerals or bacterial contamination exceed the values in Table 2, the water needs to be treated accordingly (Leeson and Summers 2009). High concentrations of total dissolved solids or high salinity, predominantly high concentrations of Ca, magnesium (Mg) and sodium (Na) salts, are the most common cause of harmful effects in poultry, and guidelines are shown in Table 3. High concentrations of Na, Cl or Ca in drinking water require changes in dietary nutrient composition to avoid excessive mineral intake. Most of the literature on Ca and Mg in water for laying hens was generated in the 1970s or earlier (Scott *et al*. 1969) and, therefore, may not be appropriate for modern high-producing laying hens. For example, there is a lack of research on the impact of mineral concentrations in drinking water on mineral metabolism or bone quality. Therefore, there is a need to determine the effect of water pH and mineral (Ca, Mg and sulfate, SO_4) concentrations on the welfare of hens. Moreover, the contribution of drinking-water concentrations of Ca and P to the homeostasis of these two minerals in laying hens (discussed above) has been largely neglected.

In many countries, underground water is a common source of drinking water for poultry and is usually high in NaCl, Ca, Mg and potassium (K; Balnave and Scott 1986). For example, concentrations of Na and Cl as high as 570 and 2000 mg/L

Table 2. Drinking water quality guidelines for poultry
Adapted from Carter and Sneed (1987)

Contaminant or characteristic	Concentration considered average	Maximum acceptable concentration	Remarks
Bacteria			
Total bacteria	0/mL	100/mL	0/mL is desirable
Coliform bacteria	0/mL	50/mL	0/mL is desirable
Nitrogen compounds			
Nitrate	10 mg/L	25–45 mg/L	Concentrations from 3 to 20 mg/L may affect performance
Nitrite	0.4 mg/L	4 mg/L	
pH	6.8–7.5	–	A pH of <6.0 is not desirable. Levels below 6.3 may decrease performance
Total hardness	60–180	–	Hardness levels <60 are usually soft; those >180 are very hard
Naturally occurring chemicals			
Calcium	60 mg/L	–	Concentrations as low as 14 mg/L may be detrimental if the sodium concentration is >50 mg/L
Chloride	14 mg/L	250 mg/L	
Copper	0.002 mg/L	0.6 mg/L	Higher concentrations produce a bad odour and taste
Iron	0.2 mg/L	0.3 mg/L	Higher concentrations produce a bad odour and taste
Lead	–	0.2 mg/L	Higher concentrations are toxic
Magnesium	14 mg/L	125 mg/L	Higher concentrations have a laxative effect. Concentrations >50 mg/L may affect performance if the sulfate concentration is high
Sodium	32 mg/L	–	Concentrations >50 mg/L may affect performance if the sulfate or chloride concentration is high
Sulfate	125 mg/L	250 mg/L	Higher concentrations have a laxative effect. Concentrations >50 mg/L may affect performance if magnesium and chloride concentrations are high
Zinc	–	1.50 mg/L	Higher concentrations are toxic

Table 3. Suitability of water for poultry containing different concentrations of total dissolved solids (TDS)
Adapted from the NRC (1974)

TDS (mg/L)	Comments
<1000	These waters should present no serious burden to any class of poultry
1000–2999	These waters should be satisfactory for all classes of poultry. They may cause watery droppings (especially at higher levels), but should not affect health or performance
3000–4999	These are poor-quality waters for poultry, often causing watery droppings, increased mortality, and decreased growth
5000–6999	These are not acceptable waters for poultry and almost always cause some type of problem, especially at the upper limits, where decreased growth and production or increased mortality may occur
7000–10 000	These waters are unfit for poultry, but may be suitable for other livestock
>10 000	These waters should not be used for any livestock or poultry

respectively, have been recorded in Australian bore water (Zhang 1993). This can be of concern since saline drinking water has been shown to have an adverse effect on eggshell quality, while having little effect on feed intake, egg production or egg weight (Zhang 1993). Similar effects of saline drinking water on eggshell quality were reported in Israel (Yoselewitz *et al*. 1993), Iran (Pourreza *et al*. 1994) and Germany (Khalafalla and Bessei 1997). However, research conducted in the USA (Maurice 1989; Damron 1998) did not find reduced eggshell quality when hens drank saline drinking water. Different responses may be due to genetic sensitivity to NaCl, as Yoselewitz and Balnave (1990) showed different sensitivities among six Australian layer strains. Furthermore, the degree of sensitivity observed among strains also existed among hens of the same strain. A reduction in bicarbonate ions in the shell gland (Balnave *et al*. 1989; Roberts and Balnave 1992), and not Ca availability (Brackpool *et al*. 1996), appears to explain the poor shell quality in hens receiving saline drinking water. Therefore, measures that increase blood bicarbonate concentrations may improve eggshell quality. Supplying ammonium bicarbonate, ascorbic acid and various zinc supplements to hens receiving saline drinking water improved shell quality (Zhang 1993). Presumably, the beneficial response to these supplements also had a positive effect on hen welfare as it would have affected acid-base homeostasis.

Gut microbiota and microbiome

Microbial activity in the gastrointestinal tract has a significant influence on bird metabolism and wellbeing. The relationship between host and microbiota can be either commensal, symbiotic or pathogenic. Microbiota refers to the community of microorganisms within the gastrointestinal environment, while microbiome describes the collective genomes of all these microbial species. The intestinal microbiota is dynamic as a result of interactions with the host, diet and environment, including the bedding material microbiome (Pan and Yu 2014); dietary components are the main modulators of the gut microbiome. Nutritional interactions that occur between the intestinal wall and gut microbes play an important role in modulating gut colonisation by either beneficial, commensal or pathogenic bacteria, as they are consistently competing for nutrients and gut attachment sites. It has been established that the gut microbiota can have a profound effect on the development of the immune system and, thus, strongly influence optimal health and productivity (Shang *et al*. 2018). Understanding these relationships is important for promoting hen health and productivity.

Many benefits have been attributed to gut microbiota for both nutrition and protection of poultry health, including a reduction of pathogen colonisation (competitive exclusion), production of B and K vitamins, volatile fatty acids (acetic acid, butyric acid and propionic acid), organic acids (e.g. lactic acid), and antimicrobial compounds (e.g. bacteriocins), induction of non-pathogenic immune (Shang *et al*. 2018) and endocrine (Villageliu and Lyte 2017) responses, and modulation of behaviour and welfare (Kraimi *et al*. 2019). Many gastrointestinal bacteria are able to metabolise otherwise indigestible β-linked dietary polysaccharides, which are then fermented to volatile fatty acids (Annison *et al*. 1968). These can be utilised by the host as energy and carbon sources (Koutsos and Arias 2006; Tellez *et al*. 2006); butyrate is an important energy source for the gut wall. Gastrointestinal microbiota also contribute to the host's nitrogen metabolism as some bacterial protein can be a source of amino acids for the host (Metges 2000). However, the degree to which microbes might influence avian nutrition and metabolism is poorly understood. It is doubtful that all of the end products of microbial metabolism will be available to the host, as most microbial activity occurs in the caeca and despite the bird's ability to cause retrograde movement of digesta to sites of absorption in the avian gut. A high inter-individual variation of intestinal microbiota can be observed, which may result in a high individual variation of nutrient metabolism or feed utilisation (Sonnenburg and Bäckhed 2016).

The gut microbiome of all animals can be affected by diet composition, including the major nutrients, antinutritive factors and feed additives, especially probiotics (Bajagal *et al*. 2016). Different dietary interventions directed at the gut microbiota are now used to enhance hen production and reduce infection risk, while maximising feed utilisation. There is recent evidence supporting the use of probiotics for laying hens to improve productivity and egg quality (Mahdavi *et al*. 2005; Mátéová *et al*. 2009), prevent gut and reproductive infections (Shini *et al*. 2013), and improve performance and bone mineralisation (Yan *et al*. 2019). Probiotics contain live bacterial cultures, but how they induce their effects remains unclear. However, as we

unravel the interactions between diet and the gut microbiota, it should be possible to refine layer diets and further improve hen wellbeing.

Nutritional physiology

The laying hen life cycle can be conveniently divided into pre- and post-attainment of sexual maturity. In this section, the impact of nutrition during pullet growth and development, and during egg production, is disussed. Many authors, including Janczak and Riber (2015) and Jongman (2021), have discussed the many factors that determine the development of the laying hen, and came to the conclusion that pullet rearing has a significant impact on subsequent egg production and welfare of the mature laying hen.

Growth and development

A strong correlation exists between body development during rearing and laying performance and also the ability to extend lay beyond 80 weeks of age (Pottgüter 2016). Key targets of pullet rearing include reaching specified BWs at specific ages, flock uniformity of at least 85%, a well developed digestive tract, and the establishment of good feeding behaviour patterns. Feeding techniques and the nutrients provided are key factors in achieving these targets. Factors that adversely affect pullet welfare may compromise body composition and reduce flock uniformity. Control of BW and flock uniformity are, therefore, crucial aspects for the successful growth and development of the laying hen.

It is common practice to feed layer chickens in four phases, reflecting the different developmental stages of the pullet, for optimum pullet development. A starter diet is offered for the first 4 weeks post-hatch, with a relatively high energy content (12.3–12.4 MJ/kg), ideally as crumbles, and with an increased Ca (1.05–1.10%) concentration to promote skeletal growth (Gous and Morris 2001; Frikha *et al*. 2009). Depending on the chick's BW, the starter diet may be extended to week 6 post-hatch. Grower diets are usually fed from 4 to10 weeks of age and contain slightly less energy (11.9–12.0 MJ/kg) and Ca (0.9–1.10%) than do starter diets. Adequate P concentration must be supplied, since inadequate P intake during the first half of pullet rearing results in an increased prevalence of osteoporosis in mature hens; ~95% of skeletal development is completed during the first half of pullet rearing (Pines and Reshef 2015). At 10 weeks of age, pullets are able to increase voluntary feed intake and should be offered low energy density diets to reduce the incidence of overweight birds and increase gut holding capacity. At this age, a diet to promote gastrointestinal organ development is introduced. Feeding a low energy mash (11.5 MJ/kg) encourages pullets to increase voluntary feed intake, stimulates gizzard activity, and increases digestive enzyme secretion (Yokhana *et al*. 2016). For this purpose, the crude fibre concentration of the developer diet may be as high as 5.5% (Pottgüter 2016). This is important for caged hens, and critical for free-range hens that may experience uncontrolled pasture intake (Ruhnke *et al*. 2015*b*).

Dietary fibre and an adequate feed particle size are key factors for gastrointestinal development (Hetland *et al*. 2003*a*; Svihus *et al*. 2004; Frikha *et al*. 2011). Coarse feed particles are especially important for the gizzard, pancreas and liver development (Engberg *et al*. 2002; Svihus 2011; Ruhnke *et al*. 2015*a*). Heavier gizzard and pancreas mass are associated with increased bile secretion and pancreatic enzyme activity respectively (Engberg *et al*. 2004; Svihus *et al*. 2004).

The onset of maturity in pullets begins at ~16 weeks of age. Commencement of egg production is determined by a combination of age, BW, body fat content, and increased light exposure, together with the influence of oestrogen. The duration of the pre-lay or transition period is ~10 days and, during this time, medullary bone develops, providing labile Ca storage while pullets continue to gain weight. Moderate increases of Ca, P and protein concentrations in the pre-lay diet during the transition period allow hens to meet the metabolic adjustment to lay, while maintaining overall feed intake (Leeson and Summers 2009; Thiele 2012) in conjunction with a dietary Ca supply of 2–2.5%. This diet should be offered for a further 10–14 days or until the flock reaches 2% hen house production (Coelho 2001; Pottgüter 2016).

Young layers of low BW are unable to consume sufficient feed to reach or maintain peak production. These birds use their body reserves in an attempt to meet genetically determined egg output, resulting in poor hen condition and reduced flock uniformity. The use of pre-lay diets helps offset this problem and is, therefore, essential in all production systems for optimum hen production and welfare.

Egg production and eggshell quality

Among the many factors that determine egg production and eggshell quality, with corresponding implications for hen welfare, nutrition is an important modulator (Roberts 2004). Egg weight is positively correlated with hen weight; for every 100 g live pullet weight, egg weight increases by 0.7 g during the onset of lay, with lasting, but less pronounced effects, for the duration of the laying period (Bouvarel *et al*. 2011). Moreover, at a given hen weight, egg weight can be increased by promoting dietary energy intake, resulting in an average of 0.96 g egg weight gain per 42 kJ additional energy intake (Jalal *et al*. 2006; Bouvarel *et al*. 2011).

Nutrition can be a tool to control egg size in aging flocks. By manipulating the diet to limit the total protein concentration to 13%, and restricting the amount of sulfur-containing amino acids, a reduction of egg size can be achieved (Calderon and Jensen 1990; Leeson and Summers 2009). Gradually decreasing the energy density of the diet during lay can control egg size, while compensatory feed intake will balance for the overall requirements. However, while the minimum demand for the essential fatty acid linoleic acid is ~1% of the diet, it is common practice to feed layers with a Phase 1 diet that contains up to 3% linoleic acid, and gradually decrease its concentration to 1% by the end of lay (Grobas *et al*. 1999*a*, 1999*b*, 2001; Pottgüter 2016). This gradual reduction of linoleic acid will result in consisitent egg size without promoting compensatory feed intake.

The lipid composition of egg yolk reflects dietary fatty acids and is of interest for the production of speciality or

designer eggs as nutraceuticals or functional foods (Surai and Sparks 2001). Omega-3-enriched eggs with increased quantities of α-linolenic acid, eicosapentanoeic acid and docosahexaenoic acid or conjugated linoleic acid (CLA) are of interest to health-conscious consumers. For each 1% CLA added to the hen's diet, 50 mg CLA is transferred into the egg. In order to achieve the the target of 300 mg omega 3 fatty acids per egg, the feed should contain 10% flaxseed (Caston and Leeson 1990). Interestingly, the health benefits from consuming polyunsaturated fatty acids are well documented for humans and, presumably, the hen receives similar benefits but this does not appear to have been documented. The concentration of cholesterol in the egg (180 mg/egg) remains relatively constant.

Dietary lipids and bile acids influence carotenoid absorption and, therefore, yolk colour. However, as nearly all β-carotenes are converted into vitamin A, its contribution to yolk pigmentation is negligible (Hencken 1992). As a result, layers accumulate mainly xanthophylls such as lutein, zeaxanthin and capsanthin (Nys 2000; Surai *et al*. 2001; Sinanoglou *et al*. 2011). The accumulation of xanthophylls is crucial to meet consumer expectations of egg yolk colour. The most common plant sources used for yolk colouring include corn, corn gluten, lucerne, marigold and capsicum (Britton 1996; Lootens *et al*. 2004). The ingestion of pasture by free-range hens also increases the deposition of lutein and zeaxanthin in the egg yolk significantly, resulting in darker yolk colour (Karunajeewa 1978; Iqbal *et al*. 2018).

Yolk mottling is associated with a low intake of carotenoids, and also a lack of yellow pigments while red pigments are increased (Leeson and Summers 2009). This occurs predominantly in spring due to variable pasture availability and as a result of prolonged feed storage. Other factors that cause yolk mottling include the ingestion of gossypol, tannins, nicarbacin, mycotoxins, coccidiosis and viral diseases (i.e. Newcastle disease, infectious bronchitis) that all impair xanthophyll absorption and deposition, resulting in pale yolks (Karunajeewa *et al*. 1984). The smell and taste of eggs can be negatively influenced by the accumulation of aldehydes or trimethylamines. This limits the dietary inclusion of canola to less than 20%, the use of fishmeal to less than 1% and also needs to be taken into account when adding betaine or choline to layer diets.

The quality of albumen is rarely influenced by nutrition but the administration of gossypol can result in pink albumen coloration. The availability of ascorbic acid (vitamin C) has positively influenced albumen height and the Haugh unit value in heat stressed hens. This is most likely due to a reduction in free radicals that would otherwise damage the quaternary and tertiary structure of the albumen protein (Benabdeljelil *et al*. 1990; Ajakaiye *et al*. 2011).

Eggshell quality remains an ongoing concern for the egg industry. Dietary Ca and P concentrations are the key determinants of eggshell breaking strength and shell weight (Hamilton 1982). Vitamin D_3 activity, which regulates Ca uptake, is modulated by hydroxylases in both the liver and kidneys. In older birds, reduced α_1-hydroxylase activity in the kidney is one of the key factors considered responsible for reduced Ca utilisation (Li *et al*. 2017). Interference with Ca deposition may also occur due to excess Cl intake in drinking water, as discussed above. The availability of Ca for eggshell formation depends on its particle size and gizzard activity. Approximately 65% of dietary Ca should be provided as coarse (>1–2.5 mm) particles (Rao and Roland 1990; Zhang and Coon 1997; Bouvarel *et al*. 2011).

Modern layers utilise 2 g Ca daily for eggshell formation, with a third of the Ca being provided from medullary bone and remainder coming directly from the gastrointestinal tract (Nys and Guyot 2011). Bone quality is negatively correlated with egg mass and eggshell quality (Riczu *et al*. 2004; Kim *et al*. 2005). Therefore, providing a pre-lay diet is essential to support medullary bone formation and is a major contributing factor to ensure adequate eggshell quality throughout production (Elaroussi *et al*. 1994; Summers and Leeson 1994) This reduces the incidence of bone fractures and osteoporosis (discussed below), thus maintaining hen health and welfare.

Stress and metabolism

Stress is a complex phenomenon and the body's response to stressors that may be internal or external. It is an attempt to maintain homeostatic equilibrium. This is achieved through complex interactions of cell metabolism and the neuroendocrine and immune axes (Husband 1995). It is in this way that nutrients are partitioned in the body to meet differing metabolic demands (Husband and Bryden 1996; Klasing 2007). Extra energy and micronutrients may be required to meet the biological costs of an immune or neuroendocrine response to stressors (such as non-infectious disease or metabolic disorders, exposure to feed contaminants, temperature fluctuations and social interactions). If these nutritional needs are not satisfied, hen welfare will be compromised.

Commercial layers often encounter social and environmental stressors. Layers kept in different housing systems have similar nutrient needs for egg production; however, exposure to stressors and disease will vary among housing systems and influence nutrition requirements (Lay *et al*. 2011). Determination of stress is difficult, but there are physiological and behavioural indices of stress in laying hens that can be quantified and used to assess hen well being (Scanes 2016; Tilbrook and Fisher 2020). Nutritional stressors including fasting, feed restriction, and dietary nutrient deficiency, can influence plasma concentrations of corticosterone, other glucocorticoids, corticosteroid binding protein, epinephrine, and norepinephrine, and together with the blood heterophil to leukocyte (H : L) ratio, can be helpful to assess hen welfare (Shini *et al*. 2010). In this section, some of the stressors experienced by laying hens will be discussed in relation to changes in nutrient responses by the hen.

Immunity and inflammation

The acute phase response is a key defence mechanism of the immune system and involves increased production of pro-inflammatory cytokines, eicosanoids and other molecules that mediate a variety of physiological and metabolic changes, to create unfavourable conditions for pathogens (Kogut 2009). Inflammation is one of the body's first non-specific responses

to infection, and overactive immune responses can cause chronic inflammation, which promotes co-infection with other pathogens. In laying hens, the acute phase response can result in unspecific clinical signs such as decreased feed intake and activity, thermogenic shivering, unphysiologic feather lifting, and increased resting times. Adaptive immunity, in contrast, is mediated by lymphocytes, and responses are specific to the pathogen. These include the production and activation of antibodies, as well as cytotoxic killing through the release of antimicrobial substances.

The diet of a laying hen can affect the development, maintenance and response of the immune system. While nutritional deficiencies of linoleic acid, vitamin A, iron, selenium, and several of the B vitamins impair immune function and increase susceptibility to infectious diseases, the requirements of these nutrients are elevated during the acute or chronic stages of disease and should be increased as required (Klasing 1998). Importantly, nutrients or compounds that modulate the immune response are active at concentrations well above the established NRC requirements for growth and productivity (Koutsos and Klasing 2014). Some nutrients and compounds, including fatty acids, vitamin E, vitamin A, and vitamin D, have anti-inflammatory properties, while others have immunomodulatory properties, such as carotenoids, flavonoids, vitamin C and various long-chain polyunsaturated fatty acids (Meriwether *et al*. 2010; Korver 2012; Wils-Plotz and Klasing 2017).

Immune responses are not only energy demanding but require an investment of various nutrient resources. This is achieved by diverting or partitioning nutrients away from other productive activities such as ovulation and growth (Klasing 2007). The costs of different modes of immune competence (innate and adaptive immunity) can be estimated by examining the amount of protein needed for the cellular and secretory processes involved in each component of immunity relative to all other processes in an adult bird at maintenance (Koutsos and Klasing 2014). Diets with inadequate levels of protein or amino acids can compromise immune function and increase infection risk.

Heat stress

Heat stress can be chronic or acute and is a complex phenomenon involving interactions among bird genetics, ambient temperature, relative humidity, feather cover, water and feed intake and acclimatisation; it has been the subject of Australian research for many years (Yeates *et al*. 1941; Lee *et al*. 1945; Balnave 2004). Together, these factors demonstrate the difficulty of predicting responses to heat load with different nutritional mitigation strategies. Heat stress can be associated with reduced egg production and egg quality due to decreased feed and water intake and, subsequently, reduced nutrient utilisation as well as increased energy demands for metabolic (cooling) activity (Lin *et al*. 2006; Lara and Rostagno 2013). Moreover, heat-wave conditions or abrupt changes in heat load increase hen mortality (Daniel and Balnave 1981). Obviously, heat stress has welfare implications for laying hens, but these can be aggravated or mitigated by diet.

Many strategies are available to alleviate heat stress (Lin *et al*. 2006) and nutrition is always an important consideration. The ability of a nutritionist to provide a single set of nutrient specifications to satisfy all possible interacting factors is challenging and expensive (Balnave 2004). Obviously, the immediate need is to overcome the impact of reduced feed intake and adjust the energy and protein density. However, there is much conjecture in the literature on which is the appropriate approach (Balnave and Brake 2005). It is advisable that hens subject to heat stress are fed a diet where energy density is primarily achieved by adding fat rather than protein to the diet, as the metabolic heat produced to digest protein exceeds the energy required to digest fat. Furthermore, offering a pelleted diet allows for a higher energy density and can also increase the feed intake significantly. However, sudden changes of the physical form of the diet should be avoided to prevent diet refusal. It is, therefore, crucial that the farm management is well prepared and hens are adapted to heat conditions in advance. Specific diet recommendations may also include a higher dietary arginine : lysine ratio (Balnave and Brake 2002). Applying additives to cooled drinking water may be the best approach to ensure additive intake.

Glatz (2001) reported that 59–66-week-old layers housed at 30°C consumed more feed and produced eggs with better shell quality when provided with drinking water at 5°C, 10°C or 15°C, compared with uncooled water at 30°C. An improvement in feed consumption and egg production was also observed due to the supplementation of vitamin C in the drinking water (Khan and Sardar 2005). Betaine has been also suggested to alleviate the adverse effects of heat stress on laying hens. Betaine acts as an osmolyte (Saeed *et al*. 2017) and has been shown to improve egg production, egg quality traits and immune indices (Attia *et al*. 2016). Acute heat exposure causes oxidative damage and dietary supplementation with selenium for laying hens improved feed intake, feed efficiency, egg production and quality, and antioxidant status (Habibian *et al*. 2015). Most importantly, the hen's panting activity results in repiratory alkalosis that impairs Ca carbonate deposition in the eggshell. Therefore, maintaining the electrolyte balance of those hens is the key factor to keep eggshell quality at an acceptable level and several commercial products are available for drinking water modification (Roberts 2004).

Moulting

Moulting is a normal physiological event in the life cycle of laying hens, during which plumage is replaced with new feathers, and is accompanied by reduced feed intake, BW loss and suspended reproduction (Yousaf and Chaudhry 2008). In contrast, induced moulting, a practice that can have significant hen welfare concerns as discussed by Glatz and Tilbrook (2020), is determined by economic considerations. Feed and water withdrawal was the classical method to induce moulting, but has serious welfare implications and is no longer permitted. Moult is now induced by feeding low energy diets such as barley or oats at least 40–60 g/bird.day with 1% Ca for up to 3 weeks (Glatz and Tilbrook 2020). Other practices that have successfully induced moult include feeding high

concentrations of zinc, aluminium or potassium or the oral administration of thyroxine (Teixeira *et al*. 2006; Bass *et al*. 2007). Induced moulting is a reaction to stress and it demonstrates that perturbations in nutrient supply or balance can modify metabolism and impair bird welfare.

Metabolic disorders and feed contaminants

Metabolic disorders, often of complex aetiology, are any condition that reduces wellbeing and productivity and is associated with some abnormality in metabolism. Exceptions are that the condition is not of infective origin, does not arise from a specific genetic defect, or is not simply a dietary deficiency. In other instances, poor bird performance may result from feed containing anti-nutritive factors or toxin contamination.

Fatty liver haemorrhagic syndrome

Fatty liver haemorrhagic syndrome (FLHS) is a metabolic disease of laying hens that is characterised by excessive accumulation of fat in the liver and abdominal cavity, liver rupture and haemorrhage, and was first described by Couch (1956). FLHS occurs in commercial layers in high production and is frequently the major cause of death in otherwise healthy flocks and has been described in Australia on several occasions (Grimes 1975; Neill *et al*. 1975; Shini *et al*. 2019). In a recent Queensland study (Shini *et al*. 2019), it was obseved that the condition was a major cause of mortality in caged hens but not in free-range hens. The difficulty with the condition is that it can be diagnosed only at necropsy. These cases are acute and it is not possible to gauge the welfare implications of chronic FLHS where a bird has an episode but recovers.

The condition has been reviewed by several authors (Butler 1976; Annison 1983; Squires and Leeson 1988; Julian 2005; Crespo and Shivaprasad 2003) who have agreed that nutrition plays a role in the aetiology of FLHS. Excessive liver fat infiltration appears to occur in laying hens under conditions of excess energy intake due to increased feed intake coupled with little exercise and high circulating oestrogen concentrations. Husbandry factors that appear to predispose hens to FLHS include dietary ingredients that stimulate lipogenesis, genetics, environmental conditions, and stress in general. Dietary factors other than excessive caloric intake, including rapeseed meal (Pearson *et al*. 1978), aflatoxin (Hamilton and Garlic 1971; Bryden *et al*. 1979) and chelated minerals (Branton *et al*.1995), have been suggested as possible factors in the aetiology of FLHS.

A fatty liver is normal in laying hens, as increased hepatic lipogenesis and deposition is essential for lipid transfer to the egg during yolk formation (Annison 1983). Most previous research on FLHS has concentrated on liver lipid accumulation rather than the factors that precipitate hepatic haemorrhage. We have recently shown, using an oestrogen model, that an inflammatory response due to disturbed lipid metabolism is involved in the pathogenesis of FLHS (Shini *et al*. 2020*a*, 2020*b*). It appears that, under field conditions, high-producing laying hens with elevated circulating oestrogen concentrations, when exposed to an inflammatory insult, are more susceptible to the development of FLHS.

Osteoporosis

Osteoporosis is a metabolic condition of laying hens associated with the progressive loss of structural (trabecular and cortical) bone, resulting in increased susceptibility to fractures (Campbell 2020). There is no single cause of osteoporosis, and multiple factors are involved in the pathogenesis of osteopenia (Whitehead 2004). It is caused by a combination of genetic, environmental and nutritional factors affecting bone health. Birds are unique and produce medullary bone, which serves as a labile reserve of Ca for eggshell formation (Nys Le Roy 2018). Hence, bones of laying hens undergo continuous and rapid remodelling (Johnsson *et al*. 2015). The amount of medullary bone builds up shortly before first oviposition, and the structural bone content of the hen declines over the remainder of the laying period, increasing the risk of fracture, which is a significant welfare issue.

Osteoporosis is not a Ca deficiency *per se*, but may involve the inability of hens to metabolise sufficient Ca because of inadequate dietary Ca, vitamin D_3 or P supply (Whitehead and Fleming 2000; de Matos 2008). Structural bone loss is also accelerated by the relative inactivity of conventionally caged birds (Newman and Leeson 1998). Good nutrition can help minimise osteoporosis, but is unable to prevent it. The timing of nutritional intervention is critical; if dietary changes are left until sexual maturity, the effect will be seen in medullary bone. Adequate dietary amounts of Ca, P and vitamins D and K are particularly important during the rearing period, before the excess resorption encountered during the laying period (Fleming *et al*. 1998). Feeding limestone in particulate form benefits skeletal and eggshell quality (Fleming 2008). Since both Ca and P are withdrawn and replaced during the shelling–non-shelling cycle, P is also required to rebuild medullary bone. Phosphorus deficiency-induced osteoporosis in high producing cage layers is called cage-layer fatigue (Riddell *et al*. 1969). Vitamin D_3 deficiency will contribute to the development of osteoporosis in laying hens because it affects Ca and P metabolism (Whitehead and Fleming 2000).

Biogenic amines

Feed-borne biogenic amines are formed in animal protein meals by microbial decarboxylation of amino acids, and have important physiological roles, but are also potential toxins. The most common biogenic amines include histamine, cadaverine, tryptamine, putrescine, spermidine, tyramine and phenylalanine (Feddern *et al*. 2019). An extensive survey of the concentrations of putrescine, cadaverine and histamine in Australian fish and animal by-product meals was undertaken by den Brinker *et al*. (2003), who found the highest concentrations in fish meals. Most published work regarding the effects of biogenic amines has focussed on broilers where poor performance was often associated with gizzard erosion and proventriculus ulcers due to gizzerosine (Sugahara 1995; Barnes *et al*. 2001).

There is a lack of published data regarding the physiological role or toxicity of biogenic amines in laying hens. The supplementation of laying hen diets with putrescine significantly improved eggshell quality. It was recommended

that, when eggshell quality is a concern, there may be economic benefits from adding small amounts of dietary putrescine (Smith *et al*. 2000). Interestingly, putrescine has been shown to be a key factor in the mode of action of vitamin D_3 in the chick intestine, which may explain the effects observed (Shinki *et al*. 1991).

Mycotoxins, anti-nutritive factors and other feed toxins

Poultry diets can be contaminated with various toxic compounds (see Cheeke 1998), including mycotoxins, pesticides, organochlorine compounds, petroleum hydrocarbons, minerals, and anti-nutritive compounds found in some feed ingredients, all of which can affect bird performance and welfare, but most toxins are rarely encountered. Plants may contain secondary plant metabolites and anti-nutritive factors such as protease inhibitors (soy, lupins, peas), lectins (soy, lupins, peas), tannins (faba beans, peas, cotton seed), phytate (cereal grains, legume seeds, soy, canola), glucosinolates (cruciferes especially brassica *spp*), saponins (lucerne, clovers, legume seeds), alkaloids (lupins), cyanogenic glycosides (cassava, linseed) and phytoestrogens (soy, legumes). The effects of these compounds are mitigated by heat during processing and accounted for in diet formulation by restricting the inclusion level of the responsible feed ingredient or supplementing the diet with an appropriate feed enzyme.

Mycotoxins, or fungal secondary metabolites, are of concern because they can contaminate feed throughout the feed supply chain and often a feed commodity is contaminated with more than one toxin of the major toxins, namely, aflatoxin, fumonisins, deoxynivalenol, ochratoxin, zearalenone (Bryden 2009). The major risk to poultry from mycotoxin exposure is chronic mycotoxicoses that result in impaired productivity, with hens experiencing reduced egg production, poor egg and shell quality, and increased morbidity and mortality, and can be difficult to diagnose (Bryden 2012*a*). Importantly, immunity is often suppressed before other impacts of the toxic insult are apparent. This can reduce the effectiveness of vaccination and has obvious welfare implications. The mycotoxicoses that are likely to be encountered in Australia have been described (Bryden 2012*b*) and because of Australia's dry climate, mycotoxin related problems occur much less frequently than they do in North America. The reliance on corn in North American poultry diets, as opposed to wheat and sorghum in Australia poultry diets, is also a contributing factor.

Feeding management

Birds are maintained in husdandry systems where much of the opportunity to determine what they eat and how much has been replaced by scientifically formuated diets. This ensures freedom from hunger and thirst, but eliminates the pleasure of seeking and anticipating food. Moreover, hunger and thirst are basic motivating forces and central to maintaining life. It is, therefore, not surprising that the body maintains very complex, multilayered control of both feed and water intake. Some of the factors modulating feed and water intake are discussed in this section, along with their management.

Feeding and behaviour

Birds select their feed on the basis of particle size, colour and form, with a preference for coarse, yellow to orange coloured structures (Amerah *et al*. 2007). If the feed particles are too fine (e.g. <500 μm), feed uptake is reduced, respiratory signs due to dust exposure may appear, and feed wastage increases, resulting in suboptimal nutrition and reduced bird welfare (Safaa *et al*. 2009). Care needs to be taken that the ability of hens to select and favour certain feed particles does not result in an unbalanced nutrient intake. This may occur with hens that are not beak trimmed, as their sharp tips are more capable of picking individual grain ingredients, compared with the more scooping feeding behaviour of hens with shorter beaks (Portella *et al*. 1988; Glatz 2003; Persyn *et al*. 2004; Iqbal *et al*. 2019*b*). While pellets prevent feed selection and unintentional ingredient seggregation, they result in a high-density nutrient intake. In contrast, mash diets require hens to spend more time feeding. Hens allocating a greater portion of their time budget to feeding are less aggressive and express significantly fewer unwanted behaviours such as severe feather pecking and stereotype behaviour (Vilariño *et al*. 1996; Huber-Eicher and Wechsler 1998; Hartini *et al*. 2002; van Krimpen *et al*. 2008). A decreased incidence of severe feather pecking can also be achieved using low energy diets and feed with higher levels of non-starch polysaccharides (van Krimpen *et al*. 2008). It appears that the time spent feeding is crucial to satisfy the hens' pecking and foraging behaviour that would otherwise be redirected into inter-hen aggression. Beak trimming is used extensively to reduce the incidence of aggressive pecking and can affect feed and water intake if poorly executed (Glatz and Underwood 2020).

Inter-hen aggression depends also on the availability of resources such as feeder space. At least 14-cm trough length per hen is required to ensure adequate feed intake, without any negative impact on flock behaviour (Sirovnik *et al*. 2018). However, hen behaviour in non-cage systems can result in the development of flock subpopulations that demonstrate individual preferences regarding physical location and access of resources (Ruhnke *et al*. 2019). For example, while 10-cm feed chain per hen was provided in a three-tier aviary system, the majority of hens accessed the feeder area located at the tier close to the ground for an average of 7.29 h/hen.day. In comparison, the feed resources on top of the aviary system were utilised on average 4.29 h/hen.day (Sibanda *et al*. 2020*a*). These observations question current feed strategies where all feeder chains are run for the same amount of time and offer the same diet quantity. Precision feeding that considers the outcomes of hen behaviour such as body condition, feather cover and metabolic needs of hen subpopulations might allow a more efficient use of feed resources while providing more optimised support to under or high performing hens (Fig. 2). As indicated above, not only hen location but also physical appearance including feather cover can be an important determinant of energy requirements.

Fig. 2. Two hens from the same flock at the end of lay; the birds were of the same breed and had been reared and housed together since hatching. The different feather cover indicates different energy requirements of these hens that were both in full production. This underlines the importance of flock uniformity, but also the need to re-think current feeding practices so as to take into account the different requirements of flock subpopulations.

Moreover, diet composition can influence plumage cover (Hetland *et al*. 2004).

Associations between hen behaviour and nutrient utilisation are observed. Low efficiency hens demonstrated more walking, pacing, escape and aggressive behaviours and had reduced feather cover compared with high-efficiency hens, which slept and rested more frequently (Braastad and Katle 1989). In conclusion, feed can provide a powerful tool to encourage desired hen behaviour critical for laying hen welfare. This is especially relevant when flock longevity is of increasing concern, where layers perform until 100 weeks of age or for the duration of a second laying cycle.

Housing system and feed intake

In an Australian study, laying hens, all of the same strain and hatch, were maintained in five different housing systems during egg production (Stewart *et al*. 2006) and data from the study are shown in Fig. 3. All birds received the same diet during lay and the nutrient density was calculated for an intake of 110 g/bird.day. Data on feed intake showed that the more productive hens had the highest consumption, such as, for example, hens in conventional cages (3 hens/cage); in contrast, the more active the hens (hens in free-range and barn systems), the higher was the feed conversion ratio (Stewart *et al*. 2006). Hens kept in cages in a controlled environment had the highest feed intake and a moderate FCR, resulting in the highest BW at 70 weeks. There were significant differences in feed consumption among birds in different housing systems, with hens in barn having the lowest feed intake and hens in three-bird cages consuming more feed. Average FCR demonstrated that birds in all other systems utilised feed significantly better than birds in the barn, and this may reflect the higher incidence of cannibalism in this system (Stewart *et al*. 2006). Birds in free-range housing utilised feed at the same level as hens caged in a controlled environment (6 hens/cage) or in conventional cages without environmental control (3 hens/cage). Presumably, the way that hens were fed affected the amount of feed consumed or wasted. Hens in free-range and barn environments were fed from automatic chain feeders (operating 6 times per day); hens in cages were all fed *ad libitum*, with feed troughs being topped up manually as required (Stewart *et al*. 2006). These factors may have affected the outcomes observed.

Feed form

Most of laying hens are fed complete diets formulated commercially that can be offered as mash, crumbles or pellets. Reasons to choose one above the other may include the feed production cost, availability, and physical factors such as feed particle size, number and shape that influence the uniformity and, subsequently, the segregation of feed ingredients (Axe 1995). The physical form of feed can result in unbalanced nutrient uptake and poor performance if not managed adequately (Hetland *et al*. 2003*a*). However, offering mash to layers is most common due to its beneficial effects on feeding time and, subsequently, hen behaviour (as outlined above) and lower production costs. Heavy BW is a concern for laying hens, due to its direct impact on egg size, eggshell quality and health. The benefit of feeding mash on lowering the energy density of the diet is, therefore, of advantage to producers who are able to deliver the feed via automated feeding systems that prevent particle selection. However, egg producers whose hens are exposed to uncontrolled pasture intake, and who do not have the technical advantage of automated feed delivery, may benefit from offering pelleted feed. The advantages of feeding pellets

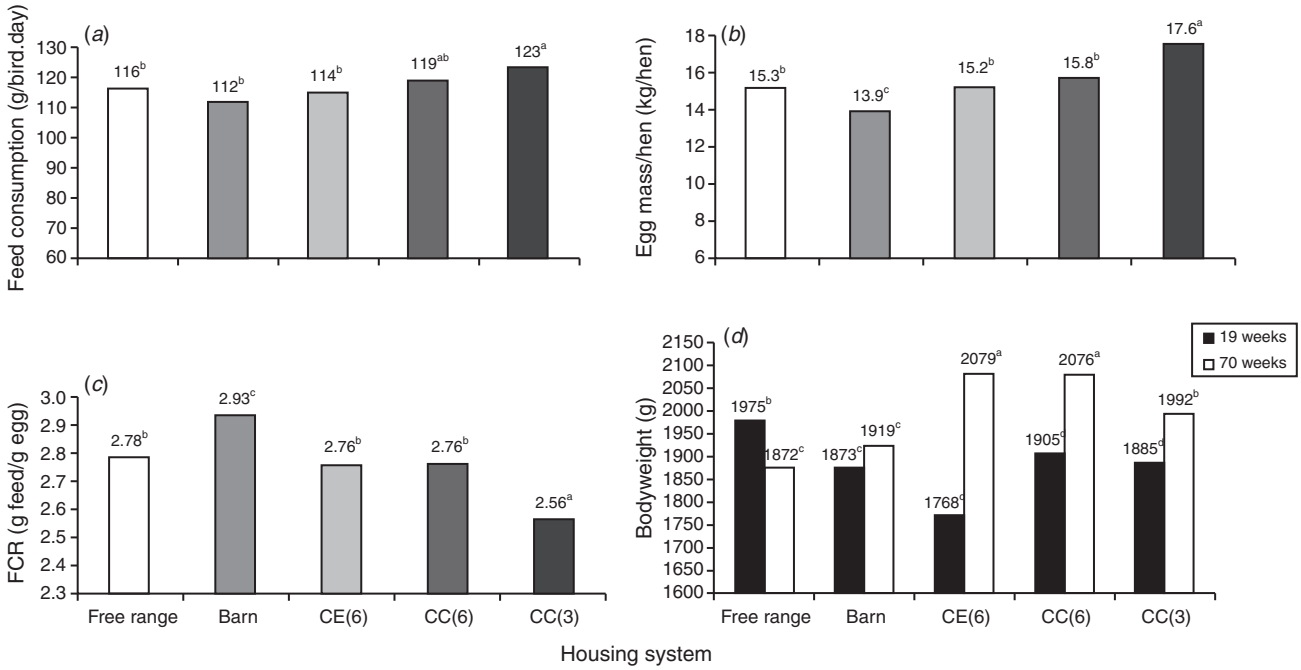

Fig. 3. The average production (n = 3 replicates) of laying hens housed in five different production systems (free range; barn; CE(6), controlled environment cages with 6 birds/cage; CC(6), conventional cages with 6 birds/cage; CC(3), conventional cages with 3 birds/cage;) was assessed from 19 to 70 weeks of age. All birds were of the same strain and hatch, and were reared under the same housing type as their laying conditions. The diet and management were comparable for the different housing systems through growth and development and lay (from Stewart *et al*. 2006). (*a*) Average daily feed consumption (g/bird.day) in all systems. (*b*) Average egg mass per hen housed in all systems. (*c*) Average feed conversion ratio (FCR) for all systems. (*d*) Average bodyweight of hens for all systems. Columns with different letters are significantly different at P = 0.05.

include reduced dust and microbial exposure, reduced feed-ingredient segregation, minimised feed wastage and a higher nutrient density (Behnke 2001), and prevention of hens from feed selection.

While pelleting or crumbling is standard for meat birds, the increased energy intake associated with a nutrient dense pellet is a disadvantage for most commercial laying hens, resulting in over-consumption and unfavourable BW gain (Safaa *et al*. 2009). However, in a recent Australian survey, 41 free-range egg farmers indicated that the majority fed mash (30.0%), followed by pellets (17.5%; Singh *et al*. 2017). Other feed practices included feeding whole grains (15.0%), coarse ground (25.0%), and fine ground feed (17.5%). Interestingly, 80% of farmers offered a complete diet, 12.5% used combined feeding, and 7.5% choice feeding. Regardless of the physical form provided, it is important to ensure that the diet contains adequate insoluble fibre to promote gizzard activity (Hetland *et al*. 2005).

Hens are able to select feed to meet their nutrient requirements, but need to be offered a reasonable choice (Emmans 1977; Summers and Leeson 1978). It is crucial to consider specific feed characteristics such as feed colour, taste and feed location, and to provide only a limited number of choices (Pousga *et al*. 2005). While successful implementation of choice-feeding has been demonstrated under research conditions, it has not been validated in large commercial flocks. So, while the feed choices offered need to be carefully considered, it cannot be assumed that hens will always select the feed ingredients required for optimum egg performance. For example, the curiosity of hens and the ability to choose their feed intake can result in over consumption of pasture and lead to grass impaction (Ruhnke *et al*. 2015*b*). This can severely affect calculated diet intake and, in such instances, offering pellets with a high nutrient density is recommended.

The structure of a diet affects gut health and hen performance (Yegani and Korver 2008). The grinding action of the gizzard is mostly responsible for the positive impact of large feed particles on improved gastrointestinal epithelial barrier function, increased ileal nutrient digestibility, and an overall better hen performance (Svihus and Hetland 2001; Svihus 2006). A well developed gastrointestinal barrier function is crucial to minimise exposure, colonisation and damage from pathogens and decrease the likelihood of concurrent diseases, thus ensuring optimum gut function and absorption (Röhe *et al*. 2014). Particle size, therefore, affects food and product safety. Large feed particles promote gastrointestinal development and, as a consequence, reduce concentrations of *Salmonella* spp. and *Clostridium* spp. in the avian intestinal tract (Bjerrum *et al*. 2005; Huang *et al*. 2006; Santos *et al*. 2008; Yegani and Korver 2008; Moen *et al*. 2012).

Particle size research has been predominantly conducted with broilers and recommendations exist for these birds, but not layers (see Amerah *et al*. 2007). However, it is known that physical feed characteristics such as feed particle size affect

egg quality. Eggshell density was found to be higher in hens that were fed finely ground particles than in hens that were fed a coarse diet (Hafeez *et al*. 2015*a*, 2015*b*). With broilers, ensuring that at least 20% of the feed particles are larger than 1.5–2.0 mm is considered to be beneficial for gut health, but with layers, inclusion of whole cereals is also practised (Wu *et al*. 2004; Amerah *et al*. 2009; Svihus *et al*. 2010). The use of whole grain reduces feed processing and feed transportation costs. Other structural components commonly included in diets or available to the bird (especially free-range hens) are shell grit, hay, silage, vegetables, pasture, insects, and harvested grass (Steenfeldt *et al*. 2007; Singh *et al*. 2017). So as to prevent nutritional imbalances, these substituted feed components need to be analysed for their nutritional value and the quantities ingested need to be taken into account for diet formulation (Bennett and Classen 2003). With wheat or corn being a common diet component, whole-grain inclusion can provide a valuable structural component and improve hen health, nutrient digestibility and hen performance (Hetland *et al*. 2003*b*; Wu *et al*. 2004).

Feed and water delivery

Feed- and water-delivery systems must meet the physiologic feeding and drinking behaviour of the hen. The structure of the feed trough and drinker should prevent any contamination with excreta, bedding material, dirt or other foreign bodies. Devices that minimise feed waste are increasingly being used and include automated feeding chains, automated feeding pans, but also manually gravity-filled troughs in smaller egg-production systems (Singh *et al*. 2017). Automated feeding systems are commonly run five to eight times a day, providing an audio stimulus encouraging hens to feed. This is especially important in non-cage systems where flock subpopulations may prefer to spend their time on the range, in a winter garden or in other areas of the production system (Sibanda *et al*. 2020*a*). The restriction of feed intake or access can result in hen frustration and increase the likelihood of aggression and feather pecking, but also limit nutrient intake of top egg producing hens. Therefore, feeding *ad libitum* and formulating diets according to an estimated feed intake are recommended (Singh *et al*. 2017). In a survey of Australian egg producers, 77.5% of farmers fed hens *ad libitum* (Singh *et al*. 2017).

For biosecurity, feeding on the range is not legally allowed in many countries (see Grimes and Jackson 2015; Scott 2015). Feed and water sources on the range may attract migrating wild birds, and if these birds are carrying highly infectious diseases such as Avian Influenza, this is a serious risk to the industry (Feare 2010; Grillo *et al*. 2015; Hoque *et al*. 2015; Singh *et al*. 2017). Furthermore, open water sources such as troughs and dams used to water hens or co-grazing livestock or as water reservoirs to comply with fire regulations are potential threats to hen health and welfare as they attract wild birds (Kim *et al*. 2009; Hernández-Jover *et al*. 2015).

Pastures

During the past 20 years, there has been a renewed interest in pastures for poultry, with the rapid acceptance by industry of free-range production systems. Prior to the intensification of egg production in the decade after World War ll, hens had been largely managed in free-range conditions. For those interested in the literature that describes the nutritional management of hens at pasture pre-war, Heuser (1955) and Eyles (1963) have provided detailed accounts. Pasture species commonly grown for laying hens include rye grass (*Lolium* spp), kikuyu (*Pennisetum clandestinum*), fat hen (*Chenopodium album*), marshmallow (*Malva parviflora*), amaranth (*Amaranthus* spp), poke weed (*Phytolacca* spp), clover (*Trifolium* spps), cocksfoot (*Dactylis glomerata*), rhodes grass (*Chloris gayana*), buffalo grass (*Stenotaphrum secundatum*), couch grass (*Cynodon dactylon*) and African love grass (*Eragrostis curvula*; Singh *et al*. 2017). The nutritional value of pasture depends on its vegetative state, with young shoots containing high sugar and protein concentrations, in constast to senesencing grasses that are dominated by fibre and lignin content (Barnes *et al*. 1995; Brockman 1995; Knudsen 1997). The large variety of pasture species grown and the various factors that influence grass growth and composition, including temperature, rainfall, soil fertility and season, make it challenging for nutritionists to calculate and integrate the nutrients obtained from pasture into diet formulations for hens.

Pasture intake can imbalance nutrient intake and reduce nutrient digestibility, but is associated with additional fibre intake and this can be beneficial for gastrointestinal development and BW (Montagne *et al*. 2003). Some studies have reported that hens spending more time on the range have higher BWs (Singh *et al*. 2016; Iqbal *et al*. 2018). Foraging is one of the most common behaviours observed on the range, with 36–60% of hens exhibiting this activity (Campbell *et al*. 2017; Larsen *et al*. 2017). However, the quantity of nutrient uptake from the range in large commercial flocks housed in static sheds is usually considered to be negligible (0–5%), unless an effort is taken to promote pasture regrowth and allow for substantial recovery periods with rotational grazing or provision of harvested fodder (Walker and Gordon 2003). Some 82% of Australian free-range farms are never stripped of vegetation, indicating the importance of appropriate agronomic practices for range and pasture management (Singh *et al*. 2017).

The quantity and quality of pasture ingestion by individual hens can vary significantly and result in reduced flock uniformity (Walker and Gordon 2003; Hegelund *et al*. 2005). With average pasture intake of birds allowed unlimited grass access being as high as 30–60 g grass/hen. day, current trends promoting pasture-raised poultry ignore the fact that chickens are not grazers and have a very limited ability to utilise fibre (Fengler and Marquardt 1988; Singh and Cowieson 2013; Ruhnke *et al*. 2015*b*). Excessive availability of pasture can severely affect the nutrient intake of a formulated diet, resulting in reduced body condition and egg production, impaired welfare, and increased flock mortality. Importantly, grass impaction, a physical blockage of the gastrointestinal tract, can result from uncontrolled pasture intake (Singh and Cowieson 2013; Ruhnke *et al*. 2015*b*). Options to reduce unwanted effects associated with pasture intake may include mowing the range, so as to restrict the physical length of the grass ingested. Traditionally, the

administration of apple cider vinegar via the drinking water has been reported to reduce clinical signs of grass constipation (Ruhnke *et al*. 2015*b*). However, high concentrations or long-term administration of these organic acids may negatively affect the hen's acid–base balance, impairing eggshell quality and a successful vaccination response.

Feeding high density pellets has been shown to be beneficial, compensating for the reduced feed intake and associated nutrient dilution of pasture intake. Feed enzyme supplementation increases nutrient digestibility and improves performance of layers and broilers consumming pastures (Buchanan *et al*. 2007; Iqbal *et al*. 2018, 2019*a*). Benzoic acid and essential oils also reduce the negative impact of pasture intake and increase ileal protein and fibre digestibility in free-range layers (Weber *et al*. 2012; Iqbal *et al*. 2019*a*). Another strategy to reduce the negative impact of traditional range grasses is to plant lucerne (*Medicago sativa*), oats (*Avena sativa*), sorghum (*Sorghum bicolor*) and faba beans (*Vicia faba*) (Singh *et al*. 2017). This minimises the consequences of nutrient dilution due to pasture intake, by providing a higher nutrient feed source on the range. While this may also minimise the abrasive damage that the long structural grass species can have on the intestinal villi, or reduce the likelihood for gastrointestinal ileus and intusseption (Cassidy *et al*. 1981; Tasman-Jones *et al*. 1982), the overall diet intake may still be imbalanced and affect individual hen performance and welfare.

Forage and shade availability for free-range hens can be a requirement of various accreditation bodies (ACO 2013; FREPA 2015). When deciding which plants should be included in a pasture mix, the possibility of plant secondary metabolites and anti-nutritive factors, as mentioned above, should be considered. These compounds may reduce voluntary intake, egg production and egg quality. Meeting the need for shelter on the range can be accomplished by pasture, but it is best achieved by shrubs and bushes that are less likely to be ingested (de Koning *et al*. 2019). For example, when free-range hens were provided with old man saltbush (*Atriplex nummularia*), 5% of their dry-matter intake was from the bush (de Koning *et al*. 2019). Incorporation of river saltbush (*Atriplex amnicola*) into layer diets at up to 20% resulted in hens producing significantly darker egg yolks and thicker eggshells, while the taste of the eggs was considered superior by consumers (de Koning *et al*. 2019). However, excreta moisture content increased significantly with increased saltbush concentration in the diet. Nevertheless, alternative range vegetation such as trees, shrubs and bushes may provide enrichment while limiting the negative consequences that can be observed when exposing hens to uncontrolled pasture access.

Conclusions

Modern laying hens produce significantly more eggs than did hens 50 years ago. The application of scientific knowledge to layer breeding, nutrition, physiology and environmental control, together with improved husbandry and biosecurity, has resulted in outstanding advances in liveability, egg production, improved feed efficiency and, generally, low cost production of high quality eggs for consumers.

These advances have been accompanied by improvements in hen welfare, but little attention has been given to the relationship between the diet supplied and hen welfare. This has occurred because most laying hens are fed a complete diet formulated commercially to provide the required nutrients for optimal health and egg production. In other words, the laying hen, irrespective of her housing, does not experience hunger or thirst. Nevertheless, there are indications that hens do experience different degrees of stress in different husbandry systems. Although diet can help laying hens cope with stress and maintain wellbeing, it is not always obvious what nutritional considerations need to be made to achieve this. Further research is required on responses to nutrition, feeding and housing systems, high temperatures, feather cover, disease challenge, inflammation, nutrient imbalances, gut microbiota, toxic insult, pastures and variations in water quality.

Conflicts of interest

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