

New early-feeding strategies for the benefit of poultry production

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■ **Growth, development and robustness: should we wait for the outbreak to happen before reacting? Enriching the egg with certain nutrients of interest is a new production strategy that aims to improve the quality of the chick at hatching, increase the efficiency of its metabolism and obtain more robust adult animals that can adapt to given rearing conditions, while also controlling the development of quality products.**

Introduction

The world's population continues to grow. The need for both cereals and meat is increasing considerably. Poultry is a source of animal protein that is popular with humans, quick to produce, cheap and not affected by religious prohibitions. Poultry production is steadily increasing (+2% per year with 107 MT in 2013) and is second only to pork (114 MT) and far ahead of beef (68 MT) (box 1).

To meet this growing demand, broilers have mainly been selected for their rapid growth rate. The increase in body weight of broilers per year is about 3.3% (Zuidhof *et al.*, 2014) (Figure 1). As the chicken is slaughtered at a target live weight of 1.7_3.5 kg, the increase in growth rates have resulted in a decrease in slaughter age of about 1 day per year. In Europe, the rearing period is less than 6 weeks for "standard" broilers (Arnould

et al., 2011). The skeletal, immune and cardiovascular systems have not kept pace with the massive increase in muscle mass (Havenstein *et al.*, 2003; Tona *et al.*, 2003; Hocking, 2010).

This selection strategy has inevitably been accompanied by undesirable effects such as a higher frequency of ascites, the appearance of skeletal and meat defects, some

Box 1. Evolution of world meat production (in Million Tons) (Source: Annual report "OECD and FAO Agricultural Outlook", 2014).

In 2014, world poultry meat production was estimated at 110.5 MT, an increase of more than 3% compared to 2013 (107 MT). FAO's agricultural outlook shows that poultry meat production can be expected to increase by 1.8% per year from 2015 to 2024. By 2020, the poultry industry would then become the world's leading meat producer.

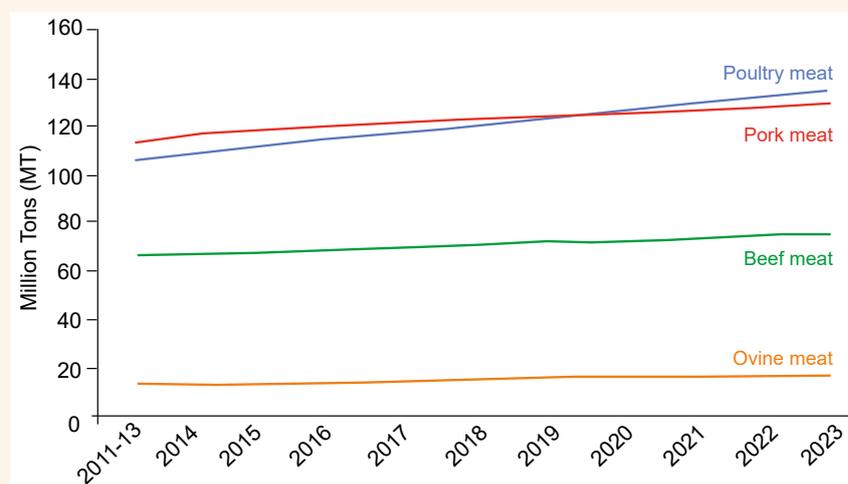
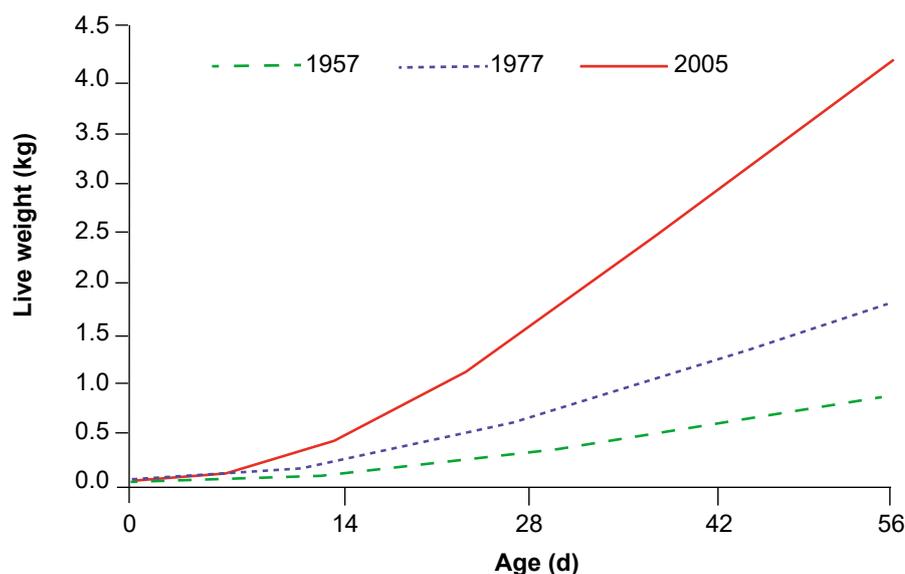


Figure 1. Changes in live weights of unselected broilers from 1957 and 1977, and Ross 308 chicken (2005) (Adapted from Zuidhof et al. 2014).



immunosuppression in animals, an increase in their susceptibility to infectious diseases and an increase in the incidence of metabolic diseases (Emmerson *et al.*, 1997; Decuypere *et al.*, 2003; Havenstein *et al.*, 2003). It should be noted that feed requirements have been established essentially on the basis of the growth performance of young animals without taking into account other functions such as immune function or, more broadly, the “health” valence. For breeding stock, *ad libitum* feeding leads to excessive obesity with very low reproductive capacity and high morbidity and mortality. This practice also has a serious impact on bird welfare (Decuypere *et al.*, 2006, 2010; De Jong and Guémené, 2011) and on animal integrity, which goes beyond health and welfare concerns (Decuypere *et al.*, 2010). The integration of the two aspects of growth and reproduction is relatively incompatible and is recognised as the “rapid growth versus reproduction — health” paradox.

In the “broiler” sector, a restriction on the diet of breeding stock is therefore carried out in order to maximise egg and chick production. Nowadays, female

breeders receive on average 25% of what they would normally consume in *ad libitum* mode. This severe dietary restriction allows the body weight trajectory to be kept within well-defined limits to ensure proper reproductive performance in adulthood. However, it has a negative impact on the offspring. In fact, restriction of breeding hen, sustained egg production levels or other stresses can cause egg composition to vary, which can then lead to eggs that are deficient in the critical nutrients needed for proper chick start-up. Under these conditions, and with a delay between hatching and rearing that can be as long as 48–72 hours, as well as transport conditions that are often sub-optimal, the starting period (1st week after hatching) is then delicate, and performance is significantly impacted.

In growing animals, any quantitative (energy and protein levels) or qualitative (type of diet, nutrient intake or distribution pattern) changes in the diet affect metabolism. This has consequences on energy consumption, feed efficiency, nutrient distribution between tissues or organs and thus on body composition and meat quality (Tesseraud *et al.*, 2014). This also applies to a developing embryo.

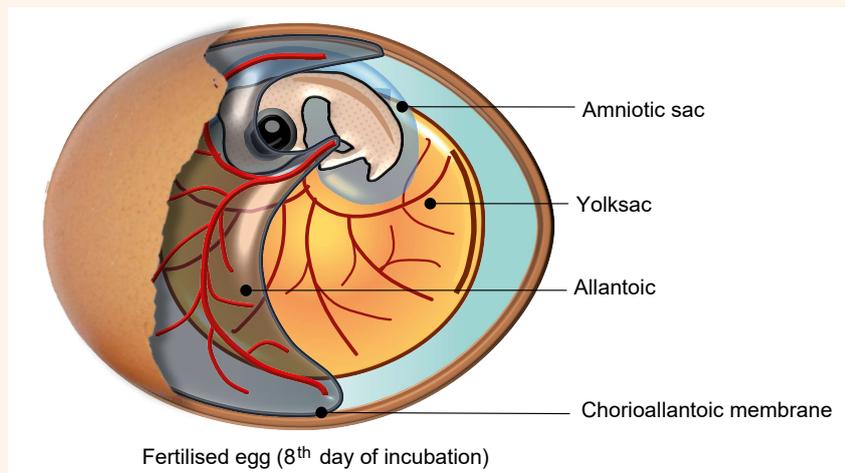
Nutrient supplementation of the egg via maternal feeding or via *in ovo* feeding are then innovative strategies to optimise the supply of nutrients to the embryo and improve the starting conditions of the chicks and their robustness. The incubation period and the first week of age are essential for their health, well-being and growth performance (Bigot *et al.*, 2003; Yassin *et al.*, 2009). The aim of such approaches is to optimise the nutritional intake of the breeding females, developing embryos and/or starter chicks in order to obtain chicks of better quality in terms of robustness, growth and/or body composition by taking advantage of the embryonic plasticity of nutrient utilisation.

1. Sources and nature of nutrients and active molecules available to the developing embryo

The duration of the embryonic development of the bird varies according to that of the life of the bird because both correspond to its growth rate. It varies from 10 days in the Cowbird (*Molothrus sp.*) to 80 days in the Royal Albatross. Ducks require 28–36 days (for the Beechnut and Barbary Albatross respectively), geese require 30–35 days and domestic chickens require 21 days. When not specified, the species being discussed in the following will be chicken. As the slaughter weight of animals is reached earlier and earlier (between 35–42 days), embryonic development accounts for 33–38% of the life of a modern broiler. When the incubation period and the perinatal period (up to 4 days after hatching) are included, together they account for more than 50% of the animal’s life (Druyan, 2010). In oviparous vertebrates (such as birds), embryonic development takes place entirely in the egg, independently of the mother. The egg then forms a natural closed

Box 2. The hatching egg and embryonic appendages.

Physical defence of the embryo is primarily ensured by the shell, which constitutes a protective envelope. Formed of calcium carbonate crystals, the shell is porous. It thus allows gas exchange but induces water losses. Three annexes allow the embryo to develop independently of the mother. The amnion delimits the cavity in which the embryo is bathed. It isolates the embryo and protects it. The yolk sac, whose wall is highly vascularized, contains the yolk reserves (yolk or vitellus). The allantoic sac serves as a reservoir for the waste products, particularly nitrogenous (uric acid), eliminated by the embryo. Its vascularized wall (chorioallantoic membrane) is the site of respiratory exchanges (via the pores of the shell). In addition, minerals from the shell can be absorbed from this allantoic sac and transferred to the embryo for the calcification of its skeleton. It is also a storage organ for many free amino acids and related compounds. These compounds are important for the nutrition of the embryo during the late incubation phase (diagram adapted from Da Silva, 2017).



enclosure that contains all the elements necessary for the survival, development and protection of an embryo when incubated under the right conditions (temperature, humidity and inversion) (box 2). The embryo uses the nutrients of the egg without the possibility of waste disposal, since only gas exchange takes place with the outside environment.

The main source of nutrients for the embryo during the first two weeks of its development is the yolk, which is composed mainly of lipids and proteins. The carbohydrate content of the egg is very limited; its concentration is less than 1% of the total nutrients and the percentage of free glucose is only about 0.3% (Campos *et al.*, 2011). In the yolk sac, with the exception of immunoglobulins, most of the proteins, triglycerides, phospholipids and cholesterol are synthesised by the liver

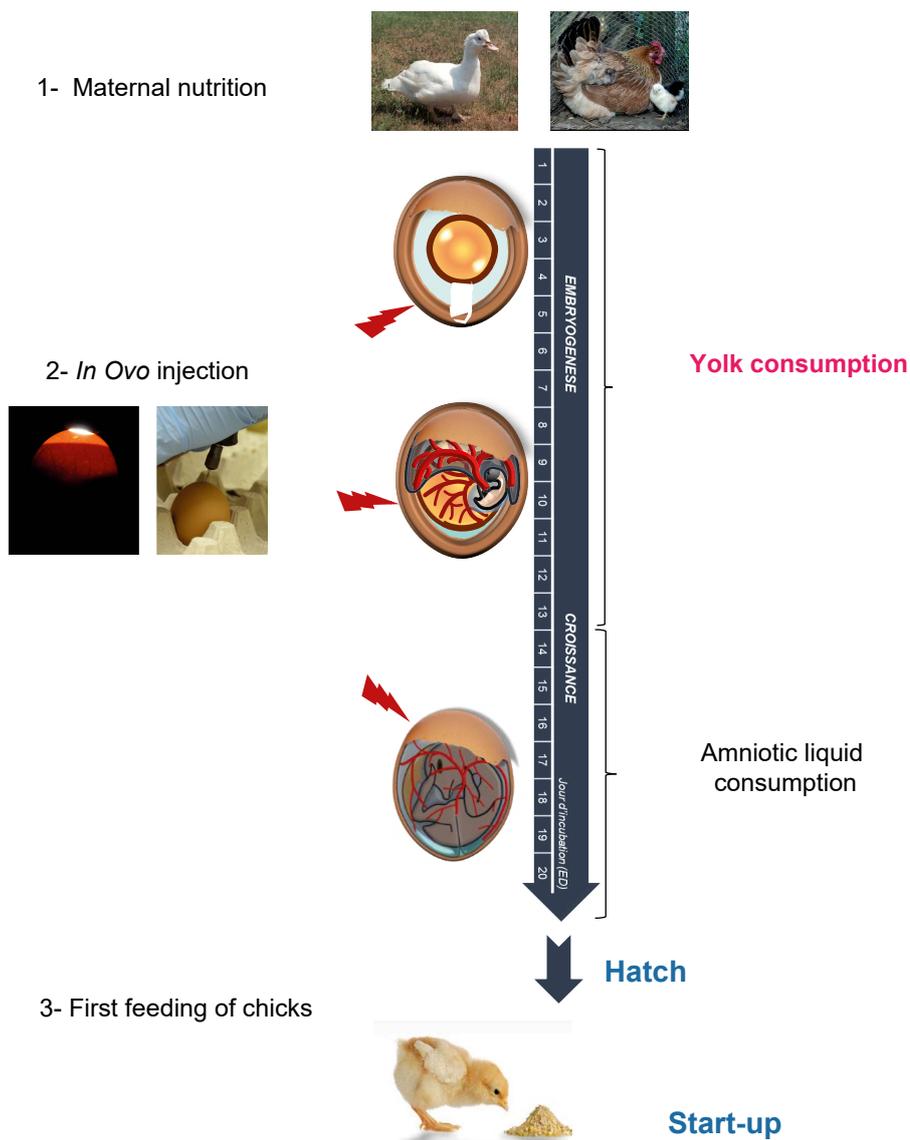
of the breeding hen. Lipids and proteins account for 62.5% and 33% of the dry matter of the yolk, respectively (Powrie *et al.*, 1986). Lipids are the main source of energy for the developing embryo (Moran, 2007; Cherian, 2015). All the proteins in the egg yolk also constitute a reserve of nutrients. In addition to their nutritional value, many proteins also have their own biological activities, such as transport and storage of vitamins ("*riboflavin-binding protein*", "*vitamin-D-binding protein*"; avidin) or metal ions (ovotransferrin, phosvitin), lipase-inhibiting activities (apovitellenin), etc. (Moran, 2007; Bourin, 2011; Cherian, 2015). Finally, yolk also contains fat-soluble molecules such as vitamins A, D, E and carotenoids. The latter come exclusively from the mother's diet. Molecules with antimicrobial potential are also present in egg yolk (Bourin *et al.*, 2011).

From 14 days of development, the embryo will be surrounded by a double envelope consisting of the amnion and the allantoid. In the final third of development, the embryo will therefore have access to other nutrients present in these two appendages (rich in water and protein). After 20 days, the chick is in its hatching position, the beak has pierced the air chamber and lung breathing has begun. After 21 days of incubation, the chick finally comes out of its shell. The allantoid, which served as its lungs, dries out because the chick uses its own lungs. During hatching, the amnion and allantoid are eliminated with the shell, the albumen has been used and the remaining yolk retracts into the abdomen of the animal. The chick then switches from a mainly lipid and protein diet to a carbohydrate diet as soon as it is transported and put in place at the breeder's with access to a cereal-rich feed.

2. Changes in the environment of the embryo via maternal feeding and consequences on the development of offspring phenotypes

The development of the embryo depends on the environment in which it is immersed and the nutrients available. One way of modulating egg composition is by altering the mother's diet (Kidd *et al.*, 2005; Rao *et al.*, 2009) (Figure 2). This environment can be modulated to influence the physiological and morphological development of embryos, with consequent effects on the development of the chick phenotype (Ho *et al.*, 2011). Links between parental nutrition, egg composition and the subsequent behaviour of the animals (Aigueperse *et al.*, 2013),

Figure 2. Early nutritional events that may modulate metabolic programming and animal phenotype (Adapted from Da Silva, 2017 and Métayer-Coustard et al., 2017).



their performance (Bergoug *et al.*, 2013), and their susceptibility to disease have been established. The mechanisms underlying these maternal effects are not always fully deciphered or understood.

■ 2.1. Changes in the phenotype of chicks as a result of rearing practices experienced by their mothers

In addition to the severity of the dietary restriction applied to the breeding females, the timing and

duration of dietary restriction during the rearing period is a determining factor in subsequent egg-laying performance and the development of the phenotype of the offspring. For example, feed restriction before the onset of sexual maturity can affect a range of circulating hormones such as thyroid or somatotrophic hormones (Bruggeman *et al.*, 1999). These hormones influence the subsequent performance of offspring.

Protein deficiency or fasting also alters the endocrine status of ani-

mals (Scanes and Griminger, 1990). Hormonal changes in the egg have been demonstrated in low-protein diet distribution programs (Rao *et al.*, 2009). This diet not only altered both the egg laying rate and egg weight but also the amount of leptin in the yolk and the expression of a number of genes expressed in the yolk sac, hypothalamus or muscle of the offspring. The chicks had lower hatching weights but faster post-hatching growth. More recently, it has been shown that protein diets administered to females negatively impacted reproductive performance but improved offspring performance (Lesuisse *et al.*, 2017). The authors were also able to demonstrate multigenerational effects of this type of diet on offspring performance (Lesuisse *et al.*, 2018a and 2018b; Li *et al.*, 2018).

Maternal effects have also been described on the development and body composition of chicks from restricted hens. These offspring, fed *ad libitum*, showed less growth and greater adiposity compared to animals with less restricted hens (van der Waaij *et al.*, 2011). This management resulted in significant economic losses due to reduced body mass and feed efficiency. Spratt and Leeson (1987) also showed differences in protein deposition and carcass adiposity in animals from breeding hens fed diets with different energy and protein content.

In addition to their nutritional value, the diets of female reproducers may have an olfactory signature that the embryos are able to pick up. The odours present during incubation, from the early stages of development of the olfactory system when the embryos have not yet adopted air breathing, may then influence the future food preferences of the young (Bertin *et al.*, 2012; Aigueperse *et al.*, 2013).

■ 2.2 Changes in the egg composition and the environment of the embryo through maternal feeding

The composition of the egg is fairly stable and the hen's diet does not affect the composition of the egg's major constituents (lipids or proteins). However, the levels of essential nutrients such as fatty acids, vitamins, trace elements, carotenoids or certain amino acids can be modulated in the egg via the mother's diet and induce changes in the performance of the offspring (for reviews, see Kidd, 2003; Calini and Sirri, 2007; Rühl, 2007). Some examples, which are not exhaustive, are detailed below.

a. Lipids and polyunsaturated fatty acids

Lipids are the major nutrients in the yolk that are available to the developing embryo. The oxidation of fatty acids (FA) covers practically all the energy demands of the embryo. Fatty acids are therefore essential for embryonic development, bird growth, the development of the central nervous system and the immune system (Noble *et al.*, 1984; Ding and Lilburn, 1996; Cherian, 2015). In hens, Menge *et al.* (1974) showed that depletions of essential fatty acids caused later hatching and that offspring showed slower growth compared to a control group.

The structure of lipoproteins is stable but the balance of fatty acids can be modulated via the mother's diet. Polyunsaturated fatty acids (PUFA) will vary according to the lipid source added to the diet. The n-6 fatty acids (FA ω 6), linoleic (C18:2) or arachidonic (C20:4), are mainly found in soybean, sunflower or safflower oil. The linoleic acid content can thus be multiplied by a factor of 2_2.5. The n-3 fatty acids (FA ω 3), linolenic C18:3, eicosapentaenoic (EPA) C20:5,

docosahexaenoic (DHA) C22:6, are found in fish, flax, millet, rapeseed oils or in microalgae. The levels of the total ω 3 can be multiplied by 6_7 times in the egg. Linolenic acid can be increased by a factor of 25, especially with linseed oil and DHA can be increased by a factor of 10 with fish oil. Variations in fatty acid composition and balance in the yolk can not only affect the hatchability, growth and performance of the offspring (Koppenol *et al.*, 2015) but also their passive immunity (Wang *et al.*, 2004). Several studies have shown that a maternal diet rich in omega 3 has olfactory properties that are transmitted to the egg (Leeson *et al.*, 1998; Gonzales-Esquerra and Leeson, 2000) and are perceived by the embryos. These odours represent an interesting tool to reduce food neophobia in offspring (Aigueperse *et al.*, 2013).

Numerous studies in humans and mouse models have shown a positive effect of long-chain FA ω 3 (FA ω 3 LC) on brain development and function. Ducks can express deleterious behaviours such as nervousness and pecking when reared. Enrichment of eggs and thus embryos with FA ω 3 LC can be achieved by feeding ducks a diet containing DHA and linolenic acid (microalgae and linseed oil) (Baéza *et al.*, 2017). This diet does not affect their egg-laying and reproductive performance and has no effect on the weight and lipid content of egg yolks. On the other hand, yolk lipids are enriched with FA ω 3 and ducklings from ducks fed an FA ω 3-enriched diet have a live weight greater at hatch (D0), D28 and D56 and a lower feed conversion ratio for the growing period. Enrichment of the duck feed with FA ω 3 LC also reduces the frequency and severity of pecking in ducklings and reduces the duration of tonic immobility tested at 19 days of age. Reduced hyperactivity and reduced stress responsiveness

in ducklings from these ducks was also observed.

b. Vitamins and minerals

Mineral and vitamin supplementation via the mother's diet has often been studied to solve skeletal mineralisation and leg problems. Avitaminoses in birds mainly result in a decrease in egg laying and a drop in hatching rate (Adrian, 1958). Vitamin A deficiency doubles the frequency of embryo malpositioning in the egg (Polk and Sipe, 1940). Vitamin E deficiency will result primarily in disintegration of the blood vessels of the blastoderm (Adamstone, 1931; Adamstone, 1941). Around the fourth day of development, a ring may also form in the blastoderm which interrupts or inhibits yolk circulation and causes the death of the embryo. A reduction in body, leg and wing length may also be noted (Ferguson *et al.*, 1954). Vitamin D3 regulates calcium flow through the chorioallantoic membrane (CAM) in a unidirectional and active process. A vitamin D-deficient diet leads to a decrease in Ca⁺⁺ transport across the CAM and a decrease in Ca⁺⁺ accumulation in the embryo (rickets in the young, bone deformation), as well as an increase in late embryonic mortality (malposition, beak unable to pierce the shell).

Fat-soluble vitamins (vitamin A, E or D) as well as water-soluble vitamins (riboflavin, B12, thiamine, biotin, folic acid, etc...) can be enriched in the egg via the females' diet. Vitamins are provided in the form of supplements in the feed according to calculated needs, which are dated and no longer necessarily correspond to the needs of today's animals. Vitamin A is produced by the hen from carotenoids found in the feed. Carotenoid contents will vary according to the plant sources used. Their yield of deposition in the egg yolk is variable from one source to another.

These carotenoids will give the egg yolk its colour, which depends on the quantity of carotenoids ingested, their colouring capacity and their stability. They will also have antioxidant properties. Vitamin D is only effective if the diet contains sufficient calcium and phosphorus. Unlike vitamin D3, vitamin D2 is not very useful for the bird (effectiveness of less than 10% compared to vitamin D3). Vitamin E, essential for fertility and brain development, is only effective if selenium is present in sufficient quantities. Minerals such as iodine, selenium, magnesium, zinc, copper or manganese can also be enriched in eggs (Surai and Parks, 2001; Jiakui and Xialong, 2004; Chinrasri *et al.* 2013; Favero *et al.* 2013; Saunders-Blades and Korver, 2015; Torres and Korver, 2018). Selenium can be increased by a factor of 5_10 and its content will be higher in egg white. This element is essential for antioxidant control in birds.

c. Amino acids

Amino acid requirements of female breeders had historically been defined by measuring the response of animals in terms of egg production. With increasing evidence of the effects of amino acids on offspring phenotype, these requirements are now being re-evaluated. Studies testing different levels of digestible lysine (Ciacciariello and Tyler, 2013) or arginine (Muller Fernandes *et al.*, 2014) in maternal diet have thus shown positive effects of supplementation on offspring (e.g. optimisation of performance, carcass yield, abdominal fat content and bone quality for arginine supplementation). The mechanisms involved are still little studied but in the case of methionine, an essential amino acid for the bird, it was concluded that the metabolism of the offspring was altered in response to the diets of the breeders. Indeed, Brun *et al.* (2013 and 2015) showed

that a diet restricted specifically to methionine distributed to ducks modified the hepatic metabolism of their offspring and could therefore induce different phenotypes in ducklings. A significant result of this study was the significant interaction between maternal diet and sex on the "foie gras" weight of the offspring. The 20% increase in male "foie gras" weight in this study could be used to reduce feeding time and associated costs, thus partly meeting a societal expectation to improve the welfare of farmed animals.

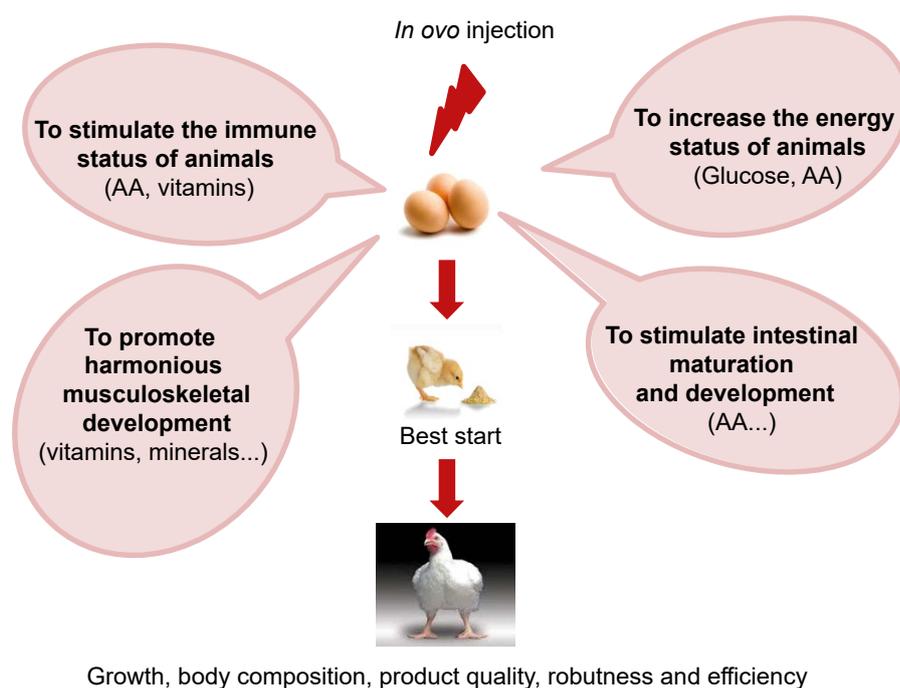
3. Egg supplementation by in ovo feeding

■ 3.1 General information on in ovo nutrition

Considering that the formation and development of tissues (such as muscle) take place during the 21 days of embryonic development, which accounts for 33_38% of an animal's total life, it is likely that a physiological or metabolic

disorder during this early development in the bird could significantly alter post-hatching weight gain and carcass characteristics (Velleman, 2007; Grodzik *et al.*, 2013). The rapid development and growth of currently selected bird strains may result in insufficient nutrients in the egg for optimal tissue development (Grodzik *et al.*, 2013). It is therefore necessary to act as early as possible on the metabolic programming of embryos in order to optimise their subsequent performance. Innovative nutritional strategies such as *in ovo* feeding by injecting nutrients directly into the egg have recently been developed to ensure a supply of readily available nutrients to support and accelerate the maturation of the digestive tract, optimise chick development and growth and thus obtain better quality chicks (Kadam *et al.*, 2013; Roto *et al.*, 2016; Gao *et al.*, 2017; Ghanaatparast-Rashti *et al.*, 2018; Peebles, 2018) (figure 3). Supplementation of the egg with different types of compounds is therefore aimed at providing additional nutrients to enable the chicks to cope with

Figure 3. In ovo injections: which ones and for what effects?



post-hatching delays of up to 48_72h and to modulate major functions. These nutritional strategies thus aim, for example, to respond to new challenges in the sector for which the use of antibiotics is strongly denounced and new practices aim to reduce or even eliminate their use in farms, which leads to production and profits losses for the breeders (Castanon, 2007).

The hatching percentage and the weight of the chicks depend on the substance injected, the site and time of injection of the nutrients into the egg. In early development, compounds are injected as close as possible to the germinal disc (Ebrahimi *et al.*, 2012). Later, the nutrients are administered into the yolk sac, which has a large surface area capable of absorbing nutrients. However, it appears that even with completely safe components, hatching is affected when injections are given early in the first two weeks of embryonic development. After 17 days of incubation, when the yolk sac is resorbed, injections are made into the air chamber or amnion. These later injections often have less adverse impact on hatchability and embryonic mortality. The amnion is the preferred site since at the end of incubation the embryos ingest the liquid present there. These injections are often carried out at the time of egg transfer from the incubator to the hatching pen at the same time as *in ovo* vaccinations (around 18 days of incubation). *In ovo* vaccinations, carried out at the end of the incubation period, make it possible to vaccinate animals early without affecting their survival or hatchability (Breedlove *et al.*, 2011). Handling of hatching animals is thus limited and the stress generated in the post-natal period is minimised. These practices are faster and allow for greater uniformity in the delivery of the vaccine dose

or nutrient solution. Nutrient solutions injected in practice are often complex.

Because the bird is oviparous, it is an ideal model for distinguishing direct nutrient effects from maternal effects. Indeed, maternal nutrition can influence both the environment in which the embryo develops (the main source of nutrients for the bird) but also the way in which genes regulating metabolism are expressed via the transmission of maternal epigenetic markers (Frésard *et al.*, 2013). This avian model thus makes it possible to understand and control both the direct (via *in ovo* injections) and indirect (via the mother) effects of nutrition on the regulation of metabolism and its long-term effects on growth, body composition and development of the offspring's phenotype.

Recent work has shown that it is thus possible to modify animal metabolism by exogenous inputs of various substances directly into the egg, including amino acids, energy substrates, electrolyte solutions, hormones, nucleotides and vitamins at key moments of embryogenesis by *in ovo* injections (Gore and Qureshi, 1997; Henry and Burke, 1999; Ohta *et al.*, 1999; Kocamis *et al.*, 1999 and 2000; Tako *et al.*, 2004; Uni *et al.*, 2005; Kadam *et al.*, 2008; Zhai *et al.*, 2011c; Mc Gruder *et al.*, 2011; Kornasio *et al.*, 2011; Bakyaraj *et al.*, 2012; Li *et al.*, 2016; Neves *et al.*, 2017; Gao *et al.*, 2017). These practices induce differences in chick performance at hatching (weight, glycogen storage, net yield), which can be maintained until slaughter age. However, the mechanisms of early guidance of metabolism at these periods remain largely unknown.

■ 3.2 Enrichment of eggs with energy substrates

Hatching is a critical milestone in a bird's life. This energy-intensive process affects hatching, morbidity and

mortality rates and, in the longer term, impacts on health, growth and the quality of the final product. At hatching, embryos preferentially use glucose rather than fatty acids for energy production. In fact, the quantity of oxygen in the egg is limited, and for the same quantity of oxygen consumed, the oxidation of glucose allows more energy to be obtained than with lipid catabolism. Because of the limited carbohydrate content in the egg, less than 1% of the total available nutrients and only 0.3% of free glucose, the maintenance of glucose homeostasis during late embryonic development depends on the amount of glucose stored as glycogen in the liver and glucose generated by gluconeogenesis from proteins (especially muscle) (Campos *et al.*, 2011). Glycerol from hepatic triglyceride metabolism (from egg yolk) is a major and indispensable substrate for glycogen synthesis in liver and muscle at the end of incubation (Sunny and Bequette, 2011). Between 15 and 19 days of incubation, the liver is the site of an active metabolism, leading to a transfer of glucose and fatty acids to the cervical muscle ("*pipping muscle*": muscle used to break the shell), which is, gradually enriched in glucose, glycogen and protein for hatching (Pulikanti *et al.*, 2010). Low liver glycogen levels are associated with later and longer hatching times and reduced hatching weight of chicks. The embryo must mobilise more muscle protein to provide gluconeogenesis-oriented amino acids, limiting growth in late hatching and during the first week of start-up (Pearce, 1971). The use of protein as an energy source will be to the detriment of muscle development. At the end of embryogenesis, pectoral muscle yield decreases significantly (Guernec *et al.*, 2003). An over-expression of atrophy-related genes such as atrogin-1 and MuRF1 observed at hatching compared to day 18 of

embryogenesis could partially explain this pectoral muscle wasting (Everaert *et al.*, 2013). To limit the use of fatty acids and the proteolysis of muscle proteins for energy purposes, injections of carbohydrates alone or combined with other nutrients of interest have been performed *in ovo* (Retes *et al.*, 2018).

In ovo supplementation has often been aimed at increasing the amount of sugars available in the egg and thus sparing the use of amino acids for gluconeogenesis (Kornasio *et al.*, 2011). In a recent study, Retes *et al.* (2018) were able to demonstrate that the results were dependent on the type of sugar injected, the injection site (yolk, albumen, amnion, allantoic fluid or air chamber), the development stage of the embryo and the genetics of interest in the studies. Uni *et al.* (2005), for example, showed that administration at 17.5 days of a solution containing carbohydrates (maltose, sucrose and dextrin) and a metabolite of leucine (β -hydroxymethylbutyrate) increased the hatching weight of chicks by 5_6%, liver glycogen stores by 2_5 times and pectoral muscle yield by 6_8% compared to control chicks whose eggs were not supplemented. These improved performances were still measurable at 25 days of age. Supplying sugars as an energy source by injection into the amnion at the end of incubation (17.5 d) may also accelerate intestinal development by increasing the size of villi and thus increasing the capacity of the gut to digest disaccharides (Tako *et al.*, 2004). This supplementation may have trophic effects on the small intestine and improve the development of calciform and mucin-secreting cells (Smirnov *et al.*, 2006), which certainly explains the higher weights of animals from supplemented eggs. Other sugars, such as fructose, are not recommended as several studies show a decrease in

hatchability of fructose-supplemented batches as well as lower animal weights (Zhai *et al.*, 2011).

Other nutritional supplements, such as amino acids (Li *et al.*, 2016), glycerol (Neves *et al.*, 2017), L-carnitine and creatine pyruvate (Zhao *et al.*, 2017) have been injected into different egg compartments at different stages of development in order to modify embryo metabolism and the phenotype of the hatching animals (quality and weight of the chick, body composition, quantity of liver glycogen, muscle yield etc.). The animals are sometimes kept beyond the hatching stage to check whether the effects observed at hatching remain perennial. With creatine pyruvate supplementation at 12 mg per egg, for example, the energy status of the animals was altered. This supplementation led to differences in the weights of the animals and *Pectoralis major* that persisted up to 21 days of age (Zhao *et al.*, 2017).

■ 3.3 Enrichment of eggs with amino acids

Embryonated eggs contain all the amino acids necessary for the growth and development of an embryo. As reported by Kucharska-Gaca *et al.* (2017), the amino acid composition of an egg has changed very little over the years while the nutrient requirements of birds during embryonic development have changed. Amino acid administration *in ovo* can provide poultry farms with an alternative method to improve hatching weights (Ohta *et al.*, 2001). This difference in hatching weight persists up to 56 days of age in some studies (Al-Murrani, 1982). Administration of amino acids may also induce an increase in lymphoid organ mass. Other strategies consist of administering a single amino acid, such as threonine (Kadam *et al.*, 2008) or DL-methionine (Coskun *et al.*, 2014).

Some injected amino acids, such as arginine or glutamine, are used for the synthesis of other amino acids. These supplements, in addition to their positive effects on animal weight, decrease hatching time (Shafey *et al.*, 2014). The contribution of amino acids alone or in association with others could stimulate protein synthesis and muscle protein accretion, but the mechanisms involved remain to be understood. Arginine, considered as an essential amino acid in birds, is involved in protein synthesis. It can also be converted to glucose and used in many metabolic pathways that produce active compounds that help maximise the potential development of an embryo by stimulating the secretion of growth hormone (Tong and Barbul, 2004). The effects of arginine have been confirmed in other birds such as quails and turkeys (Foye *et al.*, 2007) with other effects demonstrated such as an increase in the number of hatched chicks, their weight at 7 and 42 days of age, their weight gain and feed efficiency in quails and an increase in digestive enzyme activity (sucrose, maltose and leucine amino-peptidase activities) in the digestive tracts of turkeys.

■ 3.4. Enrichment of eggs with vitamins and trace elements

The efficacy of vitamins C, E, D3 and B9 (folic acid) on embryonic health and development has been reported by several teams in the literature (Peebles, 2018). As shown previously, the embryo draws its energy largely from the yolk. This use is the result of oxidative processes that produce free radicals, leading, among other things, to the degradation of polyunsaturated fatty acids in cell membranes. Vitamins, such as vitamin E or vitamin C, limit the negative effects of free radicals and protect the embryo (Surai *et al.*, 2016;

Peebles, 2018; Araújo *et al.*, 2018). Depending on doses, ages and injection sites, the reported results may be different (increased antioxidant capacity, better immunity, positive effect on growth, etc.).

Administration of vitamins A, B1, B2, B6 or E at 14 days of embryonic development may contribute positively to animal growth (vitamins B1 and B2) and/or modulate animal immunity and robustness (Goel *et al.*, 2013). The relative weight of Fabricius bursae is thus higher in 42-day-old animals that received vitamins B1, B2 and E *in ovo* and thymus weight is higher in the groups that received vitamins A, B6 and E. The group having received vitamin B1 shows a better humoral response. Vitamin E, on the other hand, will not increase the performance of the animals but will improve hatchability and stimulate the post-hatching immune status of the chicks (Salary *et al.*, 2014). Vitamin E injection will increase the resistance of chicks to diseases such as avian influenza or infectious bronchitis. In this experimental group, higher levels of IgG, IgM and IgA could be demonstrated.

To stimulate the growth and development of bone or its mineralisation, *in ovo* injections of vitamin D have been practiced. Vitamin D is involved in calcium metabolism, and previous research has shown better bone resistance (mineralisation) with higher levels of vitamin D in the diet. However, *in ovo* injection of 25-hydroxycholecalciferol on day 18 of embryogenesis in anticipation of higher bone mineralisation at hatching and 21 days of life does not always affect bone quality in hatching animals, perhaps because the chick could not find the extra calcium and minerals due to the constrained composition of the egg or the injection was made too late in the

development of the chick (Bello *et al.*, 2014). The combination of vitamin D3 and minerals injected earlier (E17) appears to have positive effects on the bone properties of hatched chicks and older chickens (Yair *et al.*, 2015). While some vitamins may appear to be ineffective in some forms, others may be toxic. For example, vitamin D3 may induce calcification of soft bones, compromising hatching and increasing embryonic death. Supplemented in the form of 25-hydroxycholecalciferol, which is biologically more active than vitamin D3 and more stable and less toxic than 1,25-dihydrocholecalciferol (Soares *et al.*, 1995), it has no deleterious effect on embryogenesis, skeletal development or the hatchability of chicks (Bello *et al.*, 2013).

Egg supplementation with selenium, an essential element in antioxidant control in birds, has been tested on the effectiveness of protecting chicks against necrotic enteritis (Lee *et al.*, 2014). An overall protective effect in chickens against experimental necrotic enteritis was demonstrated in this study. Selenium is thought to have this effect by controlling the activation of neutrophils, macrophages and B lymphocytes.

4. How nutrients can contribute to the metabolic programming of animals and the development of their phenotype.

Determining the mechanisms by which egg nutrients regulate cell metabolism, signalling, gene expression and function is essential to improve nutrient utilisation, poultry production efficiency and animal robustness. Few mechanisms have been deciphered to date in birds. The studies, which are not exhaustive and referenced, often

do not address the study of the mechanisms involved in the metabolic reprogramming of embryos. These could be metabolic regulations similar to those described in later breeding periods. The importance of certain amino acids (lysine and methionine) for the regulation of protein and energy metabolism in growing animals is well established, with marked effects on chicken growth and product quality (Berri *et al.*, 2008; Métayer *et al.*, 2008; Tesseraud *et al.*, 2009). Such regulation by amino acids used as substrates or as signal nutrients allows modulation of protein-energy metabolism (Tesseraud *et al.*, 2003; Métayer-Coustard *et al.*, 2010). In addition, epigenetic-type modifications, i.e., transmissible and reversible changes in gene expression, without altering the DNA sequence, could also be involved, particularly when molecules donating methyl groups such as methionine or other micronutrients are injected (Anderson *et al.*, 2012; Donohoe and Bultman, 2012; Veron *et al.*, 2018).

In mammals, more data are available. Maternal malnutrition leads to low birth weight of the offspring. Subsequent food abundance in the environment leads to compensatory growth (Fagerberg *et al.*, 2004). Maternal nutrition during gestation alters gene expression in offspring through epigenetic alterations such as DNA and histone modifications (Simmons, 2011). The epigenome is particularly dynamic during embryogenesis and the DNA methylation necessary for normal tissue development is established during early development. DNA methylation is an important epigenetic factor for the maintenance of genetic silence based on the provision of methyl groups. Histones are also influenced by numerous post-translational modifications (phosphorylation, ubiquitination, ADP-ribosylation, sumoylation and glyco-

sylation). Substrates and co-factors involved in the supply of methyl groups are considered particularly important during early stages of development (Sinclair *et al.*, 2007b). Thus, manipulation of the sulphur amino acid content of early diets can induce changes in cell function that have implications for the animal's development, long-term growth and health. Sulphur amino acids (Met) as well as other amino acids (Gly, His and Ser), vitamins (B6, B12 and folate) or micronutrients (betaine and choline) play a key role in providing methyl donors for DNA and histone modifications (Anderson *et al.*, 2012).

In various species, suboptimal diets of folic acid, choline, methionine and/or vitamin B12, and those deficient in methyl groups distributed around the conception, program the insulin axis and increase the carbohydrate metabolism of the offspring. Altmann *et al.* (2012 and 2013) showed that both restricted and excess protein during gestation in pigs could alter the expression of key genes involved in methionine metabolism in the liver and muscle of the offspring. These genes are important for chromosome condensation and overall DNA methylation. In rats, a diet deficient in methyl groups alters the liver proteome of the offspring in adulthood (Maloney *et al.*, 2013). For these reasons, nutritional supplementation, particularly of micronutrients, is being developed in birds in an attempt to programme the animals'

metabolism at an early stage and thus enable them to make better or different use of the nutrients available to them.

Conclusion

The very early manipulation of the environment in which the embryo develops is very promising for analysing the influence of early conditions on the development of long-term phenotypes, whether on growth criteria, body composition, tissue characteristics, robustness to challenges or adaptation to different breeding conditions. These approaches aim to increase the efficiency and quality of production (chick and meat) as well as the adaptability of animals to different environments. In addition to the developmental, health and welfare benefits for the animals, these feeding strategies can also address environmental and economic interests.

Trans-generational transmission studies are still anecdotal in the literature in birds and have yet to be developed. A new field of investigation is also opening up on the effects of fathers' diets and their repercussions on the development of the offspring's phenotypes. In mammals, epigenetic changes in offspring have recently been shown to be dependent on the diet of male broiler breeders (e.g., in mice). What about in birds?

The door is thus wide open for the future to program or redirect the

metabolism of embryos at an early stage. Nevertheless, there are still current issues. Are these feeding strategies in line with societal expectations? Intervention on the diets of female reproducers may seem more applicable and ethically acceptable than an injection into the egg. However, when female reproducers are so restricted, what assurance do we have that the enriched elements in the diet will not be diverted for their own benefit? Let us not omit a third possibility to modulate and redirect the metabolism of animals early, nutrition and management of animals during the first week of life, a period in which animals still show some metabolic plasticity. Regardless of the approach used, maternal nutrition, *in ovo* nutrition or early nutrition, it is essential not to restrict oneself to profitability criteria that always favour heavier animals obtained more rapidly. In addition to early nutrition, other strategies around egg incubation (influence of temperature, light intensity, etc.) are possible and these may influence the metabolism of embryos and the subsequent performance of the animals.

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Abstract

The development of the bird embryo depends on the environment in which it is immersed and the nutrients available. The first week post-hatching is often tricky, with sometimes significant postnatal mortalities due to feed delays and suboptimal environmental and transport conditions. Links between parental nutrition, egg composition and subsequent animal behaviour, performance and disease susceptibility are well established. Nutrient supplementations into the egg *via* maternal feeding or *in ovo* — injections ("*in ovo feeding*") are thus innovative strategies to optimise nutrient supply to the embryo. The aim of such approaches is to optimise the nutritional supply for breeders or embryos in order to obtain better chick quality in terms of robustness, growth and/or body composition by taking advantage of the embryonic plasticity for nutrient utilisation. The precocious manipulation of the environment in which the embryo develops is very promising for analysing the influence of early conditions on the development of long-term phenotypes, whether it is to meet the criteria of growth, body composition, tissue characteristics, robustness towards challenges or adaptation to different breeding conditions. In addition to the benefits observed for animals, these dietary strategies also attempt to respond to environmental and economic interests.

Résumé

Nouvelles stratégies alimentaires précoces au service de la production avicole

*Le développement de l'embryon d'oiseau dépend de l'environnement dans lequel il baigne et des nutriments disponibles. La période de démarrage (1^{re} semaine post-éclosion) est souvent délicate avec parfois des mortalités post-natales importantes dues aux délais d'alimentation et aux conditions d'ambiance et de transport sub-optimales. Les liens entre la nutrition parentale, la composition de l'œuf et le comportement ultérieur des animaux, leurs performances, et leur sensibilité aux maladies sont bien établis. Des suppléments en nutriments de l'œuf via l'alimentation maternelle ou via des injections *in ovo* (« *in ovo feeding* ») représentent alors des stratégies innovantes pour optimiser l'apport de nutriments à l'embryon. La finalité de telles approches est d'optimiser les apports nutritionnels des reproductrices ou des embryons en développement dans le but d'obtenir des poussins de meilleure qualité en termes de robustesse, croissance et/ou composition corporelle en tirant parti de la plasticité embryonnaire d'utilisation des nutriments. La manipulation très précoce de l'environnement dans lequel l'embryon se développe est très prometteuse pour analyser l'influence des conditions précoces sur l'élaboration des phénotypes à long terme que ce soit sur des critères de croissance, composition corporelle, caractéristiques tissulaires, robustesse vis-à-vis de challenges ou adaptation à des conditions d'élevage différentes. Outre les bénéfices observés pour les animaux, ces stratégies alimentaires tentent de répondre aussi à des intérêts environnementaux et économiques.*

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