



HAL
open science

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

Joël Gautron, Sophie Réhault-Godbert, T.G.H. van de Braak, I.C. Dunn

► To cite this version:

Joël Gautron, Sophie Réhault-Godbert, T.G.H. van de Braak, I.C. Dunn. Review: What are the challenges facing the table egg industry in the next decades and what can be done to address them?. *Animal*, 2021, 15, 10.1016/j.animal.2021.100282 . hal-03283630

HAL Id: hal-03283630

<https://hal.inrae.fr/hal-03283630v1>

Submitted on 8 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

26 eggs per hens in a lifetime, reducing the number of replacement layers and improving
27 the economics and sustainability. It will be a challenge for scientists to optimise the
28 genetics and the production systems to maintain the health of these hens. A major
29 ethical issue for the egg industry is the culling of male day-old chicks of layer breeds
30 as the meat of the males cannot be easily marketed. Much research has and will be
31 devoted to alternatives. Another solution is elimination of male embryos prior to
32 hatching by *in ovo* sexing approaches. The race to find a sustainable solution to early
33 stage sex determination is on. Methods based on sex chromosomes, sexually
34 dimorphic compounds and spectral properties of eggs containing male or female
35 embryos, are being researched and are reviewed in this article. Other proposed
36 solutions include the use of dual-purpose strains, where the males are bred to
37 produce meat and the females to produce eggs. The dual-purpose strains are less
38 efficient and do not compete economically in the meat or egg market, however, as
39 consumer awareness increases viable markets are emerging. These priorities are the
40 response to economic, environmental, ethical and consumer pressures that are
41 already having a strong impact on the egg industry. They will continue to evolve in
42 the next decade and if supported by a strong research and development effort, a
43 more efficient and ethical egg laying industry should emerge.

44

45 **Keywords:**

46 Egg production, Hen housing, sustainable farming, dual purpose, in-ovo sexing

47

48 **Implications**

49 Consumers are increasingly aware of the production systems for laying hens and
50 have strong opinions on what is favorable for a hen's welfare. This has resulted in a
51 move towards non-cage housing systems with favorable and some less favorable

52 consequences. Recently, several countries have banned the culling of male day-old
53 layer chicks and alternatives are being developed. These pressures will strongly
54 impact the way eggs are produced and the economy of the egg industry. Adoption of
55 ethical systems is expected to accelerate in the world and considerable research
56 effort will be needed to optimize the new systems.

57

58 **Introduction**

59 Egg production is a good example of the major changes that have occurred in the
60 agricultural sector in response to changing social demands. There is currently strong
61 consumer pressure for the consumption of healthy, high-quality animal products that
62 take into account animal welfare and sustainability. The consideration of the ethical
63 dimension in this sector has resulted in many examples of major changes to the way
64 that eggs are produced, in order to respond to societal demands. The main changes
65 to the production system concern the gradual abandonment of cage-housing
66 systems, the demand to not kill male chicks and the lengthening of the production
67 period. The latter will result in fewer birds being slaughtered each year and fewer
68 hens being required throughout the whole production system, both in breeding and in
69 production, to produce the same quantity of eggs. As a result, this method of
70 production should have less negative impact in terms of the environment and use of
71 resources. This may balance some of the negative impacts of reduced efficiency
72 resulting from other adaptations discussed. All these developments and trends will
73 have a major impact on the poultry sector, and will shape table and fertilized egg
74 production in the coming years. The objective of this review was therefore, 1) to
75 describe the issues facing the egg production chain and how the industry may evolve
76 and 2) to examine existing scientific research to address these issues, indicating the

77 current state of the art in the production of eggs for human consumption and future
78 innovations in the industry. It will finally give examples of initiatives towards more
79 ethical animal husbandry.

80

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

82 Current production methods in Europe are in line with the five freedoms of animal
83 welfare: freedom from hunger and thirst, from discomfort, from pain injury or disease,
84 to express normal behaviour and freedom from fear and distress (EFSA 2005).
85 However, among the four authorised modes of production (organic, free range, barn
86 and enriched cages), there is a growing consumer mistrust of eggs produced by
87 enriched-caged hens even though the traditional barren cage was banned since
88 01/01/2012 (Council Directive 1999/74/EC). The enriched or furnished cages that
89 replaced these classical cages, favor the expression of more natural behaviours by
90 the hens but this positive change has not been readily understood by consumers.
91 Furthermore, these changes have not solved all behavioural issues of cages as
92 highlighted by the EFSA (2005) report on laying hens' welfare. Indeed, litter supply in
93 furnished cages is still a major issue, making the hens unable to show normal
94 foraging and dustbathing behaviour (EFSA 2005). This directive has resulted in a
95 strong segmentation of the markets. In 1996, non-cage systems accounted for 8% of
96 the EU laying hen population, 30% in 2009, 46% in 2017 and 51% in 2019 (80% of
97 laying hens were in cages in 2003, 49% in 2019) (ITAVI 2019) (figure 1). The
98 proportion of hens reared in non-cage systems is currently increasing sharply,
99 although it remains very heterogeneous in Europe (from less than 10% in Spain and
100 Poland to more than 85% in the Netherlands, Germany and Austria) (Figure 1).

101 Like many of Europe's member states, French production has become increasingly
102 diversified since the late 1990s. The number of laying hens in non-cage systems was
103 19% in 2008 and has reached 46% in 2019 (Figure 1) (ITAVI 2019). In contrast, the
104 egg consumption by distribution channels did not change during the last decade
105 (Figure 2), with about 60% of eggs consumed as shelled eggs and about 40 % as
106 egg products in France (ITAVI 2019). The supermarkets and hypermarkets in France,
107 attentive to campaigns from non-governmental organizations on the welfare of laying
108 hens, have announced an end to the marketing of eggs from cage systems within the
109 next 2-5 years (Table 1). On 18th February 2018, the French Agriculture Minister
110 Stéphane Travert confirmed that the government would work on a ban of table eggs
111 from cage systems by 2022 for whole egg sales (Tassard 2018). The French
112 mediator for agricultural trade relations, in his 2017 report, points out that "the refusal
113 to market eggs produced by caged hens ... is on the way to become the norm
114 regardless of any regulatory developments".

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

121 Despite the fact that Great Britain has left the European Community, a similar trend
122 can be observed. There are four recognised forms of laying hen production following
123 EU definitions, three non-cage systems and the furnished cage. The three non-cage
124 production systems are Free-Range, organic and Barn egg production. Organic egg
125 production must also be free range, but follows different standards notably in terms of

126 stocking density, the origin of the animal feed, medication and beak trimming; Barn
127 egg production (code 2), which is similar to free range in terms of housing, but with
128 the notable exception that the hens do not have access to the outdoors. In the UK in
129 2019, there was around 2%, 3%, 53% and 42% of eggs packed from barn, organic,
130 free range and enriched cages systems, respectively (DEFRA, 2020). Both organic
131 and barn systems are increasing, but from a low starting point. It is believed that, as
132 retailers commit to going cage free, barn production will be increased to give a low-
133 price alternative to free range eggs, although with the lack of consumer
134 understanding of barn production that may be a challenge (Porter 2020). Indeed, as
135 in France, some retailers have already said that they will not sell barn eggs (White
136 2019). Much of the egg production in the UK (~90%) is part of the Lion Quality Code
137 of Practice that has enhanced requirements for welfare in terms of stocking densities
138 for free range hens, nest box space, lighting and the handling of end-of-lay hens. It
139 also focuses heavily on egg hygiene and microbiological quality, which was the initial
140 impetus for its creation. This Code of Practice can be a major driver for change if its
141 promoters choose to adopt new standards in terms of welfare. For the free-range
142 sector, research will continue on how to design the indoor environment to better allow
143 natural behaviour but reduce damage from collisions (Stratmann et al. 2015).
144 Initiatives to tackle the problems of injurious pecking are also underway (Nicol et al.
145 2013). Another important issue is the way to reduce poultry pandemics and others
146 diseases that can be transmitted to humans. Breeding systems with outdoor access
147 increase the risk of exposure to wild animals including other birds. The recent
148 COVID-19 pandemic, where wild animals are suspected to be the reservoir of virus,
149 has reinforced this concern, although there is no evidence of birds being involved in
150 the transmission in this case. Poultry pandemics (like Avian Influenza), raise the

151 question of the relocation of bird farms producing eggs from high-risk areas to areas
152 that are less prone for migrating birds carrying Avian Influenza (ANSES 2021).

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

162 **Extending the production period**

163 Currently, laying hens start laying at around 18 weeks of age and peak laying is
164 around 25 weeks of age. Eggs are well valued from the moment that the weight of
165 the egg has reached a threshold value (e.g. 53 g in EU and 49.3 g in USA). The
166 majority of the flocks are depopulated when the laying rate is reducing and, most
167 importantly the incidence of downgraded eggs increases to unprofitable levels. At
168 this point there are still many saleable eggs but the high variability in egg quality
169 means a threshold is reached where less than about 75% of the eggs are
170 marketable, although the threshold can vary depending on prevailing economics. In
171 2016, this often occurred at around 72 weeks in free-range flocks in the UK, i.e. after
172 one year of production (Bain et al. 2016), but already a Belgian study indicated that
173 the end of production could vary from 74 to 92 weeks, with an egg laying rate of 79%
174 (Molnar et al. 2016). Reproduction in birds is controlled by the hypothalamus
175 according to different environmental and endocrine stimuli. As the hen ages,

176 environmental stimuli that were previously stimulatory no longer have that effect and
177 potential inhibitory factors may increase, which results in reduced hypothalamic
178 activity of the cells driving reproduction (Dunn et al. 2009). The consequence is a
179 loss of weight and functionality of the oviduct, which leads to an increase in the
180 number of days of rest (without laying) and defective eggs (Solomon 2002). However,
181 selection for egg production has successfully reduced the effects of age on the
182 reproductive system, with the result of reducing the amount of variation in egg
183 production rate towards the end of the traditional laying period. Essentially the
184 majority of hens were maintaining production of one egg a day. To perform selection
185 and improve the sustainability of the industry, poultry breeders have extended the
186 laying periods of their pure line birds to make variation visible (pers. comm. Teun van
187 de Braak). It is now possible to select those individuals that are observed in older
188 flocks that can maintain high oviposition rates with good shell quality (Molnar et al.
189 2016). Accessing this variation has allowed an increase in the persistence of egg
190 production and maintenance of egg quality at an advanced age (Bain et al. 2016).
191 Breeders indicated 10 years ago, that they wanted to select strains of laying hens
192 capable of laying sustainability up to 100 weeks of age, for a total production of
193 almost 500 eggs per birds by 2020 (Bain et al. 2016), which has been achieved. Bain
194 et al (2016) indicate that an additional production of 25 eggs per hen could potentially
195 reduce the laying hen flock in Great Britain by 2.5 million hens. This cumulative effect
196 is obtained as shown in figure 3, due to the pyramidal structure of production in the
197 sector. An increase of 10 weeks of production would preserve 1 g of potentially
198 polluting nitrogen per dozen eggs produced (Molnar et al. 2016).

199 However, any improvement in egg-laying persistence must be achieved with
200 consistent egg quality. Egg weight increases by 70 mg per week between 60 and 80

201 weeks of age (Molnar et al. 2016). While the other egg constituents remain constant,
202 there is a decrease in shell thickness (-0.23 μm per week) and a decrease in
203 breaking strength with increasing age of the hen. Haugh units are an indicator of egg
204 white quality. They measure the height of the egg white after breaking the egg, which
205 is related to the viscosity of the colloidal gel of the egg white. This gel-like structure
206 limits bacterial proliferation and migration towards the yolk and is essential for
207 maintaining the hygienic quality of the egg. However, Haugh units reduce over the
208 course of the production period (Whitehead 2004, Bain et al. 2016, Molnar et al.
209 2016). There may be other consequences of keeping hens for longer, like moulting
210 that should result in an absence of eggs during several weeks and an economic loss
211 because hens are still fed while they do not produce eggs. It is essential to maintain
212 good bone quality in the laying hen, especially when the hen is getting older. A laying
213 hen requires between 2 and 2.5 g of calcium daily for the production of an eggshell.
214 About 2/3 of this calcium is provided directly by the feed, the remaining 1/3 comes
215 from storage by demineralisation of the medullary bone. The calcium from the
216 medullary bone is necessary in the second part of the shell mineralisation process.
217 This occurs at night, when the hen has no access to feed although birds do store
218 food in the crop for several hours. Medullary bone is capable of rapid absorption and
219 renewal (Whitehead 2004), which can be optimised by dietary calcium sources
220 (content, quality and particle size). Even with a perfectly controlled diet, bone
221 demineralization is a natural phenomenon that can also affect the structural bone,
222 ultimately leading to osteoporosis. This pathology, which can be prevalent in old
223 hens, leads to bone fragility and keel bone fractures that severely impact the welfare
224 of laying hens (Armstrong et al. 2020). Bone quality and fractures in these hens are
225 currently a major issue in the table egg sector (Sandilands 2011). It is easy to

226 observe hens in a flock with very fragile or very strong eggs and the same applies to
227 bones. However, there is relatively little knowledge about whether hens with bone
228 defects are those that lay eggs with fragile or strong shells.

229 In studies where genetic correlations between egg quality and bone quality have
230 been examined, there is little evidence that the two traits are linked, with only the keel
231 bone density in one line being significantly correlated with egg breaking strength
232 (Dunn et al. 2021). Whilst there were no significant associations for the tibia or
233 humerus strength or density with egg quality, there was a genetic correlation with
234 onset of lay and early egg number, but only in one line. Although not significant, there
235 was a suggestion that genetic loci explaining variation in egg quality might be present
236 at the same loci as one for bone quality (Dunn et al. 2020) but in a related study,
237 lines of hen successfully selected to have differences in bone strength did not have
238 differences in egg quality (Fleming et al. 2006). This is not to say that egg laying is
239 not related to issues of bone quality in laying hens, if hens do not lay eggs at all they
240 have better bone quality (Eusemann et al. 2020). However, the quality of the egg or
241 the persistency of lay do not seem to be critical components in determining bone
242 quality of a hen. Rather, it is possible that the onset of lay, which is intrinsically
243 related to the body weight of the hen and the genetic factors that directly affect bone
244 quality, appear to be most important (Dunn et al. 2020). Research is ongoing to
245 resolve some of the questions on the relationship between persistent egg production
246 and bone quality and other welfare issues, and how the physiology, nutrition and
247 welfare of the older hen will be affected. Programmes conducted in partnership with
248 researchers in the sector are underway to ensure best practice which will help
249 support the longer laying period (Toscano et al. 2020).

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

273 The accelerated change in housing systems and the prohibitions on management
274 practices such as beak trimming in several EU countries (Austria, Germany,

275 Netherlands, etc.) is the most recent manifestation of a trend that has resulted in
276 changes in selection criteria for laying hens. The traditional approach that was merely
277 focused on the economic aspects of egg production has shifted. Today, breeding
278 programs and selection indexes include more poultry health and welfare traits than
279 ever before. Next to relatively well investigated traits such as livability, bone strength,
280 disease resistance and feather cover, new traits related to behavior, such as negative
281 social interactions between birds, and behavior in cage free housing systems have
282 been adopted by the breeding companies (Brinker et al. 2018).

283 Not only have health and welfare received increased attention, breeding companies
284 are showing their commitment and making their contribution to set the standard for
285 sustainable egg production. The environmental impact per kilogram of eggs produced
286 has significantly decreased during the past decades. The review by Pelletier et al
287 (2014) showed a comparison of the environmental footprint of the egg industry in the
288 United States in 1960 and 2010. They showed an enormous reduction in the
289 environmental footprint per kilogram of eggs produced: the environmental footprint for
290 2010 was 65% lower in acidifying emissions, 71% lower in eutrophying emissions,
291 71% lower in greenhouse gas emissions, and 31% lower in cumulative energy
292 demand compared to 1960. Despite the 30% higher table egg production in 2010, the
293 total environmental footprint was significantly lower compared to 1960. Pelletier et al
294 (2014) estimated that 28 to 43% of this improvement in lower environmental footprint
295 could be attributed to the improvements in the performance of the birds. Abín et al
296 (2018) used a Spanish case study for an environmental assessment of intensive egg
297 production. Their study showed that after the production of the hen feed, the
298 purchase of new laying hens to replace the old flock contributed most to the harmful
299 environmental impact of intensive egg production. By extending the productive

300 lifetime of the laying hens in the breeding pyramid and not just at the production
301 level, the environmental impact could be further reduced, as fewer replacements
302 flocks are needed.

303

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

305 A major issue that has raised ethical concerns within the poultry sector is the fate of
306 male chicks from laying strains. Although chickens can be used for both meat
307 production and egg production, there is a trade off between the two traits (Wolc et al.
308 2012, Giersberg and Kemper 2018). As a result, there has been specialised breeding
309 of chickens for either egg production or for meat production (Leenstra et al. 2016,
310 Sakomura et al. 2019). These strains of hens are very efficient at either only egg
311 production or only meat production, but do not compete economically in each other's
312 market. Hens selected to produce eggs for human consumption have a low body
313 weight of around 1.7kg that is reached at around 20 weeks of age (sexual maturity),
314 where they start to convert their feed into egg nutrients very efficiently (Ahammed et
315 al. 2014, Bain et al. 2016). Hens at the end of lay are used for human consumption,
316 but the males have limited added-value as they grow slowly and the live weight and
317 meat yield on the carcass do not meet the meat quality/yield criteria to be marketed
318 (Giersberg and Kemper 2018). As only the females lay eggs, half of the hatched
319 chicks are therefore non-marketable males. Day-old chicks are sexed at hatch using
320 cloacal or vent sexing, or via sex linked feather features (feather color or covert
321 length). Only a limited number of males are bred to allow the reproduction of future
322 offspring. As a result, billions of male chicks have no commercial value (Weissmann
323 et al. 2013, Galli et al. 2016, Giersberg and Kemper 2018), and are culled rapidly
324 after hatch by asphyxiation or maceration. These authorized practices elicit legitimate

325 questions in terms of animal welfare and the ethics of hatching eggs without an
326 agricultural output. The joint announcement by the French and German ministers to
327 ban the culling of one-day old male chicks by the end of 2021 has given some
328 urgency to finding solutions and several methods are close to market while some of
329 them are being used by hatcheries. The three main alternative approaches to the
330 practice of killing male chicks are; 1) to identify mechanisms that can, ideally
331 completely, imbalance the sex ratio in favor of females, 2) to develop tools that would
332 allow the determination of the sex of the embryo *in ovo* prior to hatch, 3) to develop
333 dual-purpose strains where female chicks would be reared as future egg-laying hens
334 and male chicks for meat. Ultimately the systems developed will also need to be
335 acceptable to consumers.

336 ***Skewing primary sex-ratio or hatching sex-ratio***

337 It is well-known that the primary avian sex-ratio can be affected by environmental
338 factors such as diet, physiology, hormonal status and conditions as well as the
339 genetic background. However, the effects are relatively modest and can change over
340 the lifetime of a hen (Klein and Grossmann, 2008). Thus, it is quite difficult to know
341 how sex ratios could be reliably altered by poultry breeders using current knowledge
342 without gene editing or transgenesis. Research into the basic mechanisms of sex
343 determination in birds certainly continues to be warranted. Another level of
344 complexity is that the primary sex-ratio of fertilized eggs does not necessarily reflect
345 the sex ratio at hatch, which suggests that a sex-dependant selection during
346 incubation depending on the genetic background and age of laying hens may occur
347 (Klein and Grossmann 2008). The Israeli company SOOS (Soos 2020) used this
348 concept to develop an incubation system combining different incubation parameters
349 that seem to reverse males into female chicks. According to their claims, resulting

350 animals can efficiently lay eggs at sexual maturity. However, to date, scientific
351 information about the underlying mechanisms and short/long term impacts on
352 feminized chickens are missing and need to be addressed before this approach can
353 be validated by the scientific community and, ultimately, authorities and society. In
354 the light of this, it seems necessary to develop other strategies until realistic methods
355 to skew the primary sex ratio are available.

356

357 ***Sexing eggs***

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

374 *In ovo* sexing methods are based on the initial postulate that male embryos and
375 female embryos exhibit specific features (anatomical, physiological, molecular and
376 genetic) that should allow for the discrimination between sexes during incubation of
377 fertilized eggs. Some of these methods are universal while others have been
378 specifically designed for selected genotypes. A review of the various *in ovo* sexing
379 techniques that were close to market was published in 2018 (Hein 2018). Of these,
380 only two are commercialized and in use by the poultry sector (Seleggt 2019, Plantegg
381 2020). Some require the sampling of embryonic cells or embryo-derived cells while
382 others rely on the sampling of extra-embryonic fluids such as the allantoic fluid.

383 *Dimorphic chromosomes*

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

392 Another approach is based on the analysis of the DNA content of the embryonic
393 cells, considering that the Z chromosome has 200% more DNA content than the W
394 chromosome (Mendonça et al. 2010) and male cells containing the ZZ chromosomes
395 are about 2% bigger than female ZW containing cells. Using infrared spectroscopic
396 imaging on fertilized eggs prior to incubation, Steiner et al. (2011) corroborated that
397 male blastoderm cells have a higher DNA content than female blastoderm (Steiner et
398 al. 2011). Using this method, gender determination is possible at very early stages, is

399 rapid (few seconds) and accurate. However, it requires germinal disc sampling with
400 possible long-term effect on the development of the embryo or the chicks after
401 hatching. Some other authors also use the length of the sex chromosomes to
402 develop *in ovo* sexing methods based on Raman spectroscopy (Galli et al. 2016,
403 Galli et al. 2017, Galli et al. 2018). These authors focused on bloods cells, all of
404 which are nucleated in birds, and analyzed the spectra of blood fluorescence.
405 Differentiation between males and females was shown to be 90% from the 4th day of
406 incubation. According to the authors, this technique has no visible effect on the
407 hatched chick, but decreased the hatchability by about 10%. The invasiveness was
408 further reduced by keeping the eggshell membrane intact (Galli et al. 2018) leading
409 to the development of a prototype (Muller-Niegsch 2017). However, there is no
410 current information on the state of progress in terms of commercialization. Similarly,
411 the AAT group developed a Raman-spectroscopic method that would allow for sexing
412 eggs after 4 days of incubation. This approach remains semi-invasive as a small
413 piece of the eggshell needs to be removed to access the embryo and the
414 surrounding yolk sac vascularization, prior to Raman spectroscopy measurements
415 (AAT 2020).

416 *Dimorphic compounds*

417 Besides chromosome-based strategies, techniques that use reported differences in
418 the hormonal and metabolic status between male and female embryos have been
419 proposed. In 2013, Weissman et al., published that the allantoic fluid from female
420 embryos displayed significantly higher estrone sulphate (female hormone) levels than
421 males and that this difference was detectable as soon as 9 days of incubation
422 (Weissmann et al. 2013). The discovery led to the development of a prototype by
423 SELEGGT with a sexing accuracy of 97% (Seleggt 2019). The eggs resulting from

424 this approach are called “respeggt” eggs and were successfully introduced in 2018 in
425 Germany. They are available in boxes of six at all 5 500 REWE and PENNY stores.
426 For all these techniques, sampling embryonic-derived cells or extraembryonic fluids
427 implies invasive or semi-invasive technique (egg opening/eggshell drilling) that would
428 increase the risk of low viability/impaired hatchability afterwards. A non-invasive
429 technique would likely supplant all of the above approaches.

430 Besides hormones, it has been shown that glucose, choline and some amino-acids
431 (valine) are more concentrated in allantoic fluid from females (Bruins and Stutterheim
432 2014) while the same authors also found that butylated hydroxytoluene is a
433 particularly relevant volatile dimorphic biomarker (Bruins and Stutterheim 2017).
434 Such dimorphic volatile compounds are promising as they can diffuse through the
435 eggshell pores and may be detectable at the surface of the eggshell (Webster et al.
436 2015, Costanzo et al. 2016, Knepper et al. 2019). Taken together, these data were
437 probably the initial step for the development of a prototype by In Ovo, a spin-off of
438 Leiden University (InOvo 2020). Since 2017, they have been developing their current
439 Alpha prototype, realizing a throughput of 1 800 eggs per hour with 95% accuracy. In
440 the InOvotive project that started in June 2020 (CORDIS 2020), they plan to scale
441 this prototype to reach more than 10 000 eggs per hour to meet hatchery needs.

442 *Spectral dimorphism of the whole egg*

443 The most promising non-invasive technologies developed to date are based on
444 spectroscopic methods applied to the whole egg, such as hyperspectral imaging
445 (Canadian Hypereye company and Agri Advanced Technologies (AAT, Germany))
446 and the combination of spectroscopy and biosensors (SOO project, French company
447 Tronico and the French National Centre for Scientific research). The technology of
448 Hypereye uses hyper-spectral technology to acquire a specific signature through

449 mathematical algorithms to determine the gender of the embryo from the day of lay
450 onwards. Commercialization date and progress are not known. Several
451 announcements had been made and a prototype was expected for 2018, with a
452 throughput of 50 000 eggs per hour. However, since then, there is no public
453 information about their progress in this field. A similar strategy has been developed
454 by AAT technology (AAT 2020). Using a specific genotype (Lohmann Tierzucht
455 GmbH) where female chicks have brown down feathers and the males have yellow
456 down feathers, Gohler et al (2017) describe a non-destructive optical method for sex
457 determination. This hyperspectral method has been shown to reach 97% efficiency
458 between 11 and 14 days of embryonic development and is expected to be
459 implemented to allow the sexing of eggs at earlier stages (7th day of incubation).
460 According to the website (AAT 2020), the technique is 95% accurate and 20 000
461 eggs can be tested per hour per machine. Hyperspectral measurement technology
462 has been improved for large-scale practical use and French egg suppliers have
463 started to use this technology since the beginning of 2020.

464 In 2017, French Agriculture Minister Stéphane Le Foll granted Project SOO (“Sexage
465 des Oeufs d’Oiseaux” in French or “sexing avian eggs”) with 4.3 million euros to
466 finance the development of new *in ovo* sexing methods. This project, with the French
467 Tronico company and the French National Centre for Scientific research, focuses on
468 a method that combines spectroscopy (response to a light pulse) and the use of
469 biosensors, that it is claimed will be 90% reliable in sexing eggs as soon as 9 days of
470 incubation. The prototype was initially expected at the end of 2019. Although the
471 current technologies and prototypes need to be improved, it has to be mentioned that
472 ovosexing provides another advantage as compared with current manual methods for
473 sexing as it will avoid the manipulation of chicks at hatch and thus will limit additional

474 stress for animals. They may also be transposed to other bird species of industrial
475 interest, such as foie-gras production where only males are reared (female ducks are
476 culled at hatch as their liver is too small and contains many veins).

477 *Sex manipulation by genome editing*

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

497

498

Dual purpose breeds and/or growing layer male chicks

499 The development or revival of dual-purpose strains, with females producing eggs and
500 males producing enough quality meat to be marketed, is currently being examined
501 and will likely form a segment of the future market. If killing male day-old chicks is not
502 seen as an ethically defensible position, then the use of dual-purpose chickens
503 seems a straightforward proposition. Even if male chicks are killed humanely and
504 they are consumed, albeit for pet and zoo animals, the industry struggles with the
505 ethics of producing animals that do not live a full life (Bruijnjs et al., 2015). The dual-
506 purpose chicken allows the male to be reared for meat production, growing faster
507 with more saleable meat than the male of chickens bred purely for laying. Currently in
508 the available laying strains the growth of males does not meet the requirements for
509 the production of quality meat at a competitive cost (Koenig et al. 2012, Gremmen et
510 al. 2018). However, even if the resulting meat is comparable with meat from broilers,
511 the production remains less competitive in economic terms, but also in terms of
512 resources and environmental pollution (Koenig et al. 2012). A number of approaches
513 have been adopted to produce dual-purpose birds, from the use of lines of chicken
514 that have served traditional markets to specific breeding programmes, which produce
515 chickens where the males can almost compete with slow growing breeds of broiler
516 chicken, although still often with 10% less meat product. In many cases, these are a
517 cross between broiler and layer parent stock. In general, although the males take
518 longer to get to a reasonable slaughter weight than a slow growing broiler, meat
519 quality and acceptance seem to compare well with broiler meat (Mueller et al. 2018).
520 The amount of leg meat is larger in dual purpose breeds but this can be favourable in
521 some markets. The main disadvantage of dual-purpose breeds is the lower yield in
522 breast meat, therefore dual-purpose breeds are often sold as a complete carcass.

523 The same acceptance is true for the eggs from the females, which compare
524 favourably in some studies with the possible exception that brown egg dual-purpose
525 breeds produce eggs that are lighter than from a pure brown egg layer. However,
526 there are reports of poorer egg quality in some lines, in both external (shell strength,
527 shell color) and internal (Haugh units, blood and meat spots) parameters. There may
528 be other benefits of the dual-purpose bird as there is some evidence that dual-
529 purpose chickens suffer less from issues such as mortality from injurious pecking.

530 The real issue comes from the economics and environmental impact of the dual-
531 purpose breeds; indeed, it has been suggested that the current system that has
532 evolved has produced a 'lock in' (Bruijnis et al. 2015). Essentially the current
533 production system has developed to be so efficient with a comparatively small
534 environmental impact that it is impossible for any competing systems to be
535 established, either ethically or indeed economically. The biological and economic
536 consequences for different options of dual-purpose chickens have been examined in
537 several studies (Leenstra et al. 2011). This demonstrated that dual purpose chickens
538 might serve as a niche market, but a total shift to dual purpose chickens in order to
539 solve the problem of killing day-old males would not be realistic when looking at the
540 environmental burden and the economics. Alternative systems such as dual-purpose
541 chickens have higher environmental costs, taking more resources to produce the
542 same amount of food, with ratios for the conversion of feed to meat lying above four,
543 while for broilers it can be around 1.6 (Giersberg and Kemper 2018). Egg production
544 from dual-purpose hens also has greater environmental impact as they lay typically
545 around 50 fewer eggs in a year but consume similar or more food to do so. It is
546 difficult to argue that the system is ethically superior compared to current strains if
547 negative environmental impacts increase. It is argued that the 'Responsible

548 Innovation' approach, which balances economic, socio-cultural and environmental
549 aspects of any new system, would need to be promoted to shift production and get
550 consumers to accept on a larger scale the products from dual purpose rearing
551 systems or, indeed, any alternative system that improves the ethical dimensions of
552 production (Bruijn et al. 2015). In the absence of legislative changes, the use of
553 dual-purpose hens will likely remain an expanding but niche product requiring strong
554 marketing (Busse et al. 2019).

555 A German dual-purpose initiative, which includes the rearing of day-old layer male
556 chicks is again working in a niche market, where an increasing number of day-old
557 males are kept for meat production. In Germany, they have introduced this as the
558 Bruderhahn (Brother cockerel) initiative (BID 2020). Eggs are sold for a premium
559 price in order to compensate for potential economic losses when growing the males.
560 Because of their inefficient feed conversion, and different characteristics (they differ
561 in breast meat yield, taste and tenderness compared to conventional broilers), the
562 cost of production is higher with a smaller market. It is forecast that the entire organic
563 egg market will adopt the principle of growing the male layer chicks, as they do not
564 see *in-ovo* sexing as an acceptable solution, as the male embryos are still discarded.
565 A specific example of a system utilising 'Les Bleues' chicken has been used in the 'ei
566 care' project in north eastern Germany (eiCare). Both males and females are raised
567 for meat and eggs, respectively and are marketed with the 'ei care' branding to
568 organic shops and supermarkets. The project is a partner in a research program for
569 sustainable development and currently has 4 farms producing eggs and meat sold
570 relatively locally.

571 There is an urgent need for research and development of new dual-purpose strains
572 by crossing selected genotypes to optimize their productivity under realistic farm

573 conditions as well as optimising the quality of derived products (eggs and meat). It is
574 also necessary to understand the behaviour of these new strains in different farming
575 systems and under different environmental conditions, all of which should lead
576 ultimately to the best scenario/trade-off in terms of health for the hen and of costs for
577 farmers. To complement this, there is also a need to understand consumer attitudes
578 to the proposed systems. It is also important to consider that some consumers prefer
579 brown eggs while others prefer white eggs (cultural habits). It is forecast that we will
580 need to have several crosses as options, depending on countries, to meet peoples
581 cultural requirements.

582

583 **Towards more ethical animal husbandry?**

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

595 This method of production generates a number of zootechnical constraints such as
596 the control of moulting. Indeed, a hen after 15-16 months of production undergoes a

597 moult that is characterised by a regeneration of the reproductive tissues. One month
598 after the start of moulting, the hen will again lay good quality eggs, but the laying time
599 will rapidly decrease as the cycles progress. This moult may be caused by a
600 decrease in light and energy rationing strategies in the feed. Poulehouse objective is
601 to achieve 3 moulting periods separated by production periods of 9-12 months. At the
602 moment, the oldest flock is 3-4 years old. Health management of older flocks will also
603 be a challenge when the flocks will be at the end of their life.

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

614 Another initiative can be found in the Netherlands, which is the Kipster farm concept
615 (Kipster). In this concept it is all about sustainability, and they try to include all
616 elements with respect to sustainability, not only the ethics involved, but also with a
617 large focus on the impact of farming on the environment. At the Kipster farm, they
618 focus on closed loop farming, and they try to limit the waste generated during the
619 production process. Together with LIDL supermarkets, they have developed new
620 products based on the meat coming from the processed spent hens and layer males.
621 This allows them to create value out of the products that would otherwise be

622 considered as waste/products with low economic value. The eggs are sold with a
623 premium price, and no involvement of an egg packing station; in this way the egg
624 producer can benefit more from exploiting this innovative concept. High standards of
625 animal welfare are combined with extremely transparent farming. This to reduce the
626 growing gap between producers and consumers, and educate the consumers on the
627 origin of their food. At Kipster they have deliberately chosen white egg layers, as they
628 have a lower ecological footprint compared to the brown egg layer (Mollenhorst and
629 Haas 2019). The main reason for this lower ecological footprint in white egg layers is
630 their ability to be kept for longer production periods compared to the brown egg layer,
631 resulting in more saleable eggs produced per hen housed, higher total egg mass
632 produced and better feed efficiency. That the popularity of the white egg layers is on
633 the rise can be clearly seen in the Netherlands, where the brown to white egg layer
634 ratio went down from 60 - 40 in 2012, to 35 - 65 in 2018 (IEC 2018).

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

644

645 **Conclusion**

646 In the next decade, the egg sector will have to deal with the evolution of the systems
647 of egg production in cages vs non-cages to consider welfare and sustainability. We
648 have described recent developments in science, technology and production
649 strategies that are intended to tackle ethical issues in layer hen systems. They
650 included the extension of the laying period and the development of more recent
651 production methods that try to be ethical. Another important challenge for the sector
652 is the use of alternatives to the culling of male day-old chicks of layer lines. The
653 development of genotypes to obtain dual-purpose strains is in progress, but the
654 current genotypes do not yet meet the requirement for the production of quality meat
655 and egg. Introducing *in ovo* sexing techniques in hatcheries implies additional costs
656 for producers who will have to reorganize logistics, but also for consumers who will
657 pay more for eggs. Because of the drive to achieve the goal of all-female chicks, in
658 addition to properly researched proposals rooted in biology, the field has attracted
659 some unlikely 'snake oil' solutions which industry and funders should be aware of. To
660 date, most technologies are not efficient to determine the sex of the embryo at the
661 day of lay. They require 1) the removal of eggs from incubators for sexing, 2) re-
662 incubation of female eggs potentially increasing the risk of embryonic mortality 3) the
663 elimination and new valorization of male eggs as high-quality feed (SELEGGT option)
664 or other uses, and the management of male chicks if the method is not 100% reliable.
665 In the future, the accuracy of methods may be greatly improved by combining several
666 dimorphic features to get to the level of accuracy desired of near 100% and by using
667 artificial intelligence tools to integrate data. However, it is likely that if the method
668 selected is not 100% accurate, chicks will have to be re-examined by sexers at hatch
669 to avoid the introduction of males in female flocks. Already *in ovo* immunisation is
670 performed routinely, which suggests it is possible to do at scale without detriment. In

671 parallel, several strategies based on sex manipulation (transgenesis, genome-
672 editing) have shown a high potential and efficiency but the ethical and social
673 acceptability of such approaches remains very poor. Although consumers are willing
674 to pay more for eggs from non-cage systems in many parts of Europe, the more
675 widespread adoption of the systems described in this review will increase the cost of
676 eggs and egg products.

677 It should be noted that the solutions are still very marginal in terms of production or
678 remain untested and are not the dominant production model. No one knows whether
679 these modes of production will expand or remain a niche market in the future. What
680 also seems imminent, is there will be increased costs associated with the change that
681 may only partly be offset by increased efficiency. Most alternatives for sexing are
682 predicted to result in an extra-cost of 1 to 5 cents per egg for consumers. This makes
683 investment decisions difficult for farmers. However, it seems that some of the
684 changes are inevitable, at least in European markets.

685

686 **Ethics approval**

687 This review did not require any animal handling or procedures.

688

689 **Data and model availability statement**

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

691

692 **Author ORCIDs**

693 Joël Gautron: 0000-0001-7800-0578

694 Sophie Réhault-Godbert: 0000-0003-2800-3417

695 Ian Dunn: 0000-0003-3630-0120

696 Teun Van De Braak : None

697

698 **Author contributions**

699 Joël GAUTRON: Conceptualization, Investigation, Writing - Original Draft, Writing -
700 Review & Editing

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

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

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

705

706 **Declaration of interest**

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

714

715 **Acknowledgements**

716 A part of this bibliographic review was realized within the framework of a scientific
717 and collective expertise (ESCo INRAe) on the quality of food of animal origin
718 according to production and processing conditions. The bibliographic review related
719 to “Alternatives to the culling of one-day-old male chicks” was realized under the
720 framework of the Poultry and Pig Low-input and Organic production systems' Welfare
721 (PPILOW) program. Part of this review was written in close cooperation with Institut
722 de Sélection Animale B.V., a Hendrix Genetics Company.

723

724 **Financial support statement**

725 JG and SRG were supported by the European Commission (PPILOW project, grant
726 Number 816172). ICD and the Roslin Institute are funded by a BBSRC Institute
727 strategic program (grant BB/P013759/1). ICD is also funded by the Foundation for
728 Food and Agricultural Research (grant ID 550396) ICD and TVB are part of a COST
729 action CA15224 Keel Bone Damage.

730

731 **References**

732 AAT, 2020. In ovo sex determination of layer chicks in the egg. Retrieved on 05
733 March 2021 from <https://www.agri-at.com/en/products/in-ovo-sex-determination>.

734 Abin, R., Laca, A., Laca, A. and Diaz, M., 2018. Environmental assesment of
735 intensive egg production: A Spanish case study. Journal of Cleaner Production 179:
736 160-168.

737 Ahammed, M., Chae, B. J., Lohakare, J., Keohavong, B., Lee, M. H., Lee, S. J., Kim,
738 D. M., Lee, J. Y. and Ohh, S. J., 2014. Comparison of Aviary, Barn and Conventional
739 Cage Raising of Chickens on Laying Performance and Egg Quality. Asian-
740 Australasian Journal of Animal Sciences 27: 1196-1203.

741 Anderson, K. E., Havenstein, G. B., Jenkins, P. K. and Osborne, J., 2013. Changes
742 in commercial laying stock performance, 1958-2011: thirty-seven flocks of the North
743 Carolina random sample and subsequent layer performance and management tests.
744 Worlds Poultry Science Journal 69: 489-513.

745 ANSES, 2021. L'influenza aviaire en 6 questions. Retrieved on 05 March 2021 from
746 <https://www.anses.fr/fr/content/linfluenza-aviaire-en-6-questions>.

747 Armstrong, E. A., Rufener, C., Toscano, M. J., Eastham, J. E., Guy, J. H.,
748 Sandilands, V., Boswell, T. and Smulders, T. V., 2020. Keel bone fractures induce a
749 depressive-like state in laying hens. Scientific Reports 10, 3007

750 Bain, M. M., Nys, Y. and Dunn, I. C., 2016. Increasing persistency in lay and
751 stabilising egg quality in longer laying cycles. What are the challenges? British
752 Poultry Science 57: 330-338.

753 Beck, M., 2019. MEG - Marktbilanz Eier und Geflügel 2019. Verlag Eugen Ulmer,
754 Stuttgart, Germany.

755 BID, 2020. Huhn, Hahn und Ei: Was jetzt zu tun ist. Retrieved 15 January 21, from
756 <https://www.bruderhahn.de/>.

757 Brinker, T., Bijma, P., Vereijken, A. and Ellen, E. D., 2018. The genetic architecture of
758 socially-affected traits: a GWAS for direct and indirect genetic effects on survival time
759 in laying hens showing cannibalism. Genetics Selection Evolution 50: 38.

760 Bruijnis, M. R. N., Blok, V., Stassen, E. N. and Gremmen, H. G. J., 2015. Moral “lock-
761 in” in responsible innovation: The ethical and social aspects of killing day-old chicks
762 and its alternatives. *Journal of Agricultural & Environmental Ethics* 28: 939-960.

763 Bruins, W. S. and Stutterheim, W. M., 2014. Gender, viability and/or developmental
764 stage determination of avian embryos in ovo (NL). Patent WO 2014021715A2/EP
765 2880440 B1 20181031 (EN).

766 Bruins, W. S. and Stutterheim, W. M., 2017. Method and system for the non-
767 destructive in ovo determination of fowl gender (NL). Patent WO 2017/204636 A3.

768 Busse, M., Kernecker, M. L., Zscheischler, J., Zoll, F. and Siebert, R., 2019. Ethical
769 Concerns in Poultry Production: A German Consumer Survey About Dual Purpose
770 Chickens. *Journal of Agricultural & Environmental Ethics* 32: 905-925.

771 Clinton, M., 1994. A rapid protocol for sexing chick embryos (*Gallus g. domesticus*).
772 *Animal Genetics* 25: 361-362.

773 CORDIS, 2020. High-throughput solution for in-egg selection of laying hens.
774 Retrieved 15/01/2021 from <https://cordis.europa.eu/project/id/959321/fr>.

775 Costanzo, A., Panseri, S., Giorgi, A., Romano, A., Caprioli, M. and Saino, N., 2016.
776 The odour of sex: sex-related differences in volatile compound composition among
777 barn swallow eggs carrying embryos of either sex. *PLoS One* 11: e0165055.

778 DEFRA, 2020. United Kingdom Egg Statistics – Quarter 1, 2020. Retrieved on 05
779 March 2021 from
780 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachm
781 ent_data/file/905045/eggs-statsnotice-30apr20.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/905045/eggs-statsnotice-30apr20.pdf).

782 de Olde, E. M., van der Linden, A., olde Bolhaar, L. D. and de Boer, I. J. M., 2020.
783 Sustainability challenges and innovations in the Dutch egg sector. *Journal of Cleaner
784 Production* 258: 120974.

785 Douglas, C. and Turner, J. M. A., 2020. Advances and challenges in genetic
786 technologies to produce single-sex litters. *PLOS Genetics* 16: e1008898.

787 Dunn, I. C., Ciccone, N. A. and Joseph, N. T., 2009. Endocrinology and Genetics of
788 the Hypothalamic-Pituitary-Gonadal Axis. In *Biology of Breeding Poultry* (ed. P. M.
789 Hocking). CABI Publishing, Wallingford, UK, pp. 61-88.

790 Dunn, I. C., De Koning, D. J., McCormack, H. A., Fleming, R. H., Wilson, P. W.,
791 Andersson, B., Schmutz, M., Benavides, C., Dominguez-Gasca, N., Sanchez-
792 Rodriguez, E. and Rodriguez-Nvarro, A. B., 2020. Lack of genetic correlation
793 between bone quality and egg number in the laying hen suggests increased egg
794 production may not be responsible for reduction in bone quality. *Genetics Selection
795 Evolution*, In press.

796 Dunn, I. C., De Koning, D. J., McCormack, H. A., Fleming, R. H., Wilson, P. W.,
797 Andersson, B., Schmutz, M., Benavides, C., Dominguez-Gasca, N., Sanchez-
798 Rodriguez, E. and Rodriguez-Nvarro, A. B., 2021. No evidence that selection for egg
799 production persistency causes loss of bone quality in laying hens. *Genetic Selection
800 Evolution*, In press.

801 EFSA, 2005. Welfare aspects of various systems for keeping laying hens. *The EFSA
802 Journal* 197: 1-23.

803 eggXYt, 2021. Count your chicken before they hatch Retrieved 15/01/21, from
804 <https://www.eggxyt.com/>.

805 eiCare, 2021. Neue Wege beschreiten Retrieved 15/01/21, from [http://www.aktion-
806 ei-care.de/eicare-startseite.html](http://www.aktion-ei-care.de/eicare-startseite.html).

807 Eide, A. L. and Glover, J. C., 1995. Development of the longitudinal projection
808 patterns of lumbar primary sensory afferents in the chicken-embryo. *Journal of
809 Comparative Neurology* 353: 247-259.

810 Ellegren, H., 1996. First gene on the avian W chromosome (CHD) provides a tag for
811 universal sexing of non-ratite birds. *Proceedings of The Royal Society B-Biological*
812 *Sciences* 263: 1635-1641.

813 Eusemann, B. K., Patt, A., Schrader, L., Weigend, S., Thone-Reineke, C. and Petow,
814 S., 2020. The Role of Egg Production in the Etiology of Keel Bone Damage in Laying
815 Hens. *Frontiers in Veterinary Science* 7, 81.

816 Fleming, R. H., McCormack, H. A., McTeir, L. and Whitehead, C. C., 2006.
817 Relationships between genetic, environmental and nutritional factors influencing
818 osteoporosis in laying hens. *British Poultry Science* 47: 742-755.

819 Galli, R., Preusse, G., Schnabel, C., Bartels, T., Cramer, K., Krautwald-Junghanns,
820 M. E., Koch, E. and Steiner, G., 2018. Sexing of chicken eggs by fluorescence and
821 Raman spectroscopy through the shell membrane. *Plos One* 13. e0192554

822 Galli, R., Preusse, G., Uckermann, O., Bartels, T., Krautwald-Junghanns, M. E.,
823 Koch, E. and Steiner, G., 2016. In Ovo Sexing of Domestic Chicken Eggs by Raman
824 Spectroscopy. *Analytical Chemistry* 88: 8657-8663.

825 Galli, R., Preusse, G., Uckermann, O., Bartels, T., Krautwald-Junghanns, M. E.,
826 Koch, E. and Steiner, G., 2017. In ovo sexing of chicken eggs by fluorescence
827 spectroscopy. *Analytical and Bioanalytical Chemistry* 409: 1185-1194.

828 Giersberg, M. F. and Kemper, N., 2018. Rearing Male Layer Chickens: A German
829 Perspective. *Agriculture-Basel* 8: 176

830 Göhler, D., Fischer, B. and Meissner, S., 2017. In-ovo sexing of 14-day-old chicken
831 embryos by pattern analysis in hyperspectral images (VIS/NIR spectra): A non-
832 destructive method for layer lines with gender-specific down feather color. *Poultry*
833 *Science* 96: 1-4.

834 Gremmen, B., Bruijnis, M. R. N., Blok, V. and Stassen, E. N., 2018. A Public Survey
835 on Handling Male Chicks in the Dutch Egg Sector. *Journal of Agricultural and*
836 *Environmental Ethics* 31: 93-107.

837 Hein, T., 2018. Egg sexing close to market. Retrieved on 05 March 2021, from
838 [https://www.poultryworld.net/Eggs/Articles/2018/6/Egg-sexing-close-to-market-](https://www.poultryworld.net/Eggs/Articles/2018/6/Egg-sexing-close-to-market-301797E/)
839 [301797E/](https://www.poultryworld.net/Eggs/Articles/2018/6/Egg-sexing-close-to-market-301797E/).

840 IEC, 2018. Ressources. Retrieved 15/01/21, from
841 <https://www.internationalegg.com/>.

842 InOvo, 2020. In Ovo awarded €2.5 million EU grant. Retrieved 15 January 2021, from
843 <https://inovo.nl/news/in-ovo-awarded-e2-5-million-eu-grant/>.

844 ITAVI, 2019. Situation du marché des oeufs et des ovoproduits : Edition mars.
845 Service économie ITAVI 9, 1-13

846 Kaleta, E. F. and Redmann, T., 2008. Approaches to determine the sex prior to and
847 after incubation of chicken eggs and of day-old chicks. *Worlds Poultry Science*
848 *Journal* 64: 391-399.

849 Kipster, 2021. Kipster, a new chapter in sustainable chicken farming
850 Innovation. Retrieved 05 March 2021 from <https://www.kipster.farm/>.

851 Klein, S. and Grossmann, R., 2008. Primary sex ratio in fertilized chicken eggs
852 (*Gallus gallus domesticus*) depends on reproductive age and selection. *Journal of*
853 *Experimental Zoology. Part A, Ecological genetics and physiology* 309: 35-46.

854 Knepper, P., O'Hayer, M., Hoopes, J. and Gabba, E., 2019. System and method for
855 in ovo sexing of avian embryos. Patent US 20190174726

856 Koenig, M., Hahn, G., Damme, K. and Schmutz, M., 2012. Utilization of laying-type
857 cockerels as coquelets: Influence of genotype and diet characteristics on growth
858 performance and carcass composition. *Archiv Fur Geflugelkunde* 76: 197-202.

859 Krautwald-Junghanns, M. E., Cramer, K., Fischer, B., Forster, A., Galli, R., Kremer,
860 F., Mapesa, E. U., Meissner, S., Preisinger, R., Preusse, G., Schnabel, C., Steiner,
861 G. and Bartels, T., 2018. Current approaches to avoid the culling of day-old male
862 chicks in the layer industry, with special reference to spectroscopic methods. *Poultry*
863 *Science* 97: 749-757.

864 Leenstra, F., Munnichs, G., Beekman, V., van den Heuvel-Vromans, E., Aramyan, L.
865 and Woelders, H., 2011. Killing day-old chicks? Public opinion regarding potential
866 alternatives. *Animal Welfare* 20: 37-45.

867 Leenstra, F., ten Napel, J., Visscher, J. and van Sambeek, F., 2016. Layer breeding
868 programmes in changing production environments: a historic perspective. *World's*
869 *Poultry Science Journal* 72: 21-35.

870 Mendonça, M. A., Carvalho, C. R. and Clarindo, W. R., 2010. DNA content
871 differences between male and female chicken (*Gallus gallus domesticus*) nuclei and
872 Z and W chromosomes resolved by image cytometry. *The Journal of Histochemistry*
873 *and Cytochemistry : Official Journal of the Histochemistry Society* 58: 229-235.

874 Mollenhorst, H. and Haas, Y., 2019. The contribution of breeding to reducing
875 environmental impact of animal production. Wageningen Livestock Research,
876 Wageningen, NL.

877 Molnar, A., Maertens, L., Ampe, B., Buyse, J., Kempen, I., Zoons, J. and Delezie, E.,
878 2016. Changes in egg quality traits during the last phase of production: is there
879 potential for an extended laying cycle? *British Poultry Science* 57: 842-847.

880 Mueller, S., Kreuzer, M., Siegrist, M., Mannale, K., Messikommer, R. E. and
881 Gangnat, I. D. M., 2018. Carcass and meat quality of dual-purpose chickens
882 (Lohmann Dual, Belgian Malines, Schweizerhuhn) in comparison to broiler and layer
883 chicken types. *Poultry Science* 97: 3325-3336.

884 Muller-Niegsch, D., 2017. In ovo sexing of chicken eggs (*Gallus gallus* F. Dom.) as
885 alternative for routine culling of day-old male chicks of laying hen strains. Retrieved on
886 20 January 2021 from [https://tu-](https://tu-dresden.de/med/mf/ksm/forschung/forschungsprojekte/projekt-2?set_language=en#)
887 [dresden.de/med/mf/ksm/forschung/forschungsprojekte/projekt-2?set_language=en#](https://tu-dresden.de/med/mf/ksm/forschung/forschungsprojekte/projekt-2?set_language=en#).
888 Nicol, C. J., Bestman, M., Gilani, A. M., De Haas, E. N., De Jong, I. C., Lambton, S.,
889 Wagenaar, J. P., Weeks, C. A. and Rodenburg, T. B., 2013. The prevention and
890 control of feather pecking: application to commercial systems. *World's Poultry*
891 *Science Journal* 69: 775-788.

892 Pelletier, N., Ibarburu, M. and Xin, H. W., 2014. Comparison of the environmental
893 footprint of the egg industry in the United States in 1960 and 2010. *Poultry Science*
894 93: 241-255.

895 Plantegg, 2020. In-Ovo Gender determination. Retrieved on 20 January 2021 from
896 <https://www.plantegg.de/en/>.

897 Porter, R., 2020. The new standard has been agreed upon, but there's a hefty price
898 tag attached for producers. *PoultryNews*, retrieved on 05 March 2021 from
899 [http://www.poultrynews.co.uk/production/egg-production/analysis-who-will-pay-for-](http://www.poultrynews.co.uk/production/egg-production/analysis-who-will-pay-for-the-new-barn-eggs-standards.html)
900 [the-new-barn-eggs-standards.html](http://www.poultrynews.co.uk/production/egg-production/analysis-who-will-pay-for-the-new-barn-eggs-standards.html).

901 Poulehouse, 2021. L'œuf qui ne tue pas la poule. Retrieved on 15 January 2021
902 from <https://www.poulehouse.fr/>.

903 Sakomura, N. K., Reis, M. D., Ferreira, N. T. and Gous, R. M., 2019. Modeling egg
904 production as a means of optimizing dietary nutrient contents for laying hens. *Animal*
905 *Frontiers* 9: 45-51.

906 Sandilands, V., 2011. The laying hen and bone fractures. *Veterinary Record* 169:
907 411-412.

908 Seleggt, 2019. Every year, around 300 million male chicks of the egg-laying breeds
909 are killed in the EU alone because they do not lay eggs and it is uneconomical to
910 fatten them. Retrieved 05 March 2021 from <https://www.seleggt.com/>.

911 Smith, C. A., Roeszler, K. N., Ohnesorg, T., Cummins, D. M., Farlie, P. G., Doran, T.
912 J. and Sinclair, A. H., 2009. The avian Z-linked gene DMRT1 is required for male sex
913 determination in the chicken. *Nature* 461: 267-271.

914 Solomon, S. E., 2002. The oviduct in chaos. *Worlds Poultry Science Journal* 58: 41-
915 48.

916 Soos, 2020. Egg sex determination. Retrieved on 05 March 2021 from
917 <https://www.soos.org.il/>.

918 Steiner, G., Bartels, T., Stelling, A., Krautwald-Junghanns, M. E., Fuhrmann, H.,
919 Sablinskas, V. and Koch, E., 2011. Gender determination of fertilized unincubated
920 chicken eggs by infrared spectroscopic imaging. *Analytical and Bioanalytical*
921 *Chemistry* 400: 2775-2782.

922 Stratmann, A., Frohlich, E. K. F., Gebhardt-Henrich, S. G., Harlander-Matauschek,
923 A., Wurbel, H. and Toscano, M. J., 2015. Modification of aviary design reduces
924 incidence of falls, collisions and keel bone damage in laying hens. *Applied Animal*
925 *Behaviour Science* 165: 112-123.

926 Tassard, A. S., 2018. Stéphane Travert confirme la fin de l'élevage en batterie pour
927 la production d'oeufs-coquilles. Retrieved on 10 December 2020 from
928 [https://www.sciencesetavenir.fr/animaux/animaux-d-elevage/stephane-travert-
929 confirme-la-fin-de-l-elevage-en-batterie-pour-la-production-d-oeufs_121347](https://www.sciencesetavenir.fr/animaux/animaux-d-elevage/stephane-travert-confirme-la-fin-de-l-elevage-en-batterie-pour-la-production-d-oeufs_121347).

930 Toscano, M. J., Dunn, I. C., Christensen, J. P., Petow, S., Kittelsen, K. and Ulrich, R.,
931 2020. Explanations for keel bone fractures in laying hens: are there explanations in
932 addition to elevated egg production? *Poultry Science* 99: 4183-4194.

933 Webster, B., Hayes, W. and Pike, T. W., 2015. Avian egg odour encodes information
934 on embryo sex, fertility and development. PLoS One 10: e0116345.

935 Weissmann, A., Reitemeier, S., Hahn, A., Gottschalk, J. and Einspanier, A., 2013.
936 Sexing domestic chicken before hatch: a new method for in ovo gender identification.
937 Theriogenology 80: 199-205.

938 White, K., 2019. Sainsbury's set to stop selling barn eggs in 2020. Retrieved on 15
939 December 2020 from [https://www.thegrocer.co.uk/eggs-and-poultry/sainsburys-set-](https://www.thegrocer.co.uk/eggs-and-poultry/sainsburys-set-to-stop-selling-barn-eggs-in-2020/592720.article)
940 [to-stop-selling-barn-eggs-in-2020/592720.article](https://www.thegrocer.co.uk/eggs-and-poultry/sainsburys-set-to-stop-selling-barn-eggs-in-2020/592720.article).

941 Whitehead, C. C., 2004. Overview of bone biology in the egg-laying hen. Poultry
942 Science 83: 193-199.

943 Wolc, A., Arango, J., Settar, P., O'Sullivan, N. P., Olori, V. E., White, M. S., Hill, W.
944 G. and Dekkers, J. C. M., 2012. Genetic parameters of egg defects and egg quality in
945 layer chickens. Poultry Science 91: 1292-1298.

946

947

948 **Tables**

949 Table 1: Year of the announced cessation of marketing of furnished caged eggs by retailers
950 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

951

952

953 Table 2: Influence of pullet quality on the performance at different ages of the layer¹ (Hendrix
 954 genetics, personal communication)

Item	Body weight			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	+++

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

956 ¹0 = absence of correlation, + = low correlation, ++ = middle correlation, +++ = high
 957 correlation

958

959 **Figure legends**

960 Figure 1: Percentage of laying hen numbers by production systems in 2019, in
961 individual (A) and in total (B) European-27-member states (without UK for B). (Beck,
962 2019).

963 Figure 2: Egg consumption in France in 2018 by distribution channel (ITAVI, 2019).

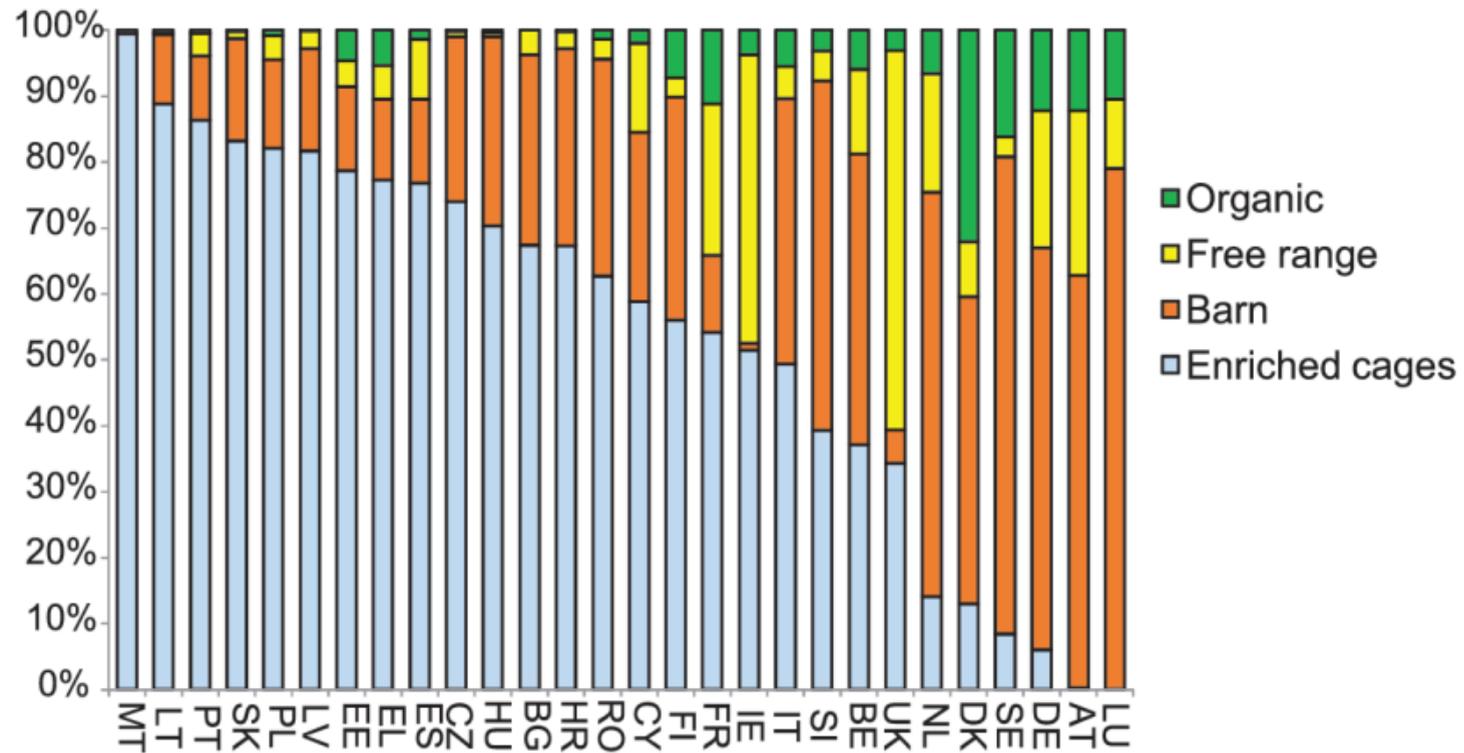
964

965 Figure 3: Schematic representation of the effect of increasing the laying cycle on
966 reducing the number of hen multipliers and layers and the consequences on the
967 production pyramid. (Modified from Bain et al., 2016).

968

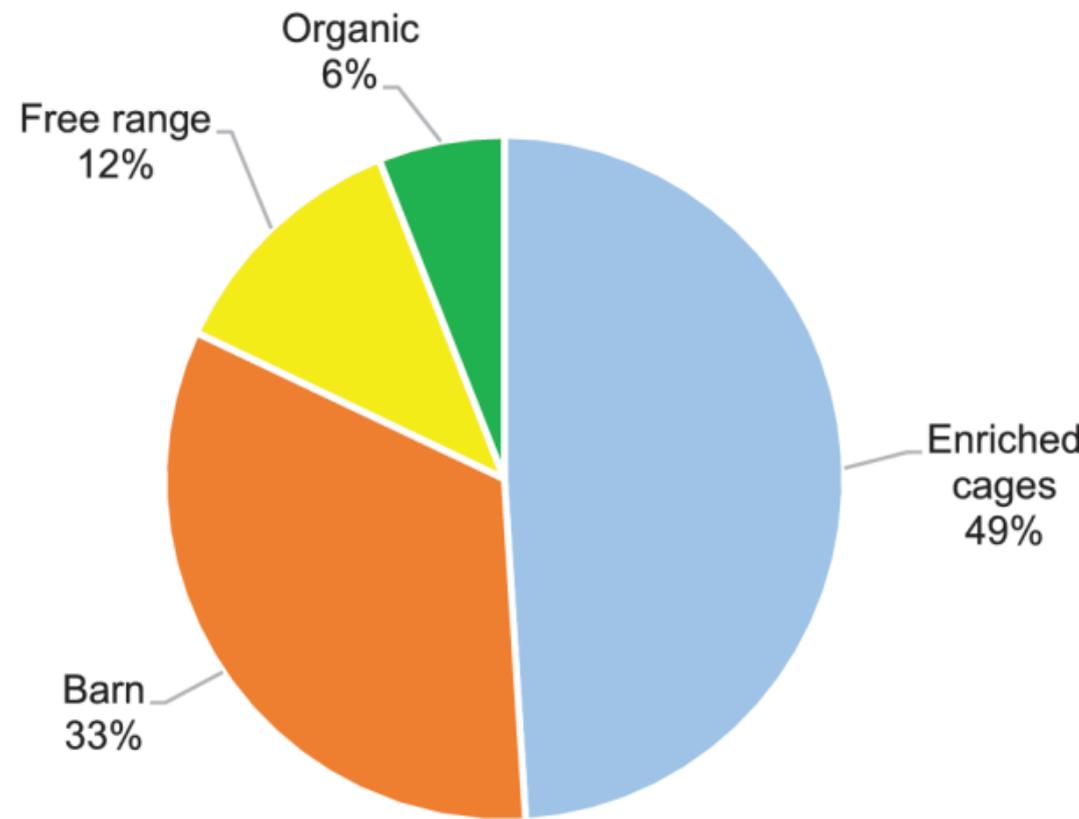
A.

Allocation of Housing Systems in the EU, 2019

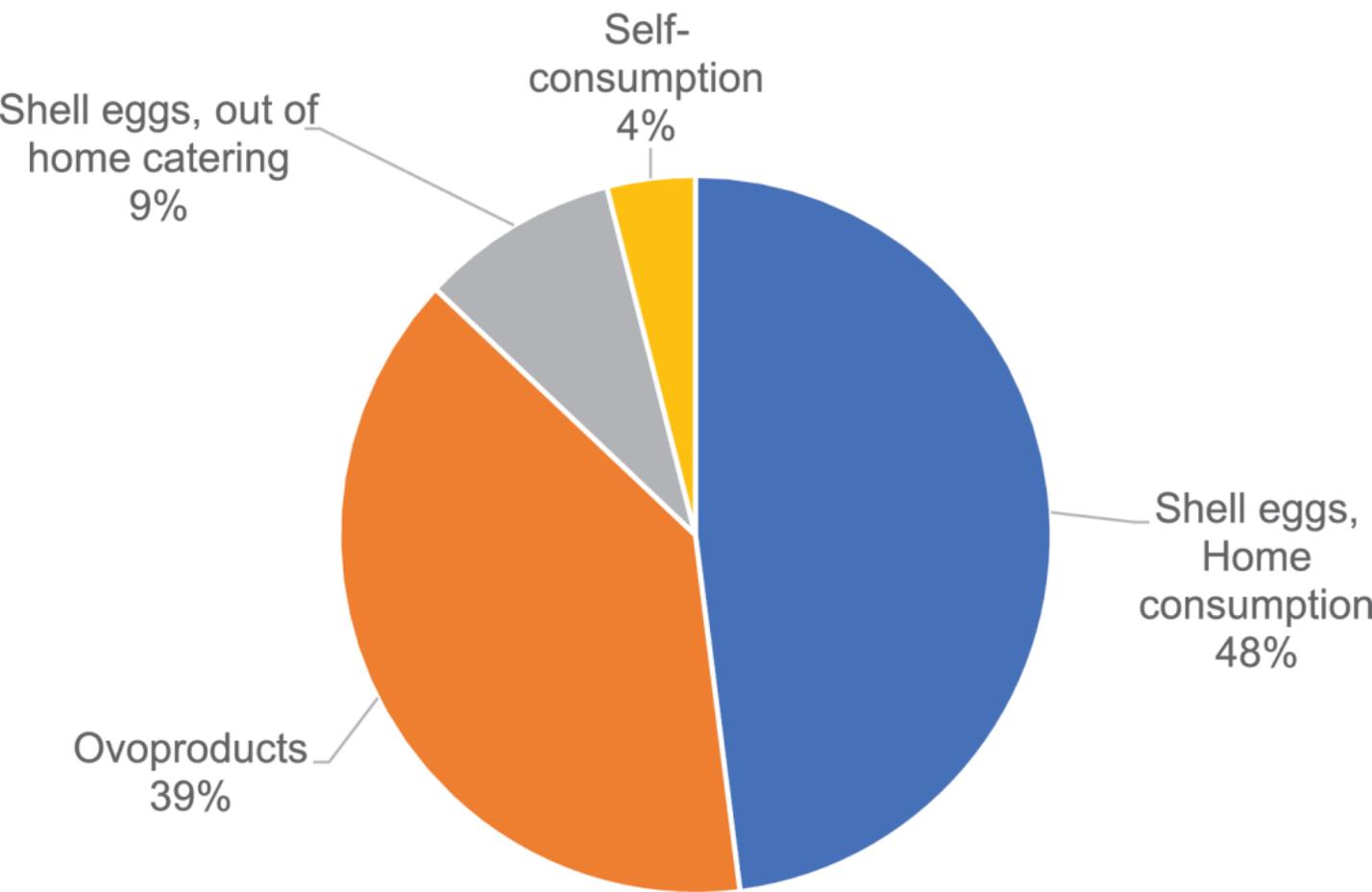


B.

Production systems for eggs in UE, 2019



Egg consumption in France in 2018



What might be achieved by increasing the time in lay on the breeding pyramid

