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What are the challenges facing the table egg industry in the next decades and what can be done to address them?

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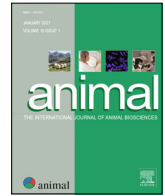




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Review: What are the challenges facing the table egg industry in the next decades and what can be done to address them?

J. Gautron^{a,*}, S. Réhault-Godbert^a, T.G.H. Van de Braak^b, I.C. Dunn^c

^aINRAE, University of Tours, BOA, 37380 Nouzilly, France

^bInstitut de Sélection Animale B.V., A Hendrix Genetics Company, 5831CK Boxmeer, the Netherlands

^cThe Roslin Institute, University of Edinburgh, EH25 9RG Scotland, UK

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ABSTRACT

There has been a strong consumer demand to take welfare into account in animal production, including table eggs. This is particularly true in Europe and North America but increasingly around the world. We review the main demands that are facing the egg industry driven by economic, societal and sustainability goals. We describe solutions already delivered by research and those that will be needed for the future. Already table egg consumption patterns have seen a major shift from cage to non-cage production systems because of societal pressures. These often feature free-range and organic production. These changes likely signal the future direction for the layer sector with the acceleration of the conversion of cage to barn and aviary systems with outdoor access. This can come with unintended consequences from bone fracture to increased disease exposure, all requiring solutions. In the near future, the laying period of hens will be routinely extended to improve the economics and environmental footprint of production. Many flocks already produce close to 500 eggs per hens in a lifetime, reducing the number of replacement layers and improving the economics and sustainability. It will be a challenge for scientists to optimize the genetics and the production systems to maintain the health of these hens. A major ethical issue for the egg industry is the culling of male day-old chicks of layer breeds as the meat of the males cannot be easily marketed. Much research has and will be devoted to alternatives. Another solution is elimination of male embryos prior to hatching by *in ovo* sexing approaches. The race to find a sustainable solution to early stage sex determination is on. Methods based on sex chromosomes, sexually dimorphic compounds and spectral properties of eggs containing male or female embryos, are being researched and are reviewed in this article. Other proposed solutions include the use of dual-purpose strains, where the males are bred to produce meat and the females to produce eggs. The dual-purpose strains are less efficient and do not compete economically in the meat or egg market; however, as consumer awareness increases viable markets are emerging. These priorities are the response to economic, environmental, ethical and consumer pressures that are already having a strong impact on the egg industry. They will continue to evolve in the next decade and if supported by a strong research and development effort, a more efficient and ethical egg-laying industry should emerge.

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Implications

Consumers are increasingly aware of the production systems for laying hens and have strong opinions on what is favourable for a hen's welfare. This has resulted in a move towards non-cage-housing systems with favourable and some less favourable consequences. Recently, several countries have banned the culling of male day-old layer chicks and alternatives are being developed.

These pressures will strongly impact the way eggs are produced and the economy of the egg industry. Adoption of ethical systems is expected to accelerate in the world and considerable research effort will be needed to optimize the new systems.

Introduction

Egg production is a good example of the major changes that have occurred in the agricultural sector in response to changing social demands. There is currently strong consumer pressure for the consumption of healthy, high-quality animal products that

* Corresponding author.

E-mail address: joel.gautron@inrae.fr (J. Gautron).

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take into account animal welfare and sustainability. The consideration of the ethical dimension in this sector has resulted in many examples of major changes to the way that eggs are produced, in order to respond to societal demands. The main changes to the production system concern the gradual abandonment of cage-housing systems, the demand to not kill male chicks and the lengthening of the production period. The latter will result in fewer birds being slaughtered each year and fewer hens being required throughout the whole production system, both in breeding and in production, to produce the same quantity of eggs. As a result, this method of production should have less negative impact in terms of the environment and use of resources. This may balance some of the negative impacts of reduced efficiency resulting from other adaptations discussed. All these developments and trends will have a major impact on the poultry sector, and will shape table and fertilized egg production in the coming years. The objective of this review was therefore, (1) to describe the issues facing the egg production chain and how the industry may evolve and (2) to examine existing scientific research to address these issues, indicating the current state of the art in the production of eggs for human consumption and future innovations in the industry. It will finally give examples of initiatives towards more ethical animal husbandry.

Evolution of the system of egg production from cage to non-cage systems

Current production methods in Europe are in line with the five freedoms of animal welfare: freedom from hunger and thirst, from discomfort, from pain injury or disease, to express normal behaviour and freedom from fear and distress (EFSA, 2005). However, among the four authorized modes of production (organic, free-range, barn and enriched cages), there is a growing consumer mistrust of eggs produced by enriched-caged hens even though the traditional barren cage was banned since 01/01/2012 (Council Directive 1999/74/EC). The enriched or furnished cages that replaced these classical cages, favour the expression of more natural behaviours by the hens but this positive change has not been readily understood by consumers. Furthermore, these changes have not solved all behavioural issues of cages as highlighted by the EFSA (2005) report on laying hens' welfare. Indeed, litter supply in furnished cages is still a major issue, making the hens unable to show normal foraging and dustbathing behaviour (EFSA, 2005). This directive has resulted in a strong segmentation of the markets. In 1996, non-cage systems accounted for 8% of the EU laying hen population, 30% in 2009, 46% in 2017 and 51% in 2019 (80% of laying hens were in cages in 2003, 49% in 2019) (ITAVI, 2019) (Fig. 1). The proportion of hens reared in non-cage systems is currently increasing sharply, although it remains very heterogeneous in Europe (from less than 10% in Spain and Poland to more than 85% in the Netherlands, Germany and Austria) (Fig. 1).

Like many of Europe's member states, French production has become increasingly diversified since the late 1990s. The number of laying hens in non-cage systems was 19% in 2008 and has reached 46% in 2019 (Fig. 1) (ITAVI, 2019). In contrast, the egg consumption by distribution channels did not change during the last decade (Fig. 2), with about 60% of eggs consumed as shelled eggs and about 40% as egg products in France (ITAVI, 2019). The supermarkets and hypermarkets in France, attentive to campaigns from non-governmental organizations on the welfare of laying hens, have announced an end to the marketing of eggs from cage systems within the next 2–5 years (Table 1). On 18th February 2018, the French Agriculture Minister Stéphane Travert confirmed that the government would work on a ban of table eggs from cage

systems by 2022 for whole egg sales (Tassard, 2018). The French mediator for agricultural trade relations, in his 2017 report, points out that “the refusal to market eggs produced by caged hens . . . is on the way to become the norm regardless of any regulatory developments”.

However, the objective of converting the existing furnished cage systems to non-cage systems is not economically or materially feasible by the end of 2020 and even 2025 according to the mediator of the French republic and ITAVI economic studies. As a result, there will be disruptions in supply and a significant increase in intra-European imports. For example, Germany has eliminated cages in favour of floor-based production, either in indoor or free-range systems, and is in a position to export.

Despite the fact that Great Britain has left the European Community, a similar trend can be observed. There are four recognized forms of laying hen production following EU definitions, three non-cage systems and the furnished cage. The three non-cage production systems are Free-Range, organic and Barn egg production. Organic egg production must also be free-range, but follows different standards notably in terms of stocking density, the origin of the animal feed, medication and beak trimming; Barn egg production (code 2), which is similar to free-range in terms of housing, but with the notable exception that the hens do not have access to the outdoors. In the UK in 2019, there was around 2%, 3%, 53% and 42% of eggs packed from barn, organic, free-range and enriched cages systems, respectively (DEFRA, 2020). Both organic and barn systems are increasing, but from a low starting point. It is believed that, as retailers commit to going cage free, barn production will be increased to give a low-price alternative to free-range eggs, although with the lack of consumer understanding of barn production that may be a challenge (Porter, 2020). Indeed, as in France, some retailers have already said that they will not sell barn eggs (White, 2019). Much of the egg production in the UK (~90%) is part of the Lion Quality Code of Practice that has enhanced requirements for welfare in terms of stocking densities for free-range hens, nest box space, lighting and the handling of end-of-lay hens. It also focuses heavily on egg hygiene and microbiological quality, which was the initial impetus for its creation. This Code of Practice can be a major driver for change if its promoters choose to adopt new standards in terms of welfare. For the free-range sector, research will continue on how to design the indoor environment to better allow natural behaviour but reduce damage from collisions (Stratmann et al., 2015). Initiatives to tackle the problems of injurious pecking are also underway (Nicol et al., 2013). Another important issue is the way to reduce poultry pandemics and other diseases that can be transmitted to humans. Breeding systems with outdoor access increase the risk of exposure to wild animals including other birds. The recent COVID-19 pandemic, where wild animals are suspected to be the reservoir of virus, has reinforced this concern, although there is no evidence of birds being involved in the transmission in this case. Poultry pandemics (like Avian Influenza), raise the question of the relocation of bird farms producing eggs from high-risk areas to areas that are less prone for migrating birds carrying Avian Influenza (ANSES, 2021).

Besides the improvement of animal welfare, the sustainability aspects of the egg industry should be further investigated to improve housing systems with respect to the needs of the laying hens, but also to lower the ecological footprint of egg production. They should permit the use of more by-products, residual waste, to improve circular egg farming (de Olde et al., 2020). Overall can new systems be developed that have benefits for the hen that do not have negative welfare consequences and are more sustainable? Perhaps the greatest challenge will be: can the consumer be educated to accept different systems which are more sustainable?

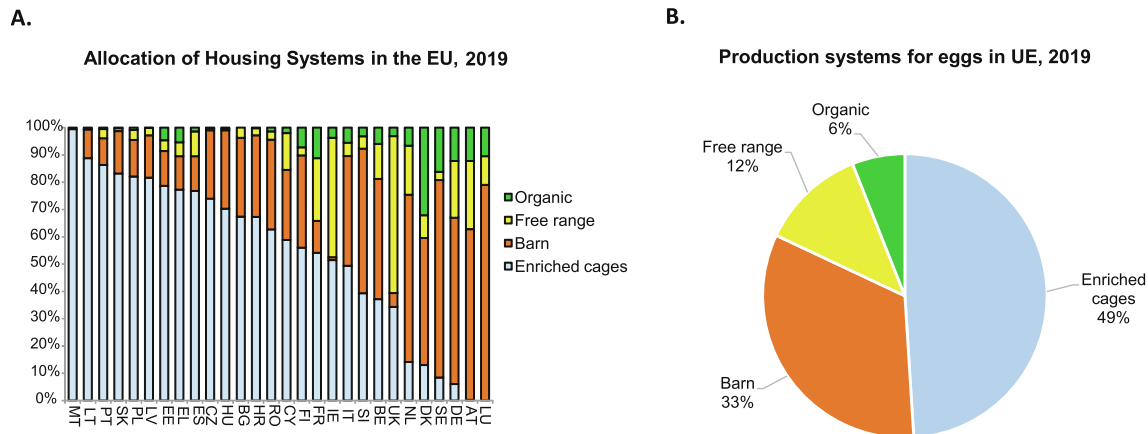


Fig. 1. Percentage of laying hen numbers by production systems in 2019, in individual (A) and in total (B) European-27-member states (without UK for B). (Beck, 2019).

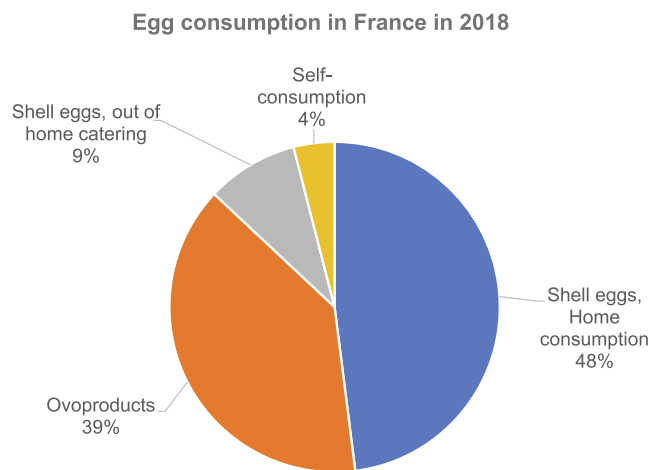


Fig. 2. Egg consumption in France in 2018 by distribution channel (ITAVI, 2019).

Extending the production period

Currently, laying hens start laying at around 18 weeks of age and peak laying is around 25 weeks of age. Eggs are well valued from the moment that the weight of the egg has reached a threshold value (e.g. 53 g in EU and 49.3 g in USA). The majority of the flocks are depopulated when the laying rate is reducing and, most importantly the incidence of downgraded eggs increases to unprofitable levels. At this point there are still many saleable eggs but the high variability in egg quality means a threshold is reached where less than about 75% of the eggs are marketable, although the threshold can vary depending on prevailing economics. In 2016, this often occurred at around 72 weeks in free-range flocks in the UK, i.e., after one year of production (Bain et al., 2016), but already a Belgian study indicated that the end of production could vary from 74 to 92 weeks, with an egg-laying rate of 79% (Molnar et al., 2016). Reproduction in birds is controlled by the hypothalamus according to different environmental and endocrine stimuli.

As the hen ages, environmental stimuli that were previously stimulatory no longer have that effect and potential inhibitory factors may increase, which results in reduced hypothalamic activity of the cells driving reproduction (Dunn et al., 2009). The consequence is a loss of weight and functionality of the oviduct, which leads to an increase in the number of days of rest (without laying) and defective eggs (Solomon, 2002). However, selection for egg production has successfully reduced the effects of age on the reproductive system, with the result of reducing the amount of variation in egg production rate towards the end of the traditional laying period. Essentially the majority of hens were maintaining production of one egg a day. To perform selection and improve the sustainability of the industry, poultry breeders have extended the laying periods of their pure line birds to make variation visible (pers. comm. Teun van de Braak). It is now possible to select those individuals that are observed in older flocks that can maintain high oviposition rates with good shell quality (Molnar et al., 2016). Accessing this variation has allowed an increase in the persistence of egg production and maintenance of egg quality at an advanced age (Bain et al., 2016). Breeders indicated 10 years ago, that they wanted to select strains of laying hens capable of laying sustainability up to 100 weeks of age, for a total production of almost 500 eggs per birds by 2020 (Bain et al., 2016), which has been achieved. Bain et al. (2016) indicate that an additional production of 25 eggs per hen could potentially reduce the laying hen flock in Great Britain by 2.5 million hens. This cumulative effect is obtained as shown in Fig. 3, due to the pyramidal structure of production in the sector. An increase of 10 weeks of production would preserve 1 g of potentially polluting nitrogen per dozen eggs produced (Molnar et al., 2016).

However, any improvement in egg-laying persistence must be achieved with consistent egg quality. Egg weight increases by 70 mg per week between 60 and 80 weeks of age (Molnar et al., 2016). While the other egg constituents remain constant, there is a decrease in shell thickness ($-0.23 \mu\text{m}$ per week) and a decrease in breaking strength with increasing age of the hen. Haugh units are an indicator of egg white quality. They measure the height of the egg white after breaking the egg, which is related to the viscosity of the colloidal gel of the egg white. This

Table 1
Year of the announced cessation of marketing of furnished caged eggs by retailers in France.

| Brand | Aldi | Auchan | Carrefour | Casino | Cora | ITM | Leclerc | Lidl | U |
|----------|----------|--------|-----------|--------|------|------|---------|----------|----------------|
| Private | 2025 | 2022 | 2020 | 2020 | 2020 | 2020 | 2020 | 2020 | 2020 |
| National | Not sold | 2025 | 2025 | 2020 | 2025 | 2025 | 2025 | Not sold | Not determined |

gel-like structure limits bacterial proliferation and migration towards the yolk and is essential for maintaining the hygienic quality of the egg. However, Haugh units reduce over the course of the production period (Whitehead, 2004; Bain et al., 2016; Molnar et al., 2016). There may be other consequences of keeping hens for longer, like moulting that should result in an absence of eggs during several weeks and an economic loss because hens are still fed while they do not produce eggs. It is essential to maintain good bone quality in the laying hen, especially when the hen is getting older. A laying hen requires between 2 and 2.5 g of calcium daily for the production of an eggshell. About 2/3 of this calcium is provided directly by the feed, the remaining 1/3 comes from storage by demineralization of the medullary bone. The calcium from the medullary bone is necessary in the second part of the shell mineralization process. This occurs at night, when the hen has no access to feed although birds do store food in the crop for several hours. Medullary bone is capable of rapid absorption and renewal (Whitehead, 2004), which can be optimized by dietary calcium sources (content, quality and particle size). Even with a perfectly controlled diet, bone demineralization is a natural phenomenon that can also affect the structural bone, ultimately leading to osteoporosis. This pathology, which can be prevalent in old hens, leads to bone fragility and keel bone fractures that severely impact the welfare of laying hens (Armstrong et al., 2020). Bone quality and fractures in these hens are currently a major issue in the table egg sector (Sandilands, 2011). It is easy to observe hens in a flock with very fragile or very strong eggs and the same applies to bones. However, there is relatively little knowledge about whether hens with bone defects are those that lay eggs with fragile or strong shells.

In studies where genetic correlations between egg quality and bone quality have been examined, there is little evidence that the two traits are linked, with only the keel bone density in one line being significantly correlated with egg breaking strength (Dunn et al., 2021). Whilst there were no significant associations for the tibia or humerus strength or density with egg quality, there was a genetic correlation with onset of lay and early egg number, but only in one line. Although not significant, there was a suggestion that genetic loci explaining variation in egg quality might be present at the same loci as one for bone quality but in a related study, lines of hen successfully selected to have differences in bone

strength did not have differences in egg quality (Fleming et al., 2006). This is not to say that egg laying is not related to issues of bone quality in laying hens, if hens do not lay eggs at all they have better bone quality (Eusemann et al., 2020). However, the quality of the egg or the persistency of lay do not seem to be critical components in determining bone quality of a hen. Rather, it is possible that the onset of lay, which is intrinsically related to the BW of the hen and the genetic factors that directly affect bone quality, appear to be most important. Research is ongoing to resolve some of the questions on the relationship between persistent egg production and bone quality and other welfare issues, and how the physiology, nutrition and welfare of the older hen will be affected. Programmes conducted in partnership with researchers in the sector are underway to ensure best practice which will help support the longer laying period (Toscano et al., 2020).

Prolonging the laying cycle can only be achieved when the health of the birds remains in good condition. For the average flock, weekly mortality figures tend to increase when the birds are getting older. Continuous genetic selection for improved livability and improved knowledge on nutrition and flock management have resulted in lower weekly mortality numbers. There is some optimism that, in a similar way that selection for saleable egg production reduces the incidence of egg defects (Wolc et al., 2012), health issues will be reduced by selection for saleable eggs as negative health traits often result in reduced egg production. As the genetics for bone quality suggests, more emphasis should be placed on the rearing period. The mindset is changing from seeing it as a period of costs to a period of investment in the bird's productive achievements later on in life. For decades, birds have been selected to come into lay at an earlier age. From the overview of 37 tests of a North Carolina random sample and subsequent layer performance and management tests (Anderson et al., 2013), it can be observed that the age at sexual maturity has been reduced by more than a month. When we look at the last, 10 tests and talking to breeding companies (communication with Hendrix Genetics), it can be observed that selection is not continuing for birds to come into lay even earlier. The long-life layer needs sufficient time to grow and develop. Bodyweights at weeks 6 and 17 of the rearing period are associated with productivity later in the bird's life, i.e., higher bodyweights at the crucial development stages are positively associated with higher peaks of production, higher egg weights and improved persistency in egg production and, as discussed previously in connection with bone quality (communication with Hendrix Genetics) (Table 2).

The accelerated change in housing systems and the prohibitions on management practices such as beak trimming in several EU countries (Austria, Germany, Netherlands, etc.) is the most recent manifestation of a trend that has resulted in changes in selection criteria for laying hens. The traditional approach that was merely focused on the economic aspects of egg production has shifted. Today, breeding programmes and selection indexes include more poultry health and welfare traits than ever before. Next to relatively well investigated traits such as livability, bone strength, disease resistance and feather cover, new traits related to behaviour, such as negative social interactions between birds, and behaviour in cage free housing systems have been adopted by the breeding companies (Brinker et al., 2018).

Not only have health and welfare received increased attention, breeding companies are showing their commitment and making their contribution to set the standard for sustainable egg production. The environmental impact per kilogram of eggs produced has significantly decreased during the past decades. The review by Pelletier et al. (2014) showed a comparison of the environmental footprint of the egg industry in the United States in 1960 and 2010. They showed an enormous reduction in the environmental footprint per kilogram of eggs produced: the environmental foot-

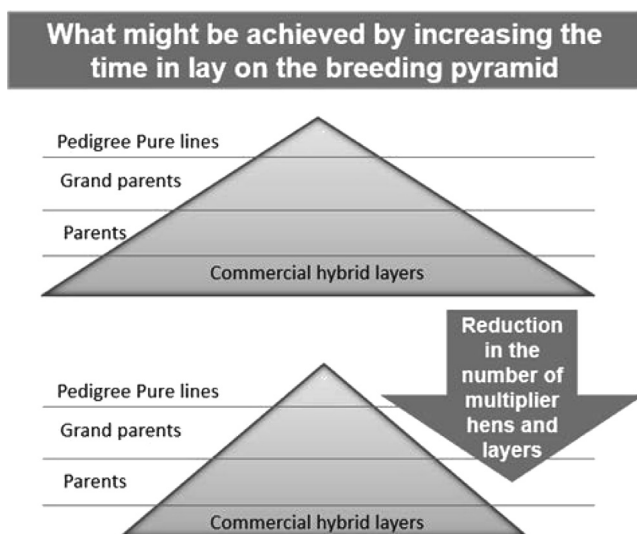


Fig. 3. Schematic representation of the effect of increasing the laying cycle on reducing the number of hen multipliers and layers and the consequences on the production pyramid (modified from Bain et al., 2016).

print for 2010 was 65% lower in acidifying emissions, 71% lower in eutrophying emissions, 71% lower in greenhouse gas emissions, and 31% lower in cumulative energy demand compared to 1960. Despite the 30% higher table egg production in 2010, the total environmental footprint was significantly lower compared to 1960. Pelletier et al. (2014) estimated that 28–43% of this improvement in lower environmental footprint could be attributed to the improvements in the performance of the birds. Abin et al. (2018) used a Spanish case study for an environmental assessment of intensive egg production. Their study showed that after the production of the hen feed, the purchase of new laying hens to replace the old flock contributed most to the harmful environmental impact of intensive egg production. By extending the productive lifetime of the laying hens in the breeding pyramid and not just at the production level, the environmental impact could be further reduced, as fewer replacements flocks are needed.

Alternatives to the culling of one-day-old male chicks

A major issue that has raised ethical concerns within the poultry sector is the fate of male chicks from laying strains. Although chickens can be used for both meat production and egg production, there is a trade-off between the two traits (Wolc et al., 2012; Giersberg and Kemper, 2018). As a result, there has been specialized breeding of chickens for either egg production or for meat production (Leenstra et al., 2016; Sakomura et al., 2019). These strains of hens are very efficient at either only egg production or only meat production, but do not compete economically in each other's market. Hens selected to produce eggs for human consumption have a low BW of around 1.7 kg that is reached at around 20 weeks of age (sexual maturity), where they start to convert their feed into egg nutrients very efficiently (Ahmed et al., 2014; Bain et al., 2016). Hens at the end of lay are used for human consumption, but the males have limited added-value as they grow slowly and the live weight and meat yield on the carcass do not meet the meat quality/yield criteria to be marketed (Giersberg and Kemper, 2018). As only the females lay eggs, half of the hatched chicks are therefore non-marketable males. Day-old chicks are sexed at hatch using cloacal or vent sexing, or via sex linked feather features (feather colour or covert length). Only a limited number of males are bred to allow the reproduction of future offspring. As a result, billions of male chicks have no commercial value (Weissmann et al., 2013; Galli et al., 2016; Giersberg and Kemper, 2018), and are culled rapidly after hatch by asphyxiation or maceration. These authorized practices elicit legitimate questions in terms of animal welfare and the ethics of hatching eggs without an agricultural output. The joint announcement by the French and German ministers to ban the culling of one-day-old male chicks by the end of

2021 has given some urgency to finding solutions and several methods are close to market while some of them are being used by hatcheries. The three main alternative approaches to the practice of killing male chicks are; (1) to identify mechanisms that can, ideally completely, imbalance the sex-ratio in favour of females, (2) to develop tools that would allow the determination of the sex of the embryo *in ovo* prior to hatch, (3) to develop dual-purpose strains where female chicks would be reared as future egg-laying hens and male chicks for meat. Ultimately the systems developed will also need to be acceptable to consumers.

Skewing primary sex-ratio or hatching sex-ratio

It is well-known that the primary avian sex-ratio can be affected by environmental factors such as diet, physiology, hormonal status and conditions as well as the genetic background. However, the effects are relatively modest and can change over the lifetime of a hen (Klein and Grossmann, 2008). Thus, it is quite difficult to know how sex-ratios could be reliably altered by poultry breeders using current knowledge without gene editing or transgenesis. Research into the basic mechanisms of sex determination in birds certainly continues to be warranted. Another level of complexity is that the primary sex-ratio of fertilized eggs does not necessarily reflect the sex-ratio at hatch, which suggests that a sex-dependent selection during incubation depending on the genetic background and age of laying hens may occur (Klein and Grossmann 2008). The Israeli company SOOS (Soos, 2020) used this concept to develop an incubation system combining different incubation parameters that seem to reverse males into female chicks. According to their claims, resulting animals can efficiently lay eggs at sexual maturity. However, to date, scientific information about the underlying mechanisms and short/long-term impacts on feminized chickens are missing and need to be addressed before this approach can be validated by the scientific community and, ultimately, authorities and society. In the light of this, it seems necessary to develop other strategies until realistic methods to skew the primary sex-ratio are available.

Sexing eggs

The second strategy is to detect and discard male eggs before hatching instead of killing one-day-old male chicks (Krautwald-Junghanns et al., 2018). This approach relies on the development of *on ovo* or *in ovo* sexing methods that are based on the detection of sexual dimorphic traits or molecules. Several approaches have been tried during the last decade to obtain a method that can be used in practice in hatcheries. There are many prerequisites to develop an operational sexing method than can be effective at an industrial level. The analysis must be rapid, inexpensive, highly accurate and have no impact on chick hatching rate, health and performance (Kaleta and Redmann, 2008). There is also one other constraint. Ovosexing methods as an alternative to the culling of one-day-old male chicks will need to meet the required social/consumer acceptability (Gremmen et al., 2018). Consumers may not differentiate the killing of an embryo from the killing of a day-old chick. With this in mind, methods used for sex determination and disposal would be best employed before the embryo feels pain, approximately 7 days before nociception appears (Eide and Glover, 1995). A consensual limit on 9 days of incubation has been proposed as there is a controversial grey zone for up to 15 days depending on studies.

In ovo sexing methods are based on the initial postulate that male embryos and female embryos exhibit specific features (anatomical, physiological, molecular and genetic) that should allow for the discrimination between sexes during incubation of fertilized eggs. Some of these methods are universal while others

Table 2

Influence of pullet quality on the performance at different ages of the layer¹ (Hendrix genetics, personal communication).

| Item | BW | | | Uniformity 16 weeks |
|---|---------|----------|----------|------------------------|
| | 5 weeks | 10 weeks | 16 weeks | |
| Early maturity (%HD prod. 24 weeks) | +++ | +++ | ++ | 0 |
| Early maturity (%HD prod. 68– 72 weeks) | +++ | 0 | 0 | ++ |
| HH eggs up to 60 weeks | +++ | ++ | 0 | +++ |
| HH eggs up to 72 weeks | +++ | 0 | 0 | +++ |
| Livability up to 72 weeks | +++ | 0 | 0 | +++ |

Abbreviations: HD = Hen day; HH = Hen Housed; prod. = production.

¹ 0 = absence of correlation, + = low correlation, ++ = middle correlation, +++ = high correlation.

have been specifically designed for selected genotypes. A review of the various *in ovo* sexing techniques that were close to market was published in 2018 (Hein, 2018). Of these, only two are commercialized and in use by the poultry sector (Seleggt, 2019; Plantegg, 2020). Some require the sampling of embryonic cells or embryo-derived cells while others rely on the sampling of extra-embryonic fluids such as the allantoic fluid.

Dimorphic chromosomes

In birds, unlike mammals, males are homogametic (two Z chromosomes), whereas females are heterogametic (Z and W chromosomes). The constraint of techniques built upon these sexual characteristics is the sampling of embryo-derived cells that bear the embryonic genome. The detection of W- or Z-specific genes by polymerase chain reaction to distinguish female embryos from male embryos is well established (Clinton, 1994; Ellegren, 1996; Smith et al., 2009). The German company PLANTEGG has developed a PCR-based method using a few drops of the allantoic fluid that contain embryonic cells (Plantegg 2020).

Another approach is based on the analysis of the DNA content of the embryonic cells, considering that the Z chromosome has 200% more DNA content than the W chromosome (Mendonça et al., 2010) and male cells containing the ZZ chromosomes are about 2% bigger than female ZW containing cells. Using infrared spectroscopic imaging on fertilized eggs prior to incubation, Steiner et al. (2011) corroborated that male blastoderm cells have a higher DNA content than female blastoderm (Steiner et al., 2011). Using this method, gender determination is possible at very early stages, is rapid (few seconds) and accurate. However, it requires germinal disc sampling with possible long-term effect on the development of the embryo or the chicks after hatching. Some other authors also use the length of the sex chromosomes to develop *in ovo* sexing methods based on Raman spectroscopy (Galli et al., 2016, 2017 and 2018). These authors focused on blood cells, all of which are nucleated in birds, and analysed the spectra of blood fluorescence. Differentiation between males and females was shown to be 90% from the 4th day of incubation. According to the authors, this technique has no visible effect on the hatched chick, but decreased the hatchability by about 10%. The invasiveness was further reduced by keeping the eggshell membrane intact (Galli et al., 2018) leading to the development of a prototype (Muller-Niegsch, 2017). However, there is no current information on the state of progress in terms of commercialization. Similarly, the AAT group developed a Raman-spectroscopic method that would allow for sexing eggs after 4 days of incubation. This approach remains semi-invasive as a small piece of the eggshell needs to be removed to access the embryo and the surrounding yolk sac vascularization, prior to Raman spectroscopy measurements (AAT, 2020).

Dimorphic compounds

Besides chromosome-based strategies, techniques that use reported differences in the hormonal and metabolic status between male and female embryos have been proposed. In 2013, Weissman et al., published that the allantoic fluid from female embryos displayed significantly higher estrone sulphate (female hormone) levels than males and that this difference was detectable as soon as 9 days of incubation (Weissmann et al., 2013). The discovery led to the development of a prototype by SELEGGT with a sexing accuracy of 97% (Seleggt, 2019). The eggs resulting from this approach are called “respeggt” eggs and were successfully introduced in 2018 in Germany. They are available in boxes of six at all 5 500 REWE and PENNY stores. For all these techniques, sampling embryonic-derived cells or extra-embryonic fluids implies invasive or semi-invasive technique (egg opening/eggshell drilling) that would increase the risk of low viability/impaired hatchability

afterwards. A non-invasive technique would likely supplant all of the above approaches.

Besides hormones, it has been shown that glucose, choline and some amino-acids (valine) are more concentrated in allantoic fluid from females (Bruins and Stutterheim, 2014) while the same authors also found that butylated hydroxytoluene is a particularly relevant volatile dimorphic biomarker (Bruins and Stutterheim, 2017). Such dimorphic volatile compounds are promising as they can diffuse through the eggshell pores and may be detectable at the surface of the eggshell (Webster et al., 2015; Costanzo et al., 2016; Knepper et al., 2019). Taken together, these data were probably the initial step for the development of a prototype by *In Ovo*, a spin-off of Leiden University (InOvo, 2020). Since 2017, they have been developing their current Alpha prototype, realizing a throughput of 1 800 eggs per hour with 95% accuracy. In the InOvo project that started in June 2020 (CORDIS, 2020), they plan to scale this prototype to reach more than 10 000 eggs per hour to meet hatchery needs.

Spectral dimorphism of the whole egg

The most promising non-invasive technologies developed to date are based on spectroscopic methods applied to the whole egg, such as hyperspectral imaging (Canadian Hypereye company and Agri Advanced Technologies (AAT, Germany)) and the combination of spectroscopy and biosensors (SOO project, French company Tronico and the French National Centre for Scientific research). The technology of Hypereye uses hyperspectral technology to acquire a specific signature through mathematical algorithms to determine the gender of the embryo from the day of lay onwards. Commercialization date and progress are not known. Several announcements had been made and a prototype was expected for 2018, with a throughput of 50 000 eggs per hour. However, since then, there is no public information about their progress in this field. A similar strategy has been developed by AAT technology (AAT, 2020). Using a specific genotype (Lohmann Tierzucht GmbH) where female chicks have brown down feathers and the males have yellow down feathers, Göhler et al. (2017) describe a non-destructive optical method for sex determination. This hyperspectral method has been shown to reach 97% efficiency between 11 and 14 days of embryonic development and is expected to be implemented to allow the sexing of eggs at earlier stages (7th day of incubation). According to the website (AAT, 2020), the technique is 95% accurate and 20 000 eggs can be tested per hour per machine. Hyperspectral measurement technology has been improved for large-scale practical use and French egg suppliers have started to use this technology since the beginning of 2020.

In 2017, French Agriculture Minister Stéphane Le Foll granted Project SOO (“Sexage des Oeufs d’Oiseaux” in French or “sexing avian eggs”) with 4.3 million euros to finance the development of new *in ovo* sexing methods. This project, with the French Tronico company and the French National Centre for Scientific research, focuses on a method that combines spectroscopy (response to a light pulse) and the use of biosensors, that it is claimed will be 90% reliable in sexing eggs as soon as 9 days of incubation. The prototype was initially expected at the end of 2019. Although the current technologies and prototypes need to be improved, it has to be mentioned that ovosexing provides another advantage as compared with current manual methods for sexing as it will avoid the manipulation of chicks at hatch and thus will limit additional stress for animals. They may also be transposed to other bird species of industrial interest, such as foie-gras production where only males are reared (female ducks are culled at hatch as their liver is too small and contains many veins).

Sex manipulation by genome editing

Although the social acceptability of such approaches remains very poor (Gremmen et al., 2018), the advent of CRISPR/Cas9 genome editing highlights a new opportunity to potentially generate a gender that would bear a specific marker, which would increase the feasibility to detect males from females at soon as the egg is laid, or to create all-female or all-male progeny using some of the imaging approaches outlined above. In this regard, the program EggXYT developed a gene edited breed of chickens based on the introduction of a fluorescent marker within the sexual chromosome (eggXYt). Males can be detected using fluorescence imaging and reliability is claimed to be 100%. Its state of commercial development is at the level of prototype 3.0, which does not yet appear fast enough for high throughput hatcheries. There are also many challenges regarding the genetic technologies that would allow the production of single-sex litters (Douglas and Turner, 2020). Although gene-drive methods can also have disadvantages (mutation, abnormalities, uncontrolled spread of synthetic gene drives), some consumers may consider the use of transgenic animals in agriculture ethically preferable to the culling of the unrequired sex (Douglas and Turner, 2020). This approach, although still unacceptable by most public authorities, still elicit interests and scientific research/development. Thus, there is an urgent need to discuss more globally about the acceptability and the potential benefit/risk balance of these genetic methodologies.

Dual-purpose breeds and/or growing layer male chicks

The development or revival of dual-purpose strains, with females producing eggs and males producing enough quality meat to be marketed, is currently being examined and will likely form a segment of the future market. If killing male day-old chicks is not seen as an ethically defensible position, then the use of dual-purpose chickens seems a straightforward proposition. Even if male chicks are killed humanely and they are consumed, albeit for pet and zoo animals, the industry struggles with the ethics of producing animals that do not live a full life (Bruijnjs et al., 2015). The dual-purpose chicken allows the male to be reared for meat production, growing faster with more saleable meat than the male of chickens bred purely for laying. Currently in the available laying strains the growth of males does not meet the requirements for the production of quality meat at a competitive cost (Koenig et al., 2012; Gremmen et al., 2018). However, even if the resulting meat is comparable with meat from broilers, the production remains less competitive in economic terms, but also in terms of resources and environmental pollution (Koenig et al., 2012). A number of approaches have been adopted to produce dual-purpose birds, from the use of lines of chicken that have served traditional markets to specific breeding programmes, which produce chickens where the males can almost compete with slow growing breeds of broiler chicken, although still often with 10% less meat product. In many cases, these are a cross between broiler and layer parent stock. In general, although the males take longer to get to a reasonable slaughter weight than a slow growing broiler, meat quality and acceptance seem to compare well with broiler meat (Mueller et al., 2018). The amount of leg meat is larger in dual-purpose breeds but this can be favourable in some markets. The main disadvantage of dual-purpose breeds is the lower yield in breast meat, therefore dual-purpose breeds are often sold as a complete carcass. The same acceptance is true for the eggs from the females, which compare favourably in some studies with the possible exception that brown egg dual-purpose breeds produce eggs that are lighter than from a pure brown egg layer. However, there are reports of poorer egg quality in some lines, in both external (shell strength, shell colour) and internal (Haugh units, blood and meat spots) parameters. There may be other benefits of the

dual-purpose bird as there is some evidence that dual-purpose chickens suffer less from issues such as mortality from injurious pecking.

The real issue comes from the economics and environmental impact of the dual-purpose breeds; indeed, it has been suggested that the current system that has evolved has produced a 'lock in' (Bruijnjs et al., 2015). Essentially the current production system has developed to be so efficient with a comparatively small environmental impact that it is impossible for any competing systems to be established, either ethically or indeed economically. The biological and economic consequences for different options of dual-purpose chickens have been examined in several studies (Leenstra et al., 2011). This demonstrated that dual-purpose chickens might serve as a niche market, but a total shift to dual-purpose chickens in order to solve the problem of killing day-old males would not be realistic when looking at the environmental burden and the economics. Alternative systems such as dual-purpose chickens have higher environmental costs, taking more resources to produce the same amount of food, with ratios for the conversion of feed to meat lying above four, while for broilers it can be around 1.6 (Giersberg and Kemper, 2018). Egg production from dual-purpose hens also has greater environmental impact as they lay typically around 50 fewer eggs in a year but consume similar or more food to do so. It is difficult to argue that the system is ethically superior compared to current strains if negative environmental impacts increase. It is argued that the 'Responsible Innovation' approach, which balances economic, socio-cultural and environmental aspects of any new system, would need to be promoted to shift production and get consumers to accept on a larger scale the products from dual-purpose rearing systems or, indeed, any alternative system that improves the ethical dimensions of production (Bruijnjs et al., 2015). In the absence of legislative changes, the use of dual-purpose hens will likely remain an expanding but niche product requiring strong marketing (Busse et al., 2019).

A German dual-purpose initiative, which includes the rearing of day-old layer male chicks is again working in a niche market, where an increasing number of day-old males are kept for meat production. In Germany, they have introduced this as the Bruderhahn (Brother cockerel) initiative (BID, 2020). Eggs are sold for a premium price in order to compensate for potential economic losses when growing the males. Because of their inefficient feed conversion, and different characteristics (they differ in breast meat yield, taste and tenderness compared to conventional broilers), the cost of production is higher with a smaller market. It is forecast that the entire organic egg market will adopt the principle of growing the male layer chicks, as they do not see *in ovo* sexing as an acceptable solution, as the male embryos are still discarded. A specific example of a system utilizing 'Les Bleues' chicken has been used in the 'ei care' project in north eastern Germany (eiCare). Both males and females are raised for meat and eggs, respectively, and are marketed with the 'ei care' branding to organic shops and supermarkets. The project is a partner in a research programme for sustainable development and currently has four farms producing eggs and meat sold relatively locally.

There is an urgent need for research and development of new dual-purpose strains by crossing selected genotypes to optimize their productivity under realistic farm conditions as well as optimizing the quality of derived products (eggs and meat). It is also necessary to understand the behaviour of these new strains in different farming systems and under different environmental conditions, all of which should lead ultimately to the best scenario/trade-off in terms of health for the hen and of costs for farmers. To complement this, there is also a need to understand consumer attitudes to the proposed systems. It is also important to consider that some consumers prefer brown eggs while others prefer white

eggs (cultural habits). It is forecast that we will need to have several crosses as options, depending on countries, to meet people's cultural requirements.

Towards more ethical animal husbandry?

Already there are some well developed systems that are marketing the concept. Poulehouse is a company founded by Fabien Saullman, Elodie Pellegrain and Sébastien Neuch (Poulehouse). The slogan of this company is "the egg that doesn't kill the hen". They define themselves as "A responsible production method. Ethical. Innovative. From production to the plate". Classically, hens are slaughtered at 70–80 weeks when their productivity becomes uneconomic. Poulehouse offers a rearing method where the hen is kept alive until its natural death, which can occur at 7–12 years of age. The hens produce shell eggs of sufficient good quality for about 3 years, which are sold at a price of about 1 euro per egg, which is three times more expensive than organic eggs. The selling price of the eggs thus makes it possible to house and feed the non-producing hens until their natural death.

This method of production generates a number of zootechnical constraints such as the control of moulting. Indeed, a hen after 15–16 months of production undergoes a moult that is characterized by a regeneration of the reproductive tissues. One month after the start of moulting, the hen will again lay good quality eggs, but the laying time will rapidly decrease as the cycles progress. This moult may be caused by a decrease in light and energy rationing strategies in the feed. Poulehouse objective is to achieve three moulting periods separated by production periods of 9–12 months. At the moment, the oldest flock is 3–4 years old. Health management of older flocks will also be a challenge when the flocks will be at the end of their life.

Poulehouse produces organic eggs (code 0), but also free-range eggs (code 1). Poulehouse has initiated a collaboration with the German start-up company Seleggt, which has developed a technique to detect the sex of the chick in the egg and thus hatch only females (see the paragraph "sexing eggs"). Two of their farms are already producing eggs that do not kill either the hen or the male chick. Eventually, they want to generalize this process to produce "eggs that do not kill the hen and the male chick". The Poulehouse company has undertaken numerous marketing campaigns to promote their products. It is still a weak market, but it does reflect a trend of producers to serve consumers who want to consume products in accordance with their convictions.

Another initiative can be found in the Netherlands, which is the Kipster farm concept (Kipster). In this concept it is all about sustainability, and they try to include all elements with respect to sustainability, not only the ethics involved, but also with a large focus on the impact of farming on the environment. At the Kipster farm, they focus on closed loop farming, and they try to limit the waste generated during the production process. Together with LIDL supermarkets, they have developed new products based on the meat coming from the processed spent hens and layer males. This allows them to create value out of the products that would otherwise be considered as waste/products with low economic value. The eggs are sold with a premium price, and no involvement of an egg packing station; in this way the egg producer can benefit more from exploiting this innovative concept. High standards of animal welfare are combined with extremely transparent farming. This is done to reduce the growing gap between producers and consumers, and educate the consumers on the origin of their food. At Kipster they have deliberately chosen white egg layers, as they have a lower ecological footprint compared to the brown egg layer (Mollenhorst and Haas, 2019). The main reason for this lower ecological footprint in white egg layers is their ability to be kept for longer production periods compared to the brown egg layer,

resulting in more saleable eggs produced per hen housed, higher total egg mass produced and better feed efficiency. That the popularity of the white egg layers is on the rise can be clearly seen in the Netherlands, where the brown to white egg layer ratio went down from 60–40 in 2012, to 35–65 in 2018 (IEC, 2018).

In the UK, because the free-range concept has been around for so long, there are considerable challenges to any other systems entering the market. Free-range is the mainstream product which customers identify as an ethical choice and to some extent this may be preventing new systems being produced, perhaps another example of a 'lock in'. Consumers identify with what free-range means. Although there might be advantages in terms of health for the hens with something as simple as a barn system for example, it has had very limited success as a product. More ambitious examples of sustainable systems as outlined in France, Netherlands and Germany have not yet emerged commercially to our knowledge.

Conclusion

In the next decade, the egg sector will have to deal with the evolution of the systems of egg production in cages vs non-cages to consider welfare and sustainability. We have described recent developments in science, technology and production strategies that are intended to tackle ethical issues in layer hen systems. They included the extension of the laying period and the development of more recent production methods that try to be ethical. Another important challenge for the sector is the use of alternatives to the culling of male day-old chicks of layer lines. The development of genotypes to obtain dual-purpose strains is in progress, but the current genotypes do not yet meet the requirement for the production of quality meat and egg. Introducing *in ovo* sexing techniques in hatcheries implies additional costs for producers who will have to reorganize logistics, but also for consumers who will pay more for eggs. Because of the drive to achieve the goal of all-female chicks, in addition to properly researched proposals rooted in biology, the field has attracted some unlikely 'snake oil' solutions which industry and funders should be aware of. To date, most technologies are not efficient to determine the sex of the embryo at the day of lay. They require (1) the removal of eggs from incubators for sexing, (2) re-incubation of female eggs potentially increasing the risk of embryonic mortality (3) the elimination and new valorization of male eggs as high-quality feed (SELEGGT option) or other uses, and the management of male chicks if the method is not 100% reliable. In the future, the accuracy of methods may be greatly improved by combining several dimorphic features to get to the level of accuracy desired of near 100% and by using artificial intelligence tools to integrate data. However, it is likely that if the method selected is not 100% accurate, chicks will have to be re-examined by sexers at hatch to avoid the introduction of males in female flocks. Already *in ovo* immunization is performed routinely, which suggests it is possible to do at scale without detriment. In parallel, several strategies based on sex manipulation (transgenesis, genome editing) have shown a high potential and efficiency but the ethical and social acceptability of such approaches remains very poor. Although consumers are willing to pay more for eggs from non-cage systems in many parts of Europe, the more widespread adoption of the systems described in this review will increase the cost of eggs and egg products.

It should be noted that the solutions are still very marginal in terms of production or remain untested and are not the dominant production model. No one knows whether these modes of production will expand or remain a niche market in the future. What also seems imminent, is there will be increased costs associated with the change that may only partly be offset by increased efficiency. Most alternatives for sexing are predicted to result in an extra-cost of 1–5 cents per egg for consumers. This makes investment

decisions difficult for farmers. However, it seems that some of the changes are inevitable, at least in European markets.

Ethics approval

This review did not require any animal handling or procedures.

Data and model availability statement

No data or model were generated as part of this study.

Author ORCIDs

Joël Gautron: <https://orcid.org/0000-0001-7800-0578>

Sophie Réhault-Godbert: <https://orcid.org/0000-0003-2800-3417>

Ian Dunn: <https://orcid.org/0000-0003-3630-0120>

Author contributions

Joël Gautron: Conceptualization, Investigation, Writing - Original Draft, Writing - Review & Editing

Sophie Réhault-Godbert: Investigation, Writing - Original Draft, Writing - Review & Editing

Ian C. Dunn: Investigation, Writing - Original Draft, Writing - Review & Editing

Teun Van de Braak: Investigation, Writing - Original Draft, Writing - Review & Editing

Declaration of interest

Sophie Réhault-Godbert, Joël Gautron and Ian Dunn declare that this review was written in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest. Teun van de Braak has a business conflict of interest as he is employed by the Institut de Sélection Animale B.V. a significant player in the global layer breeding industry. As this review is about a future prediction, there is very limited chance that ISA B.V. is affected by this specific review.

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