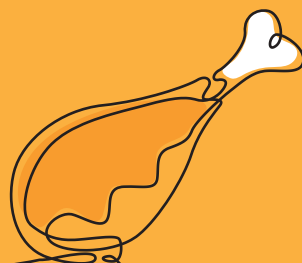


# Broiler breeder nutrition affects chick quality and offspring performance Fat or lean or in-between?

**Jesse Heijmans**



## **Propositions**

1. Hatchling quality does not predict post-hatch performance.  
(this thesis)
2. Body protein mass is more important for broiler breeder performance than body fat mass.  
(this thesis)
3. Objective statistics depend on subjective preferences.
4. Thermal heat storage is needed for a sustainable energy future.
5. A PhD defence is stealing the spotlight from a team-effort.
6. High energy prices put pressure on inspiration and innovation.
7. Pregnancy and maternity are great ways to realize deadlines and priorities.

Propositions belonging to the thesis entitled

‘Broiler breeder nutrition affects chick quality and offspring performance:

Fat or lean or in-between?’

Jesse Heijmans

Wageningen, 26<sup>th</sup> of April 2023

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Fat or lean or in-between?

**Jesse Heijmans**

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This research was conducted under the auspices of the Graduate School Wageningen Institute of Animal Sciences (WIAS)

# **Broiler breeder nutrition affects chick quality and offspring performance**

Fat or lean or in-between?

**Jesse Heijmans**

## **Thesis**

submitted in fulfilment of the requirements for the degree of doctor  
at Wageningen University  
by the authority of Rector Magnificus,  
Prof. Dr A.P.J. Mol,  
in the presence of the  
Thesis Committee appointed by the Academic Board  
to be defended in public  
on Wednesday 26 April 2023  
at 1:30 p.m. in the Omnia Auditorium.

Jesse Heijmans

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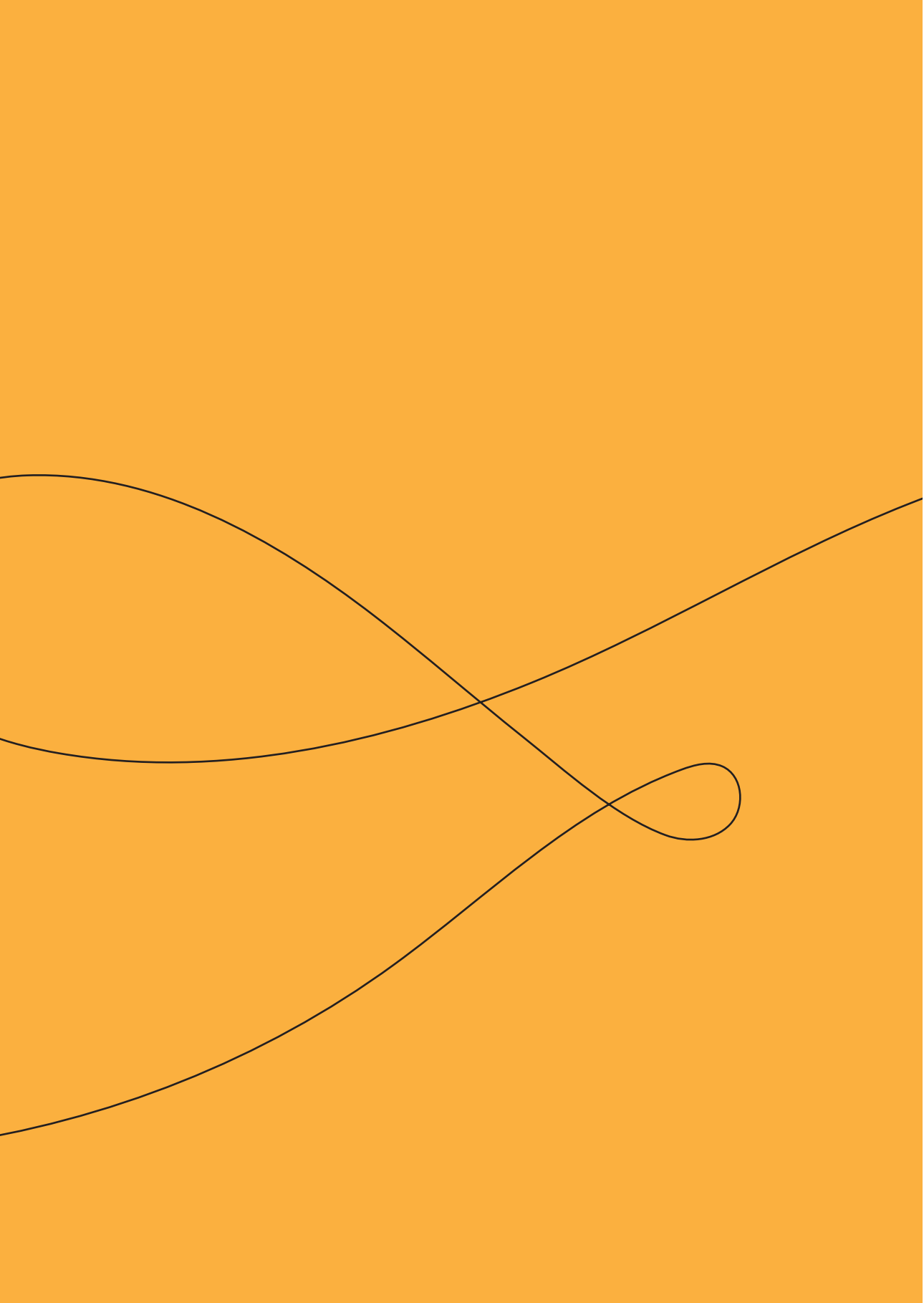
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# CHAPTER I

## General introduction

*‘Als je maar blijft trappen, kom je vanzelf boven’  
Ons pap en ons mam*

Global meat consumption is expected to increase by 14% in the next decade, mainly due to population growth in emerging economies. The associated increase in global meat production will mainly derive from growth in poultry production (OECD/FAO, 2021). This increase in meat production should be achieved in a sustainable and responsible way (FAO, 2021). This includes efficient use of resources, but also promoting welfare of animals. An important aspect of the poultry meat production chain is the quality of day-old chicks (Decuypere et al., 2001). A good day-old chick quality is crucial for performance of broilers, but also for their health and welfare (Tona et al., 2005; Van de Ven et al., 2012). Strategies aiming for an improvement in chick quality might therefore help to improve sustainability in the poultry meat production chain.

Most research on improving chick quality has focused on factors post-oviposition and during incubation, for example egg handling, egg storage or incubation temperature (Molenaar et al., 2010; Nariñ and Aydemir, 2021). Factors pre-oviposition, like maternal nutrition, potentially also affect chick quality and broiler performance (Moraes et al., 2014; Lesuisse et al., 2017, 2018b; Moraes et al., 2019; Zukiwsky et al., 2021). Information, however, of the impact of maternal nutrition on chick quality and broiler performance is scarce and the exact mechanism remains unclear.

A mother provides the first environment of an individual's life in both mammals and avian species. In this first environment, also referred to as the prenatal or pre-oviposition environment, a mother passes on both genetic and non-genetic information to her offspring. The prenatal or pre-oviposition environment can have both short and long lasting effects on offspring fitness and developmental plasticity (Mousseau and Fox, 1998). Two key mediators in non-genetic information transfer to offspring are nutrient resources (Christians, 2002; Giordano et al., 2014; Lesuisse et al., 2017) and hormonal cues (Groothuis et al., 2005) of the mother. The quantity and quality of non-genetic information transfer depends on the environment (including nutrition) the mother encounters during the rearing and reproductive phase. This allows the mother to transfer information about the local environment to her offspring. In poultry meat production, the offspring (broiler) is not housed in the same environment as the mother hen (broiler breeder). Consequently, it is important to investigate the impact of the maternal environment on offspring's phenotype in order to optimize broiler health, welfare and performance. In this thesis, I will focus on the nutrient resources of the broiler breeder hen as mediator for chick quality and offspring performance.

In this *General Introduction*, firstly, chick quality will be defined and the relationship between chick quality and broiler performance will be discussed. Secondly, the relationship between egg characteristics and chick quality will be summarized. Thirdly,

the relationship between breeder diet composition, breeder nutrient intake, breeder body composition and egg characteristics will be discussed. Finally, potential transgenerational epigenetic effects, which are not directly measurable in egg characteristics will be discussed. Figure 1 provides a schematic overview of the subjects discussed in this *General Introduction*. At the end of this *General Introduction*, the aim and outline of this thesis will be presented.

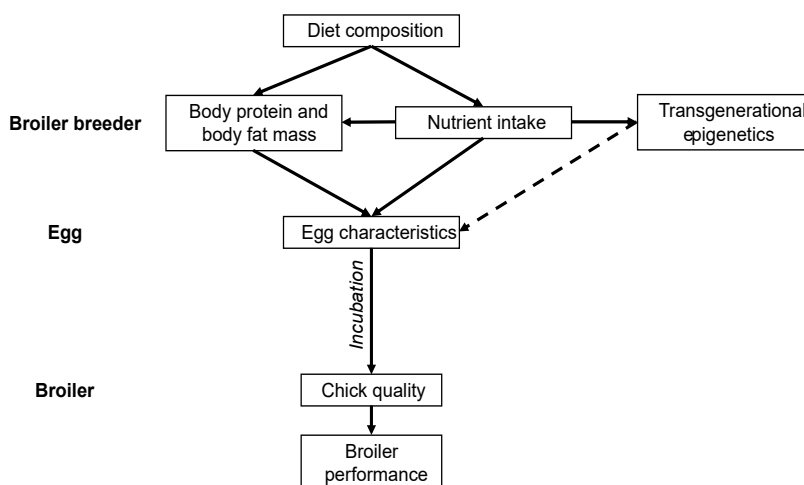


Figure 1. Schematic overview of the discussed subjects in this General Introduction. Solid lines indicate relationships between boxes. The dotted line indicates potential relationship between transgenerational epigenetics and egg characteristics.

## Chick quality

Chick quality is a difficult trait to define (Willemsen et al., 2008; Nariç and Aydemir, 2021), but is mainly focused on quality parameters directly post-hatch. Criteria for selection of high quality, first grade chicks directly post-hatch are not standardized and depend on the judgement of individual persons (Decuypere and Bruggeman, 2007). In the hatchery, chick quality is scored on a binomial scale where chicks are divided into first grade and second grade chicks at pulling. In general, a high quality, first grade chick at hatch is defined as a dry, clean chick with bright eyes, free of deformities, injury or oedema, has a completely closed navel and a fully retracted yolk, is alert and interested in the environment (Nariç and Aydemir, 2021). Chicks that don't have these properties are classified as second grade and, in practice, are culled directly at pulling. In the hatchery, chick quality of a batch of chicks is expressed as the percentage of first grade chicks relative to total hatched chicks. Several quantitative and qualitative methods have been developed to measure chick quality. Quantitative

methods include chick weight, chick length, residual yolk weight and yolk-free body mass (Narinç and Aydemir, 2021). In general, a higher value of these parameters is interpreted as a better chick quality. Qualitative methods include scoring of activity, appearance, navel condition, legs and beak (Narinç and Aydemir, 2021), where an active, clean and dry chick, with a closed navel and no abnormalities on leg or beak is interpreted as a better chick quality. The Tona-score (0-100 scale; Tona et al., 2003) and Pasgar score (0-10 scale; Boerjan, 2002) have been developed as a qualitative scoring method to score chick quality at hatch. Additionally, first week mortality post-hatch is also an important indicator for chick quality (Yassin et al., 2009).

Chick quality at hatch, however, is not a full predictor of later life health, welfare and performance. Van de Ven et al. (2012) observed a higher mortality (65.2% vs. 1.9%) and a lower slaughter weight (2270 vs. 2528 g) for second grade chicks, compared to first grade chicks. Tona et al. (2003, 2004) also reported a 200 to 300 g lower slaughter weight for chicks with anomalies (second grade), compared to first grade chicks. In terms of animal health and welfare and broiler farm profitability, culling of second grade chicks seems well-founded. When only first grade chicks are placed on farm, no relationship was observed between chick quality (Tona score; Willemsen et al., 2008; Pasgar score; Van de Ven et al., 2012) and post-hatch performance. Furthermore, it remains unclear what the relationship is between individual qualitative parameters assessed at hatch and post-hatch performance. Qualitative scoring of chick quality might be more informative for the quality of incubation rather than a predictor for post-hatch performance (Van de Ven et al., 2012). There seems to be a (weak) correlation of 0.1 to 0.35 between chick weight at hatch and broiler body weight at 42 d of age (Tona et al., 2005; Willemsen et al., 2008; Van de Ven et al., 2012).

Summarized, distinction between first and second grade chicks is a good predictor for post-hatch performance. Qualitative scoring does not predict post-hatch performance, whereas chick weight at hatch potentially explains a part in post-hatch performance. This suggests that other factors than chick quality explain a larger part of post-hatch performance. Potentially, breeder nutrition is one of these factors.

### **Egg characteristics in relation to chick quality**

At oviposition, a fixed amount of nutrients is stored within the egg. A fresh hatching egg contains approximately 50% protein, 40-43% lipids and 6% carbohydrates on a dry matter basis (Nangsuay et al., 2013). These egg nutrients are deposited in the yolk and albumen. The yolk is a major energy source and both the yolk and the albumen are major protein sources for tissue synthesis in the developing embryo (Noble and Cocchi, 1990; Willems et al., 2014a). During incubation, a broiler embryo can use these egg nutrients for growth and development. The amount of nutrients stored

within the egg may therefore affect chick quality and post-hatch performance. Furthermore, some specific nutrients (e.g. fatty acids) might also affect chick quality and post-hatch performance. This will be discussed in the following paragraphs.

### Total amount of egg nutrients

The total amount of nutrients stored within the egg mainly depends on egg size (Nangsuay et al., 2011, 2013). A larger hatching egg, within the same breeder age, contains more nutrients in total compared to a smaller hatching egg (Nangsuay et al., 2011, 2013). In turn, hatching egg weight is a dominant factor in determining hatch weight (Blueweiss et al., 1978; Wolanski et al., 2007), where a larger hatching egg results in a heavier (Lourens et al., 2006; Ulmer-Franco et al., 2010; Nangsuay et al., 2011; Iqbal et al., 2017) day-old chick, with a heavier yolk-free body mass (Lourens et al., 2006; Nangsuay et al., 2011) compared to day-old chicks from smaller hatching eggs. Furthermore, it was observed that broilers originating from larger eggs had a higher post-hatch growth performance compared to broilers originating from smaller eggs (Ulmer-Franco et al., 2010; Iqbal et al., 2017).

The yolk is a major energy and protein source for the developing embryo during incubation. At hatch, the yolk derived lipids are mainly stored in the adipose tissue or remained in the residual yolk. The residual yolk serves as main nutrient resource in the post-hatch phase until the first feed is consumed. In the first week after hatch, these lipids are used for growth of the gastrointestinal tract and as a source of energy (Boonsinchai, 2015). It can therefore be hypothesized that a higher yolk weight (absolute and relative) in a fresh hatching egg (at start of incubation) might be beneficial for chick quality.

The albumen is a major source of water and protein for tissue deposition in the developing embryo (Willems et al., 2014a). It has been observed that partial removal (3 mL) of albumen reduces prenatal protein availability. In layers, negative consequences were observed due to pre-incubation removal of albumen on body weight at hatch and body weight throughout their lifespan (Willems et al., 2015). Furthermore, it was observed that partial removal of albumen pre-incubation negatively affected reproductive performance (e.g. egg mass produced; Willems et al. (2013) and had negative consequences for physiology at hatch and in later life (e.g. lower glucose tolerance, higher sensitivity to insulin, higher T4/T3 ratio; Willems et al. (2014b)). It can thus be speculated that a lower absolute albumen weight might have negative consequences for chick quality and post-hatch performance.

## Specific egg nutrients

Fatty acid composition of the egg yolk might affect embryonic development and offspring quality. During incubation, the embryo is selective in uptake (Yalçın et al., 2008; Sahan et al., 2014), deposition (Noble et al., 1986) and most probably oxidation (Yalçın et al., 2008) of different yolk fatty acids. However, the physiological mechanism involved in this selective use of fatty acids remains unclear. To my knowledge, no information is yet available on the effect of specific proteins from the yolk or albumen on embryo physiology, chick quality or offspring performance.

Based on the above, it can be hypothesized that strategies aiming for a higher egg weight, by means of a higher yolk and/or albumen weight, show potential for a better chick quality and improved post-hatch performance.

## Relationship between broiler breeder nutrition and egg characteristics

Nutrients deposited in the egg originate either from mobilized body reserves of the breeder or directly from nutrients originating from her diet (Figure 2; Ekmay et al., 2014; Salas et al., 2017). Ekmay et al. (2014) observed that 87 to 90% of yolk proteins originate from body protein reserves of the breeder hen at a young (25 wk) and older (45 wk) age, whereas at peak production (29 wk) only 15% of yolk protein originated from body protein reserves and the remaining 85% of yolk proteins originated from dietary protein. In young breeders (25 and 29 wk), 68 to 71% of albumen proteins originated from dietary protein, whereas at older breeders (45 wk) only 47% of albumen proteins originated from dietary protein and the remaining fraction originated from body protein reserves. Salas et al. (2017) observed that fatty acids in the yolk of young breeders (25 wk) originated 99% from *de novo* lipogenesis in the liver of the breeder hen. Boonsinchai (2015) observed that approximately half of the glucose used for *de novo* lipogenesis originated from the diet and the other half from body protein mobilization via gluconeogenesis. At older ages (31 and 45 wk), Salas et al. (2017) observed that 45 to 48% of yolk fatty acids originated directly from dietary fat sources and 29 to 45% of yolk fatty acids originated from body fat reserves. The remaining fraction originated from *de novo* lipogenesis.

Consequently, it can be hypothesized that a change in breeder body composition or diet composition might influence nutrient deposition in the egg and that this nutrient deposition changes with age of the breeder hen. The potential explanation for the change in body and dietary resources for nutrient deposition in the egg in young and older breeders is further discussed below. Furthermore, in the following paragraphs, effects of body reserves, dietary protein and dietary energy on egg characteristics will be discussed.

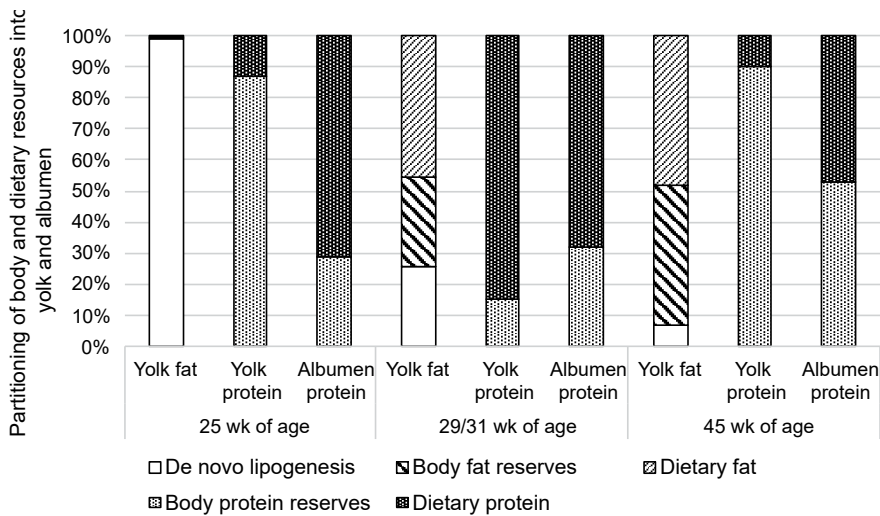


Figure 2. Relative partitioning of dietary and body resources into yolk and albumen at 3 broiler breeder ages (adapted from Ekmay et al. (2014) and Salas et al. (2017)).

## Body composition

Genetic selection for growth efficiency and carcass yield in broiler production has resulted in a significant increase in lean broiler meat production (Havenstein et al., 2003; Zuidhof et al., 2014). Consequently, modern broiler breeders also show fast muscle growth with a higher breast weight and a lower abdominal fat pad weight at entry of lay compared to broiler breeders of 1980 (Eitan et al., 2014). Body fat mass in broiler breeder hens has decreased approximately 50% over the last 30 years (Eitan et al., 2014; Zuidhof, 2018), which may have consequences for nutrient deposition in the egg (Salas et al., 2017), as discussed in the previous paragraph.

Broiler breeders pullets are restricted in their feed intake and consequently they may experience a strong drive for compensatory growth during the early laying period when feed allowance is increased. This may lead to a competition for nutrients within the body for growth and egg production. Young broiler breeders (25 wk) use dietary carbohydrates for the synthesis of fatty acids in the liver as main source of egg yolk lipids (Figure 2; Salas et al., 2017) and they may depend more on *de novo* lipogenesis for yolk lipid formation as they have a lower body fat content compared to older breeders (Caldas et al., 2018; Salas et al., 2019). Young broiler breeders may need to use the available fatty acids (synthesized *de novo* and from dietary fat) for both egg yolk and storage into body fat as they are still maturing. Spratt and Leeson (1987a) hypothesized that partitioning of energy between egg production and body tissue

growth is mainly important for young broiler breeders. In their study, broiler breeders fed a diet with a low energy content (2,167 kcal ME/kg) failed to lay adequately, while still increasing in body fat mass. This indicates that body fat deposition may occur at the expense of egg production. It is hypothesized that breeders with a higher fat reserve in the body at entry of lay can mobilize more lipids towards the yolk compared to leaner breeders. Consequently, this might be beneficial for chick quality. In peak producing breeders (31 and 45 wk), body fat is an important source for yolk fat (Figure 2; Salas et al., 2017). Using body reserves for fat deposition in the yolk might be less efficient, compared to direct use of dietary energy sources. However, evolutionary seen it might have advantages over the use of dietary energy sources. Body fat reserves can be considered as an insurance in times of variable feed (energy) resources. It can be speculated that wild birds have uncertainty about nutrient intake and therefore use a consistent flow of nutrients from the body reserves to maintain egg production. Some wild birds even rely completely on their body reserves in times of egg production and incubation (reviewed by Klasing, 1998). Broiler breeders are domesticated birds and are fed daily, in European production systems, but they are fed a restricted amount of feed. Therefore, it can be speculated that they are (partly) in a fasting state. At the moment feed is available, energy is stored in adipose tissue. Conversely, fatty acids are mobilized from adipose tissue during fasting. Origin of fat deposited in the yolk might have remained the same in wild and domesticated birds in order to maintain a constant egg production. It can be hypothesized that a minimum amount of body fat is needed to sustain a constant egg production (Li et al., 2011; Van Emous, 2015). Li et al. (2011) observed a lower egg production, but a 1.8 g higher egg weight in genetically fat breeders compared to genetically lean breeders at 7 wk of age (4.3 vs. 1.2% abdominal fat, respectively), which were pair-fed. Restricting feed intake with 25% in fat breeders led to a 1.9 g/d higher egg mass, mainly due to a 5.4% higher egg production. Furthermore, a 0.8% higher relative yolk weight and 0.9% lower relative albumen weight of fresh eggs was observed. In lean breeders, the opposite was observed, where feed restriction resulted in a 0.1 g/d lower egg mass, mainly due to a 1.7% lower production. Fresh eggs had a 0.8% lower relative yolk weight and 0.8% higher relative albumen weight. This suggests that fat and lean birds respond differently to nutritional stress. Fat breeders might be able to cope better with a restricted feed intake, as they have higher body fat reserves. Besides serving as energy storage, it can be speculated that a higher fat content in the body may decrease energy requirement for maintenance at a similar body weight and thus increase the amount of energy that can be partitioned towards the egg. Body lipid does not require maintenance energy (Gous, 2015), whereas body protein does.

Based on these results, it is hypothesized that a fatter broiler breeder might be able to partition more nutrients towards the egg, which may benefit chick quality. A higher



fat mass in the broiler breeder can be achieved by a higher breeder body weight (Van Emous et al., 2013) or by feeding a higher dietary energy-to-protein ratio (Van Emous et al., 2013; Lesuisse et al., 2017). These strategies will be discussed in the following paragraphs.

### Breeder body weight

A 8 to 43% higher breeder body weight during rearing resulted in a 25 to 227 g higher body fat mass at 20 wk of age (Van Emous et al., 2013; Van der Klein et al., 2018a; Zuidhof, 2018; Salas et al., 2019) and 29 to 65 g more breast meat (Van Emous et al., 2013; Van der Klein et al., 2018a) or 716 g more lean mass (Salas et al., 2019), as an indicator for more body protein.

Maternal body weight has a clear impact on egg weight in wild birds, where higher body weights result in higher egg weights (Blueweiss et al., 1978; Christians, 2002). In wild birds, it has been observed that a high condition mother, defined as mothers with higher body weight at each given age, invests more in her offspring, by means of more eggs or heavier eggs, as compared to a low condition mother (Price, 1998). Furthermore, egg size was also affected by feeding conditions in wild birds, where females that were given supplemental feed produced heavier eggs (Price, 1998). In current practice, broiler breeders are fed restricted quantities of feed to achieve a desired body weight at the end of rearing (Cobb Vantress, 2008; Aviagen, 2016), as *ad libitum* feeding results in a higher mortality and lower reproductive performance (Hocking et al., 2002; Heck et al., 2004; Sun et al., 2006). However, there is still a huge gap between rearing to the recommended weight of 2.2 to 2.4 kg at 20 weeks of age or rearing on an *ad libitum* feeding schedule, leading to a body weight of over 5 kg at 20 weeks of age (Heck et al., 2004).

Rearing broiler breeder pullets on a higher growth curve might be beneficial for egg weight and egg composition during production. *Ad libitum* fed broiler breeders produced 0.4 to 2.3 g heavier eggs (Hocking et al., 2002; Heck et al., 2004; Mohiti-Asli et al., 2012) with a 1.0 to 1.6% higher proportional yolk content (Chen et al., 2006; Mohiti-Asli et al., 2012) and a 1.1% lower proportional albumen content (Mohiti-Asli et al., 2012) compared to broiler breeders fed according to commercial growth curve recommendations. Other studies observed no effect of a 13 to 20% higher body weight at 20 wk of age on egg weight (Gous and Cherry, 2004; Renema et al., 2007; Ekmay et al., 2012; Van Emous et al., 2013; Van der Klein et al., 2018b; Salas et al., 2019) or a 0.9 g (Sun and Coon, 2005) to 1.9 g (Renema et al., 2001) increase in egg weight. Reasons for this discrepancies include:

- Different growth curves up to 20 wk of age were converged to a similar body weight during the laying period (Renema et al., 2007; Van Emous et al., 2013).
- Feed allowance was fixed at a similar level for different growth curves during the laying period, irrespective of body weight differences at 20 wk of age (Gous and Cherry, 2004; Sun and Coon, 2005; Ekmay et al., 2012; Salas et al., 2019).
- Body weight gain was fixed at a similar level for different growth curves during the laying period, irrespective of body weight differences at 20 wk of age (Renema et al., 2001; Van der Klein et al., 2018b)

None of the above mentioned studies have maintained a relative difference in growth curve during the laying period as well. The amount of feed available for growth and egg production depends on the amount of feed that is used for maintenance. The latter one being mainly dependent on body weight (Caldas et al., 2018; Hadinia et al., 2018) and to a lower extent on body composition (Gous, 2015). A fixed body weight, growth curve or feed allowance during the laying period probably reduced the amount of feed available for growth and egg production for heavier breeders compared to lighter breeders. Consequently, lighter breeders may benefit from this, as they have more nutrients available for egg production and thus produce similar egg weights as heavier breeders (Gous and Cherry, 2004; Sun and Coon, 2005; Renema et al., 2007; Ekmay et al., 2012; Van Emous et al., 2013; Van der Klein et al., 2018b; Salas et al., 2019). In the study of Renema et al. (2001), a relative higher feed intake for heavier breeders was maintained during production. This resulted in a 1.1 to 1.9 g heavier egg weight for heavier breeders. It is therefore hypothesized that a heavier breeder will produce heavier eggs with a larger yolk, but only when relative differences in body weight, and thus feed intake, are maintained during the laying period. This might be beneficial for chick quality and offspring performance.

## Dietary protein

Several authors have investigated effects of a lower dietary crude protein intake on broiler breeder performance and egg characteristics (Joseph et al., 2000; Mohiti-Asli et al., 2012; Van Emous et al., 2013, 2015; Steenhuisen and Gous, 2016; Lesuisse et al., 2017, 2018a; Table 1). Some studies used the pair-feeding strategy among treatment groups during the laying period (Spratt and Leeson, 1987; Joseph et al., 2000; Mohiti-Asli et al., 2012; England et al., 2014; Steenhuisen and Gous, 2016), leading to a higher body weight for breeders with a higher protein intake. Other studies used the pair-gaining strategy among treatment groups during rearing alone (Van Emous et al., 2013, 2015) or during both rearing and laying (Lesuisse et al., 2017, 2018a). If a similar body weight was maintained among treatment groups (i.e. pair-gaining), a higher feed intake was observed for broiler breeders fed a lower dietary protein level

Table 1. Effect of dietary protein content in the broiler breeder diet on egg characteristics. Studies 1 to 5 are based on pair-feeding strategies during the laying period. Studies 6 and 7 are based on pair-gaining strategies during the rearing period. Studies 8 and 9 are based on pair-gaining studies during the rearing and laying period.

Ref <sup>1</sup>	Breed	Reduction crude protein by (raw materials)	Age (wk)		n	Dietary crude protein (%)	Total lysine (%)	Energy level diet (kcal/kg)	Feed intake (g/d)	CP intake (g/d)	Energy intake (kcal/d)	kcal / g protein	Egg weight (g)	Yolk Albumen Shell		
			Diet <sup>2</sup>	Data <sup>3</sup>										% of egg weight		
Pair-feeding studies laying period																
1	Hubbard	Soybean meal	19-41	28-40	15	12.7	0.64	2578 ME	150	19.0	387	20.4		27.9	61.6	10.6
						16.7	0.91	2578 ME	150	25.0	387	15.5		27.6	61.8	10.6
2	Cobb 500	Soybean meal	20-29	24-29	32	14.8	0.67	2760 ME	133	19.7	367	18.6	50.1		63.9	
		Corn gluten meal				16.4	0.78	2830 ME	133	21.8	376	17.3	51.3		66.6	
		Meat bone meal				18.5	0.86	2840 ME	133	24.6	377	15.4	51.5		64.7	
3	Ross 308	Soybean meal	43-55	43-55	30	14.5	0.65	2800 AME	174	25.2	487	19.3	59.2	31.6	59.9	8.5
		Corn gluten				17.4	0.78	2800 AME	173	30.1	484	16.1	60.5	31.7	59.5	8.8
4	Cobb 700	Soybean meal	23-40	26-40	12	15.2	0.66 <sup>4</sup>		154	23.4	441	18.8	58.0			
						17.1	0.79 <sup>4</sup>		154	26.3	441	16.8	58.7			
5	Ross 308	Soybean meal	26-33	31-33	15	11.8	0.40	2866 AME <sub>n</sub>	156	18.5	447	24.2	57.5	29.4	61.3	9.3
		(blending of diets)				12.9	0.47	2866 AME <sub>n</sub>	160	20.6	459	22.3	60.5	29.2	61.6	9.2
						14.1	0.55	2866 AME <sub>n</sub>	159	22.4	456	20.3	61.3	29.3	61.5	9.2
						15.2	0.62	2866 AME <sub>n</sub>	159	24.2	456	18.8	61.0	29.2	61.7	9.1
						16.4	0.70	2866 AME <sub>n</sub>	155	25.4	444	17.5	62.4	29.2	61.4	9.4
						17.5	0.77	2866 AME <sub>n</sub>	152	26.6	436	16.4	64.2	29.2	61.7	9.1
Pair-gaining studies rearing period																
6	Ross 308	Soybean meal	2-22	23-40	16	12.8 <sup>5</sup>	0.47	2700 AME <sub>n</sub>	78.4	10.2	209	21.1	57.5			
		Rapeseed meal				14.0 <sup>5</sup>	0.52	2700 AME <sub>n</sub>	74.5	10.5	199	19.3	57.5			
		Corn gluten				14.9 <sup>5</sup>	0.56	2700 AME <sub>n</sub>	71.3	10.8	190	18.1	57.2			
7	Ross 308	Soybean meal	2-22	23-40	18	12.6 <sup>5</sup>	0.47	2700 AME <sub>n</sub>	83.1	10.6	222	21.4	63.7			
		Rapeseed meal				15.1 <sup>5</sup>	0.56	2700 AME <sub>n</sub>	73.6	11.0	197	17.9	63.7			



Table 1. Continued

Ref <sup>1</sup>	Breed	Reduction crude protein by (raw materials)	Age (wk)		n	Dietary crude protein (%)	Total lysine (%)	Energy level diet (kcal/kg)	Feed intake (g/d)	CP intake (g/d)	Energy intake (kcal/d)	kcal / g protein	Egg weight (g)	Yolk Albumen Shell		
			Diet <sup>2</sup>	Data <sup>3</sup>										% of egg weight		
Pair-gaining studies rearing and laying period																
8	Pure line A (F0)	Soybean meal	0-40	24-40	5	11.6	0.41	2792 AME <sub>n</sub>	169	19.6	471	24.2	53.2	29.7	56.5	13.9
						16.2	0.56	2791 AME <sub>n</sub>	156	25.2	435	17.3	57.6	29.3	57.5	13.1
9	Pure line A (F1)	Soybean meal	0-42	27-42	4	11.4	0.41	2792 AME <sub>n</sub>	160	18.2	447	24.5	66.5	30.5	55.7	13.8
						15.6	0.56	2791 AME <sub>n</sub>	143	22.3	399	17.9	67.5	30.9	56.2	12.9

<sup>1</sup>References: 1) Spratt and Leeson (1987a), 2) Joseph et al. (2000), 3) Mohiti-Asli et al. (2012), 4) England et al. (2014), 5) Steenhuisen and Gous (2016), 6) Van Emous et al. (2013), 7) Van Emous et al. (2015) , 8) Lesuisse et al. (2017), 9) Lesuisse et al. (2018a).

<sup>2</sup>Age when dietary treatments were applied. <sup>3</sup>Age when data was collected. <sup>4</sup>Digestible lysine instead of total lysine. <sup>5</sup>Diets were fed during the rearing period, diet composition based on pre-breeder diets (15-22 wk of age). Feed and nutrient intake based on rearing period (2 to 22 wk of age). Egg weight based on the laying period (23 to 40 wk of age) .

and this resulted in a higher body fat content (Van Emous et al., 2013, 2015; Lesuisse et al., 2017, 2018a).

The impact of dietary protein level on egg characteristics varied depending on feeding strategy and when the diets were fed. In summary, dietary protein intake during rearing alone appears to have a minimal effect on egg characteristics (Van Emous et al., 2013, 2015). A lower dietary protein intake during lay resulted in a lower egg weight, mainly due to a lower albumen weight (Spratt and Leeson, 1987; Joseph et al., 2000; England et al., 2014; Steenhuisen and Gous, 2016; Lesuisse et al., 2017, 2018a). Consequently, this might have negative effects on chick quality and offspring performance. This might also indicate that a higher body fat content, achieved by a lower dietary protein content, might not be beneficial for egg characteristics, chick quality and offspring performance.

### Dietary energy

Dietary fat and starch largely contribute to the energy level in a diet. In most studies, effect of a higher dietary energy level is confounded with a higher fat level or a higher starch level, which may affect nutrient partitioning towards the yolk (Salas et al., 2017). It is therefore important to consider which energy source is used in the diets. Table 2 gives an overview of the studies related to energy intake of broilers breeders. Again, studies differed in experimental approach. Some studies used a pair-feeding strategy during the laying period (Spratt and Leeson, 1987; Peebles et al., 2000; Zaghari et al., 2018), whereas another study used a pair-gaining strategy during the laying period (Van Emous et al., 2015), or neither feed intake nor body weight was similar among treatment groups (Salas et al., 2019), or information about breeder nutrient intake is lacking (Moraes, 2013). In case a pair-feeding strategy was applied, a higher energy level with a similar feed intake resulted in heavier breeders (Spratt and Leeson, 1987). In case a pair-gaining strategy was applied, a higher dietary energy level led to a lower feed intake (Van Emous et al., 2015). To-date, no studies have investigated the impact of dietary energy level during both the rearing and laying period in broiler breeders on egg characteristics.

Results on effects of energy intake on egg weight and egg composition are inconsistent (Table 2). In summary, altering dietary energy content during rearing alone appears to have minimal effects on egg characteristics (Moraes, 2013). A higher dietary energy content during laying alone might affect not affect egg weight (Moraes, 2013; Van Emous et al., 2015; Zaghari et al., 2018; Salas et al., 2019) or lead to a higher egg weight (Spratt and Leeson, 1987; Van Emous et al., 2015). A higher dietary energy level resulted in higher (Spratt and Leeson, 1987; Peebles et al., 2000), similar (Peebles et al., 2000) or lower (Moraes, 2013) yolk weight. Discrepancy between studies might

Table 2. Effect of dietary energy content in the broiler breeder diet on egg characteristics. Studies 1 to 3 are based on pair-feeding strategies during the laying period. Study 4 is based on pair-protein-feeding strategy during the laying period. Study 5 is based on pair-gaining strategy during the laying period. Exact feeding strategy and nutrient intake are unknown from study 6.

Ref <sup>1</sup>	Breed	Energy source	Raw material	Energy source	Age (wk)	n	Energy level diet (kcal/kg)	Feed intake (g/d)	Energy intake (kcal)	Crude protein intake (g/d)	kcal / g protein	% of egg weight				
												Diet <sup>2</sup>	Data <sup>3</sup>	Egg weight (g)	Yolk	Albumen
Pair-feeding studies laying period																
1	Hubbard	Starch	Corn		19-41	28-40	10	2167 ME	150	325	22.0	14.8	60.3	26.5	62.8	10.8
								2567 ME	150	385	22.0	17.5	60.9	28.1	61.3	10.6
								3000 ME	150	450	22.0	20.5	62.1	28.4	61.0	10.5
2	Arbor Acres	Fat	Poultry fat		22-47	26-47	4	2709 ME	159	430	23.0	18.7	28.5	28.5	62.6	8.9
		Starch	Corn					2709 ME	159	430	22.9	18.8	28.8	28.8	62.2	9.0
		Fat	Poultry fat					2940 ME	159	467	23.0	20.3	29.4	29.4	61.7	8.9
		Starch	Corn					2940 ME	159	467	21.6	21.6	28.7	28.7	62.4	8.9
3	Ross 308	Fat	Corn oil		40-49	40-49	4	2790 AME <sub>n</sub>	162	453	24.0	18.9	65.0			
								2800 AME <sub>n</sub>	162	463	24.0	19.3	64.5			
								2810 AME <sub>n</sub>	162	473	24.0	19.7	65.0			
								2820 AME <sub>n</sub>	162	483	24.0	20.1	64.2			
Pair-protein-feeding study laying period																
4	Cobb 500	-	-		21-65	21-65	8	2860 ME	115	330	23.9	13.8	64.5			
								2860 ME	126	360	24.0	15.0	66.2			
								2860 ME	136	390	23.9	16.3	64.2			
								2860 ME	147	420	24.1	17.4	65.1			
								2860 ME	157	450	24.0	18.8	64.5			
								2860 ME	168	480	24.0	20.0	64.1			



Table 2. Continued

Ref <sup>1</sup>	Breed	Energy source	Raw material energy source	Age (wk)		n	Energy level diet (kcal/kg)	Feed intake (g/d)	Energy intake (kcal)	Crude protein intake (g/d)	kcal / g protein	Egg weight (g)	% of egg weight			
				Diet <sup>2</sup>	Data <sup>3</sup>								Yolk	Albumen	Shell	
Pair-gaining study laying period																
5	Ross 308	Starch and fat	Corn starch and soya oil	22-45	22-45	12	2600 AME <sub>n</sub>	165	428	23.5	18.2	60.1				
							2800 AME <sub>n</sub>	153	429	22.0	19.5	60.3				
							3000 AME <sub>n</sub>	141	424	20.5	20.7	60.3				
		Starch and fat	Corn starch and palm oil	45-60	45-60	18	2800 AME <sub>n</sub>	151	424	21.5	19.8	69.1				
							3000 AME <sub>n</sub>	138	415	19.4	21.4	68.7				
Unknown feeding strategy																
6	Ross 708	Starch	Corn	3-24	29-37	5	2528 ME					59.3	29.1	62.1	8.8	
							2736 ME					59.4	29.2	62.0	8.8	
				25-37	29-37	5	2800 ME					59.2	29.3	61.9	8.8	
		Fat	Canola oil				2900 ME					59.5	29.0	62.2	8.8	

<sup>1</sup>References: 1) Spratt and Leeson (1987), 2) Peebles et al. (2000), 3) Zaghari et al. (2018), 4) Salas et al. (2019), 5) Van Emous et al. (2015), 6) Moraes (2013).

<sup>2</sup>Age when dietary treatments were applied. <sup>3</sup>Age when data was collected.

be due to a difference in breeder age, where a lower dietary energy level mainly impacted egg and yolk weight in young breeders (< 35 wk of age; Peebles et al. (2000)) or in older breeders (> 45 wk of age; Van Emous et al. (2015)). It remains unclear whether or not a higher dietary energy content during both rearing and laying affects egg characteristics. Furthermore, it is unknown whether or not a higher body fat content, achieved by a higher dietary energy content, might affect egg characteristics.

### **Potential transgenerational epigenetic effects**

Transgenerational epigenetics is the transfer of parental environmental information to the offspring by encoded changes in gene expression, without changes in the nucleotide sequence of the DNA (Choi and Friso, 2010; Berghof et al., 2013; Burton et al., 2022). These parental environmental conditions induce changes in gene expression and then influence the phenotype of the offspring in an attempt to maximize the offspring's fitness and affect their developmental plasticity (Mousseau and Fox, 1998).

In mammalian models (e.g. rodents and ovine), there is a good body of evidence that maternal feed restriction and diet composition during gestation can affect offspring's phenotype (Levin, 2008; George et al., 2012; Lagisz et al., 2014, 2015), which are mainly guided by epigenetic changes (Ford and Long, 2012). These studies demonstrate that maternal feed restriction and maternal protein restriction can lead to a higher susceptibility for metabolic disorders in their offspring, but may also benefit the offspring when raised in malnutritional environments. Unlike mammalian fetal development, whereby nutrients are supplied constantly across the placenta, deposition of nutrients into an avian egg are fixed at start of embryonic development. It is therefore unclear if a similar epigenetic mechanism occurs in avian species. A body of evidence is lacking in avian species for this mechanism, although there are some indications that a similar mechanism occurs in avian species as in mammalian species. For example, Lesuisse et al. (2018b) observed a higher nitrogen efficiency and performance in offspring originating from breeders with a 22% lower protein intake during rearing and production compared to a standard protein intake. It was speculated that a lower breeder dietary protein intake 'programmed' the offspring to be efficient with protein, as this nutrient was limited available in the maternal environment. This resulted in an improved broiler performance.

This indicates the maternal dietary energy-to-protein ratio may induce transgenerational epigenetic effects, with consequences for offspring performance. It remains unclear whether or not a change in dietary energy content may also induce transgenerational epigenetic effects, which potentially may affect chick quality and offspring performance as well. Although, these epigenetic effects cannot be ruled out in trans-



generational studies, they fall outside of the scope of this thesis. In this thesis, only the nutritional pathways via the egg will be taken into account.

## Aim of this thesis

The aim of this thesis is to investigate effects of broiler breeder nutrition on chick quality and offspring performance. In this thesis, the impact of maternal body reserves and dietary energy-to-protein ratio is investigated on egg characteristics, chick quality and broiler performance. The following hypotheses are formulated:

1. A higher broiler breeder body weight results in higher egg weights, which will improve chick quality and offspring performance.
2. A fatter broiler breeder can partition more nutrients towards the egg, resulting in better chick quality and broiler performance.

Figure 3 gives a visual overview of the hypotheses and potential interfering factors.

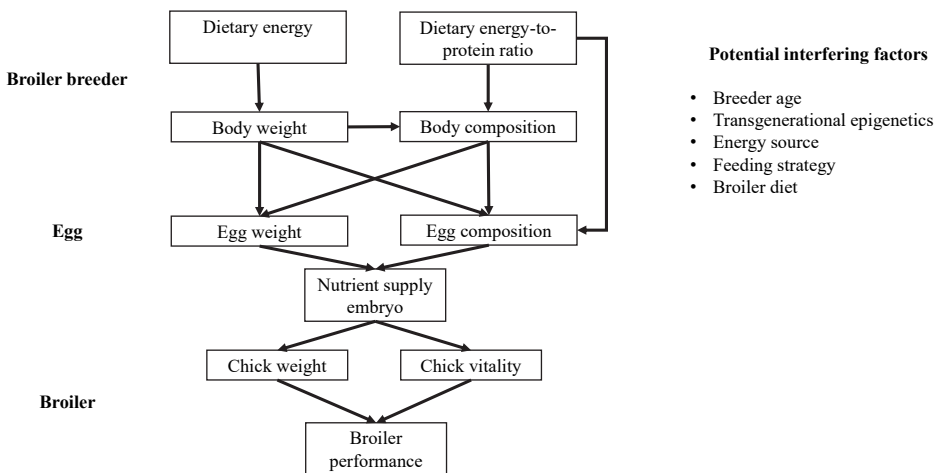


Figure 3. Hypotheses, parameters of interest and potential interfering factors evaluated within this thesis.

## Outline of this thesis

Two studies were conducted to answer these hypotheses (Figure 4). Within each study one breeder experiment and two consecutive broiler experiments were conducted. In the first study (*Chapters 2 to 5*), the impact of breeder body weight and dietary energy-to-protein ratio was investigated. In this study, breeders were assigned to 2 different growth curves, a standard growth curve and an elevated growth curve, which targeted a 15% higher body weight throughout rearing and production. Within each growth

curve, 4 dietary energy-to-protein ratios, ranging from 96% AME<sub>n</sub> to 108% AME<sub>n</sub>, were fed on a pair-gain basis. All breeder treatments were applied from hatch until 60 wk of age. At 28 and 36 wk of age, hatching eggs from these breeders were incubated. Chick quality was assessed at hatch and broiler performance followed until slaughter. *Chapter 2* describes the findings of this study on productive performance of the breeders. *Chapter 3* describes the findings on egg quality and egg composition. *Chapter 4* describes the findings on breeder body composition and energetic efficiency. *Chapter 5* describes the findings on chick quality and broiler performance.

Based on the findings of the first study, it was decided to further investigate the interaction between maternal dietary energy-to-protein and offspring dietary protein content. Furthermore, we aimed to evaluate which breeder period (rearing or laying) has a larger impact on chick quality and offspring performance. In this second study, all breeders were fed on an elevated growth curve with 2 dietary energy-to-protein ratios (96% AME<sub>n</sub> and 104% AME<sub>n</sub>) on a pair-gain basis during 2 periods (rearing and laying) in a 2 x 2 factorial approach. At 29 and 38 wk of age, hatching eggs from these breeders were incubated and chick quality was assessed at hatch. Broilers were fed 3 dietary protein contents (low, medium, high protein) and broiler performance was followed until slaughter. *Chapter 6* describes the results of this second study on chick quality and broiler performance. In *Chapter 7*, findings from both studies are combined and discussed. Furthermore, practical implications are suggested for new feeding strategies aiming for an improved chick quality and broiler performance and suggestions for future research are provided.

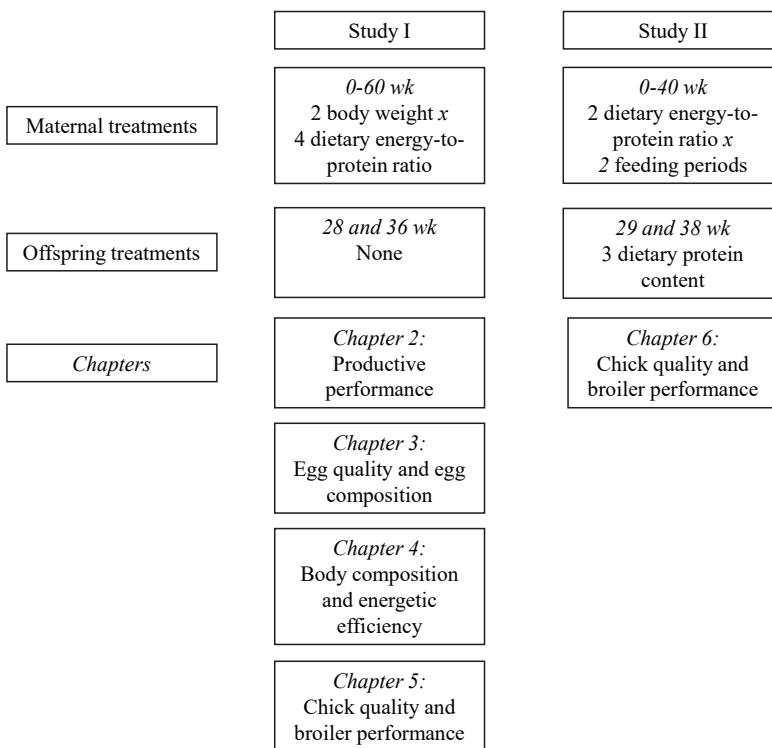


Figure 4. Schematic overview of the studies that were conducted in this thesis and themes per chapter of this thesis.

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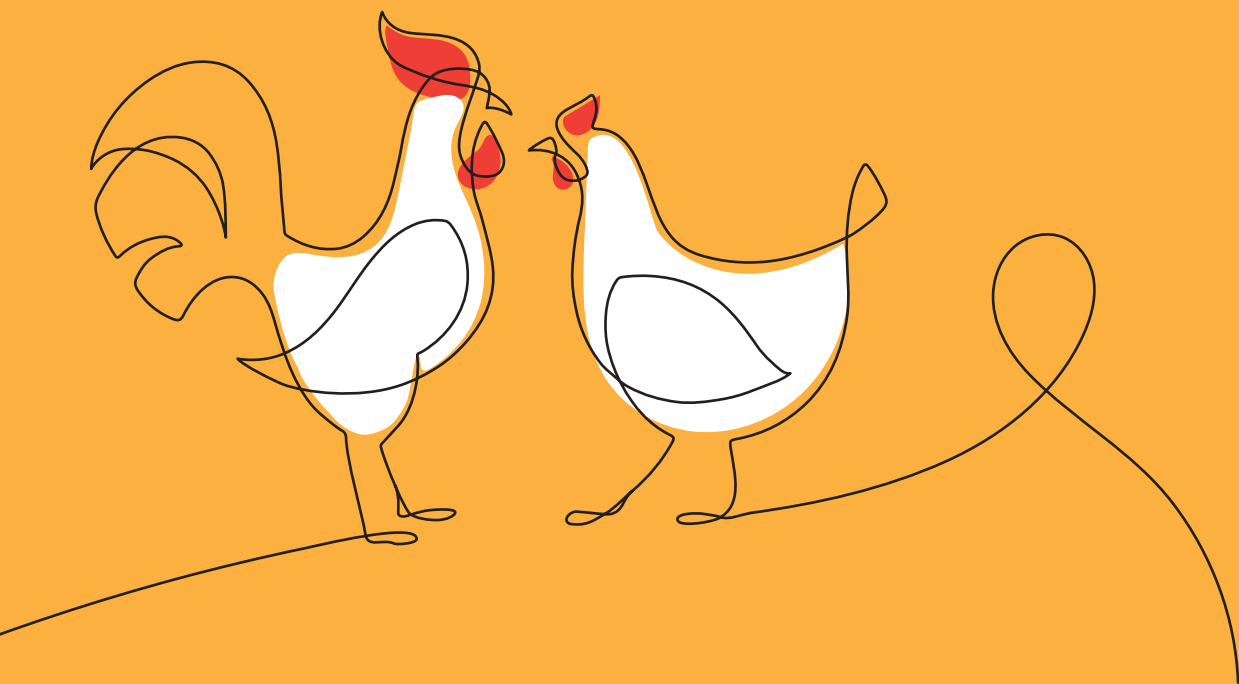
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# Chapter 2

## Impact of growth curve and dietary energy-to-protein ratio on productive performance of broiler breeders

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*‘Tok-tok’  
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## ABSTRACT

The impact of growth curve (GC) and dietary energy-to-protein ratio on productive performance of broiler breeder females was investigated from 0 to 60 wk of age. One-day-old pullets ( $n = 1,536$ ) were randomly allotted to 24 pens according to a  $2 \times 4$  factorial arrangement, with 2 GC (standard growth curve = SGC or elevated growth curve = EGC, +15%) and 4 diets, differing in energy-to-protein ratio (96%, 100%, 104%, or 108% AME<sub>n</sub>). Feed allocation per treatment was adapted weekly based on the desired GC, meaning that breeders fed the different diets within each GC were fed according to a paired-gain strategy. Linear and quadratic contrasts for energy-to-protein ratio for each GC were evaluated. Elevated growth curve breeders had an earlier sexual maturity ( $\Delta = 4.1$  d) than SGC breeders. Egg weight was higher for EGC breeders ( $\Delta = 2.3$  g) than for SGC breeders over the whole laying phase (22-60 wk). No differences between EGC and SGC breeders were observed on settable egg production. An increase in dietary energy-to-protein, at a similar BW, led to a linear increase in age at sexual maturity ( $\beta = 0.14$  d/% AME<sub>n</sub>). From 22 to 40 wk of age, an increase in dietary energy-to-protein ratio led to a linear decrease in egg weight ( $\beta = -0.06$  g/% AME<sub>n</sub>), regardless of GC. An interaction between GC and dietary energy-to-protein ratio was observed on settable egg production in this phase. An increase in dietary energy-to-protein ratio led to a linear decrease on settable egg production, which was more profound in EGC breeders ( $\beta = -0.70$  eggs/% AME<sub>n</sub>) than in SGC breeders ( $\beta = -0.19$  eggs/% AME<sub>n</sub>). From 41 to 60 wk of age, an interaction between GC and dietary energy-to-protein ratio was observed on egg weight. In the EGC, an increase in dietary energy-to-protein ratio led to a linear decrease in egg weight ( $\beta = -0.13$  g/% AME<sub>n</sub>), whereas in the SGC, a linear increase in egg weight was observed ( $\beta = 0.03$  g/% AME<sub>n</sub>). From 41 to 60 wk of age, no differences between diets were observed on settable egg production. It can be concluded that a higher GC of breeders has beneficial effects on egg weight, while maintaining settable egg production. Feeding breeders a lower dietary energy-to-protein ratio stimulated productive performance of broiler breeder hens, mainly during the first phase of lay. This effect was more profound when breeders were fed according to a higher GC.

**Key words:** broiler breeder, feed strategy, sexual maturity, egg production, egg weight

## INTRODUCTION

Broilers are genetically selected for high growth of muscle tissue and low body fat (Renema et al., 2007b; Zuidhof et al., 2014). This genetic selection has changed the body composition of broiler breeder hens as well (Eitan et al., 2014). Body fat mass in broiler breeder hens has decreased approximately 50% over the last 30 years (Eitan et al., 2014; Zuidhof, 2018). Several studies suggested that body fat mass of the broiler breeder hen plays an important role in sexual maturation (Bédécarrats et al., 2016; Zuidhof, 2018; Hadinia et al., 2020), egg production (Van der Klein et al., 2018), egg composition (Salas et al., 2017), and laying persistency (Van Emous et al., 2015). Concerns have recently been raised that a biological minimum of body fat mass may be approached or even reached in modern broiler breeder hens, which may endanger reproductive success (Van der Klein et al., 2018; Zuidhof, 2018; Hadinia et al., 2020).

In broiler breeders, changes in body composition are often due to either differences in growth curve or diet composition. A higher growth curve during rearing resulted in a higher body fat mass at 20 wk of age (Sun and Coon, 2005; Van Emous et al., 2013; Salas et al., 2019). Feeding a higher dietary energy-to-protein ratio during rearing, while maintaining a similar growth curve by pair-gaining, led to a higher body fat mass and a lower body lean mass at 20 wk of age compared to a lower dietary energy-to-protein ratio (Van Emous et al., 2013, 2015). In the indicated studies, however, contrasts in growth curve or diet composition were only maintained until 20 wk of age, resulting in breeders having the same body fat mass during production, irrespective of the initial BW and body fat mass differences at 20 wk of age (Sun and Coon, 2005; Van Emous et al., 2013, 2015; Salas et al., 2019). This may explain the absence of differences in egg production in these studies. Consequently, it can be hypothesized that differences in productive performance can only be reached when differences in growth curve or diet composition are maintained during the production phase.

This hypothesis is supported by Renema et al. (2001a) and Van der Klein et al. (2018), who maintained differences in growth curve during the production period and observed a higher total egg production when breeders were also heavier and fatter during production. Van der Klein et al. (2018) suggested that the higher productive performance of breeders on a higher growth curve was due to a higher body fat mass, but is it unclear whether or not a higher body lean mass of the heavier breeders might have played a role as well. Consequently, it remains unclear from these studies which of these variables is more dominant in determining effects on productive performance of broiler breeder hens.

Lesuisse et al. (2017, 2018) studied effects of a higher dietary energy-to-protein ratio, obtained by lowering the dietary crude protein content, during rearing and production. They observed a higher body fat mass during production when feeding a higher dietary energy-to-protein ratio, at a similar BW. In this study, a rather severe reduction of 25% in dietary crude protein led to a lower productive performance of the breeder hens. These findings suggest that a higher body fat mass, obtained by feeding less dietary crude protein, might not be beneficial for productive performance. It remains unclear whether a higher body fat mass, obtained by an increasing dietary energy content, rather than a decreasing dietary crude protein content, might affect productive performance.

The aim in the current study was to disentangle effects of body composition and growth curve on productive performance, by feeding diets differing in energy-to-protein ratio at each of the two growth curves.

## MATERIALS AND METHODS

### Experimental Design

An experiment with female Ross 308 broiler breeders was set up as a 2 x 4 factorial arrangement, with 2 growth curves (**GC**) (standard growth curve = **SGC** or elevated growth curve = **EGC**) and 4 diets, differing in energy-to-protein ratio (defined as 96%, 100%, 104% or 108% AME<sub>n</sub> diet). The experiment lasted from hatch to 60 wk of age. Feed allocation per treatment was adapted based the desired GC, meaning that breeders fed the different diets within each GC were fed according to a paired-gain strategy. All experimental protocols were approved by the Central Committee for Animal Experimentation (The Hague, the Netherlands), approval number 2018.W-0023.001.

### Breeders, Housing and Management

A total of 1,536 Ross 308 female broiler breeder day-old pullets were obtained from a 37 wk old grandparent flock (Aviagen-EPI, Roermond, The Netherlands). Pullets were randomly divided over 24 pens (64 pullets per pen), in a climate controlled room, in 3 blocks of 8 pens (n=3 per treatment). Within each block, pens were randomly assigned to one of 8 treatments. Each pen had a floor area covered with wood shavings as bedding (1.75 x 2.80 m), an elevated floor (1.75 x 2.90 m) with plastic slats, and laying nests (1.75 x 0.60 m). Until 6 wk of age, the slats were covered with rearing paper (MS Schippers, Bladel, The Netherlands) and wood shavings. Until 20 wk of age, the laying nests were covered with plastic to prevent access or sight to the nest. Pullets and breeders were fed with a track feeding system (9 m feeding length), which

was placed on the elevated floor, containing a grill to prevent roosters access to the feed (after 20 wk of age). During the first 2 wk of age, two additional feeding pans per pen were placed on the elevated floor in order to stimulate feed intake. Perches (7.2 m) were placed above the elevated floor. Water was available *ad libitum* via drinking nipples positioned above the elevated floor. Feed was provided once per day at 07.15 h from wk 0 to 21 of age and at 09.00 h from wk 21 to 60 of age. Room temperature was maintained at 36°C until 3 d of age. From 3 d of age onwards, temperature was gradually reduced to 20°C at 28 d of age and was maintained thereafter. Pullets were vaccinated according to a standard protocol (Poultry Vets, Diessen, The Netherlands) and reared at a photoperiod of 23L:1D (20 lux) at the day of arrival which gradually changed to 8L:16D (10 lux) at 21 d of age, which was maintained until 21 wk of age. Lights were on between 07.00 h and 15.00 h. At 21 wk of age, pullets were photo-stimulated by increasing the photoperiod instantly to 11L:13D (20 lux) and then gradually to 13L:11D (40 lux) at 23 wk of age. Lights were on between 03.00 h and 16.00 h.

At 20 wk of age, all pens were standardized to 45 breeders per pen closest to the average pen weight. At that age, 4 Ross 308 roosters of the same age were introduced per pen. Roosters were fed with one rooster feeding pan, which was placed above the littered area at a minimum of 50 cm height to prevent breeder access. Roosters were fed a commercial available rooster diet (2,725 kcal of AME<sub>n</sub>/kg, 134 g of CP/kg, 5 g digestible lysine/kg). Body weight, body condition, and mating activity of roosters were assessed every other week according to breeder recommendations (Aviagen, 2018). Inactive roosters were instantly replaced by sexually mature spike roosters.

## Experimental Diets and Feed Allocation

Pullets and breeders were fed according to a 5-phase feeding system. A starter 1 diet was fed from 0 to 3 wk, a starter 2 diet from 3 to 6 wk, a grower diet from 6 to 16 wk, a pre-breeder diet from 16 to 23 wk, a breeder 1 diet from 23 to 40 wk, and a breeder 2 diet from 40 to 60 wk of age. All diets were fed as mash. Feed was provided *ad libitum* from day of arrival until 2 wk of age. Thereafter, daily feed allocation was adjusted weekly to obtain 2 different GC. The SGC was according to the breeder recommendation (Aviagen, 2016a), whereas the EGC targeted to have a 15% higher BW throughout rearing and production. Within each GC, daily feed allocation was adjusted in the 96%, 104%, and 108% AME<sub>n</sub> diets to obtain pair-gaining to the 100% AME<sub>n</sub> diet. Within each phase and GC, all diets were formulated isonitrogenous. Dietary AME<sub>n</sub> levels were increased linearly from 96% to 108% in 4 steps (96%, 100%, 104%, and 108%) relative to the standard (100%; Aviagen, 2016b). The higher AME<sub>n</sub> levels were reached by exchanging cellulose and finely ground oat hulls for soy oil, lard, and maize starch. The ratio between crude fat and starch was

kept similar in all diets within each feeding phase. First, the 96% and 108% AME<sub>n</sub> diets were produced. The intermediate diets (100% and 104% AME<sub>n</sub>) were produced by homogeneous mixing 96% and 108% AME<sub>n</sub> diets in a 2:1 (100% AME<sub>n</sub>) or 1:2 (104% AME<sub>n</sub>) ratio. Diets were analyzed on CP (NEN-EN-ISO 16634-1), crude fat (NEN-EN-ISO 6492-1999), and starch (NEN-ISO 6493) content. Ingredient composition with calculated and analyzed nutrient contents of the experimental diets are presented in Table 1.

## Observations and Measurements

**Body Weight, Feed Allocation and Mortality.** Body weight was determined weekly before feeding by weighing a minimum of 20 (rearing phase) or 15 (production phase) randomly selected breeders per pen. Once every 3 (rearing phase) or 4 (production phase) wk, all breeders within a pen were weighed individually. At these moments, BW uniformity (SD and CV) was calculated for each pen. Feed allocation per pen (expressed as g/breeder per d) was recorded weekly and adjusted to reach a targeted BW gain among diets within each GC. Average daily feed allocation was calculated per pen per phase (from now on defined as rearing phase, first phase of lay, and second phase of lay; 0-21, 22-40, and 41-60 wk of age, respectively). Average daily nutrient intake per pen per phase was calculated by multiplying average feed allocation per pen per phase with the calculated nutrient content of the diet. Relative nutrient intake per phase was calculated also per pen and expressed as a percentage to the 100% AME<sub>n</sub> within GC. Mortality was recorded daily per pen and included culled breeders. Mortality during the first 2 wk of age was excluded from analysis.

**Abdominal Fat Pad.** At 12, 16, 21, 24, 28, 31, 36, 46 and 60 wk of age, 2 breeders per pen were selected before feeding within  $\pm 2.5\%$  of the average BW of both GC. Breeders were euthanized by a percussive blow on the head, followed by cervical dislocation. Breeders were defeathered, dissected and the abdominal fat pad, including fat surrounding the gizzard and proventriculus, was weighed. Abdominal fat pad percentage was calculated as a percentage of live BW.

**Egg Production Traits.** Eggs were collected daily per pen. Eggs were graded as settable or unsettable (small (< 50 g), double yolked, abnormal shell, dirty, cracked, or floor eggs). Total egg mass of all settable, unsettable, and double yolked eggs was recorded daily per pen. Average egg weight of all eggs, excluding double yolked eggs, was calculated per pen per phase. Total number of eggs, settable eggs, and unsettable eggs was calculated per pen per phase (22-40 wk, 41-60 wk, and 22-60 wk). Age at sexual maturity (ASM) was defined as age at 50% production and was determined per pen by a linear interpolation of age in days at which breeders passed 50% rate of lay. Age at first settable egg was defined as age at 50 g egg weight and was determined per



Table 1. Dietary ingredients, and calculated and analyzed nutrients of diets (g/kg, as-fed basis)

Item	Starter 1 (0-21 days)			Starter 2 (22-42 days)			Grower (43-112 days)			Pre-breeder (113-160 days)			Breeder 1 (161-280 days)			Breeder 2 (281-420 days)		
	96% AME <sub>n</sub>	108% AME <sub>n</sub>	450.0	96% AME <sub>n</sub>	108% AME <sub>n</sub>	500.0	96% AME <sub>n</sub>	108% AME <sub>n</sub>	400.0	96% AME <sub>n</sub>	108% AME <sub>n</sub>	500.0	96% AME <sub>n</sub>	108% AME <sub>n</sub>	440.0	96% AME <sub>n</sub>	108% AME <sub>n</sub>	460.0
Ingredient																		
Maize	450.0	100.0	450.0	500.0	100.0	500.0	400.0	100.0	400.0	500.0	100.0	500.0	440.0	100.0	440.0	460.0	100.0	460.0
Wheat	240.9	245.1	50.0	141.3	146.3	90.0	76.1	80.7	150.0	48.9	52.8	165.0	100.0	152.5	80.0	130.5	133.4	90.0
Soybean meal	-	-	-	-	-	-	100.0	100.0	25.0	25.0	1.0	48.0	1.0	45.2	1.0	46.6	1.0	-
Sunflower meal	50.0	50.0	50.0	90.0	90.0	90.0	150.0	150.0	50.0	46.8	1.0	5.0	4.8	10.8	29.5	23.5	32.1	76.9
Wheat middlings	-	-	-	-	-	-	100.0	100.0	5.0	7.0	10.2	96.1	-	-	-	-	-	-
Oat hulls (fine)	50.0	1.0	56.0	5.1	5.0	5.0	65.0	19.3	13.4	24.5	24.6	71.0	71.1	73.5	73.4	73.5	73.5	73.5
Cellulose	44.1	1.0	47.9	5.0	5.0	5.0	50.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Soya oil	11.1	17.8	9.5	14.3	6.8	3.3	8.0	12.0	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Lard	3.0	4.2	4.2	4.2	4.2	4.2	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Maize starch	14.0	94.5	14.3	13.8	13.9	13.3	13.3	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4
Chalk	13.9	14.1	13.8	13.8	13.9	13.3	13.3	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4
Limestone (coarse)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Monocalcium phosphate	9.8	9.2	10.5	9.9	9.9	9.9	5.4	4.9	5.4	5.8	5.2	5.8	6.0	5.5	6.0	6.5	5.9	5.9
Sodium bicarbonate	3.3	3.3	3.3	3.3	3.3	3.3	2.5	2.5	2.5	3.3	3.3	3.3	2.7	2.7	2.7	3.0	2.9	2.9
Salt	1.8	1.8	1.7	1.7	1.7	1.7	2.2	2.2	2.2	1.5	1.5	1.5	2.1	2.1	2.1	2.0	2.0	2.0
L-Lysine	1.73	1.69	1.88	1.80	1.80	1.80	0.23	0.15	0.15	1.63	1.58	1.63	0.44	0.42	0.44	0.36	0.34	0.34
L-Threonine	0.68	0.68	0.54	0.54	0.54	0.54	-	-	-	0.49	0.48	0.49	0.57	0.58	0.57	0.54	0.55	0.55
DL-Methionine	2.34	2.34	1.71	1.71	1.71	1.71	0.65	0.65	0.65	1.13	1.13	1.13	1.73	1.77	1.73	1.59	1.62	1.62
Choline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chloride-50%	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.5	1.4	1.5	1.4	1.3	1.4	1.5	1.4	1.4
Xylanase	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Phytase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Premix rearing <sup>1</sup>	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	-	-	-	-	-	-	-	-	-
Premix laying <sup>2</sup>	-	-	-	-	-	-	-	-	-	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5



Table 1. Continued

Item	Starter 1 (0-21 days)			Starter 2 (22-42 days)			Grower (43-112 days)			Pre-breeder (113-160 days)			Breeder 1 (161-280 days)			Breeder 2 (281-420 days)		
	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>
Calculated content <sup>3</sup>																		
AME <sub>n</sub> (kcal/kg)	2,570	2,890		2,570	2,890		2,545	2,865		2,640	2,970		2,735	3,080		2,735	3,080	
Crude protein	175.1	175.0		143.7	143.6		136.5	136.5		123.0	122.5		138.5	137.7		135.2	134.3	
Crude fat	41.5	49.0		42.0	49.0		40.0	47.0		38.8	45.7		60.0	71.1		61.6	72.8	
Crude fibre	77.1	37.7		88.0	48.3		111.5	71.5		105.6	64.3		81.4	42.0		85.2	43.9	
Starch	379.5	446.9		408.6	477.5		371.5	438.5		407.5	480.4		368.2	434.4		373.8	436.0	
Starch:fat	9.1	9.1		9.7	9.7		9.3	9.3		10.5	10.5		6.1	6.1		6.1	6.0	
Linoleic acid	18.0	21.0		18.0	20.3		17.0	19.0		16.3	17.4		16.8	20.0		20.0	22.0	
Digestible lysine	9.0	9.0		7.0	7.0		4.8	4.8		5.1	5.1		5.9	5.9		5.5	5.5	
Calcium	9.8	9.8		9.8	9.8		8.9	8.9		13.1	13.1		31.0	31.0		31.0	31.0	
Retainable phosphorus	4.1	4.1		4.1	4.1		3.3	3.3		3.2	3.2		3.2	3.2		3.2	3.2	
Analyzed content																		
Crude protein <sup>4</sup>	170.2	172.9		145.1	148.0		133.0	135.1		129.6	127.4		145.2	142.2		139.9	135.1	
Crude fat <sup>4</sup>	37.0	43.2		38.3	44.3		39.0	42.4		33.1	41.1		57.6	66.8		58.2	67.3	
Starch	401.0	463.0		408.0	472.0		377.0	431.0		415.6	486.3		376.4	436.8		371.7	432.5	

<sup>1</sup> Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 3.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 6.0 mg; Vitamin B<sub>6</sub> 4.0 mg; Vitamin B<sub>12</sub> 20 µg; Nicotinamide 35 mg; D-pantothenic acid 15 mg; Folic acid 1.5 mg; Biotin 0.20 mg; Iron 40 mg; Copper 16 mg; Manganese 120 mg; Zinc 90 mg; Iodine 1.25 mg; Selenium 0.3 mg.

<sup>2</sup> Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 5.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 12.0 mg; Vitamin B<sub>6</sub> 5.0 mg; Vitamin B<sub>12</sub> 40 µg; Nicotinamide 55 mg; D-pantothenic acid 15 mg; Folic acid 2.0 mg; Biotin 0.40 mg; Iron 50 mg; Copper 10 mg; Manganese 120 mg; Zinc 90 mg; Iodine 2.0 mg; Selenium 0.3 mg.

<sup>3</sup> Calculated according to CVB (2012).

<sup>4</sup> Analyzed values were within boundaries of the analytical error.

pen by a linear interpolation of age in days at which breeders passed 50 g egg weight. Peak egg production per pen was determined as a 3-wk moving average.

**Feather Development.** Feather cover score of 10 randomly selected breeders per pen was recorded at a 5-wk (rearing phase) or 10-wk (production phase) interval, starting at 5 wk of age. Feather cover was scored according to the method described by Bilkic and Keeling (1999). Scores, varying from 0 (intact feathers) to 5 (completely denuded area), were given to 4 body parts (back, wings, tail, and thighs). The average score of the 10 breeders per pen was calculated per body part. The average of 4 body parts was calculated as an average feather cover score. Feather weight, as a percentage of live BW, was determined at the same ages, of the same breeders, as abdominal fat pad weight was determined and additionally at wk 6 of age. Feather weight was calculated as the difference between live BW and defeathered BW.

## Statistical Analysis

Data on BW (plus SD and CV) and abdominal fat pad percentage were analyzed per measuring moment, due to heterogeneous variation between ages. Data on all other variables (feed allocation, nutrient intake, laying performance, feather development and mortality) were analyzed per phase or overall. All data were analyzed using the Restricted Maximum Likelihood variance component analysis procedure within a linear mixed model (Genstat 19<sup>th</sup> Edition, 2019). The model used was:

$$Y_{ijk} = \mu + GC_i + Diet_j + GC_i \times Diet_j + Block_k + e_{ijk},$$

Where  $Y_{ijk}$  = the dependent variable,  $\mu$  was the overall mean,  $GC_i$  = the growth curve ( $i$  = SGC or EGC),  $Diet_j$  = the energy-to-protein ratio in the diet ( $j$  = 96%, 100%, 104%, or 108% AME<sub>n</sub>),  $GC_i \times Diet_j$  = the interaction between GC and diet,  $Block_k$  = block within the room ( $k$  = 1, 2 or 3), and  $e_{ijk}$  = the residual error. Additionally, effects of dietary energy-to-protein ratio were analyzed as linear or quadratic contrasts, also within GC. Feather parameters were analyzed with the same model added with breeder age and interactions of the other factors with breeder age. Pen was used as experimental unit for all analyses. Distributions of means and model residuals were checked on homogeneity and normality. Not-normal distributed data (feather scores) were square root transformed before analyses. Least square differences were compared, using Fisher adjustments for multiple comparisons. Data are presented as LS means  $\pm$  SEM. For transformed data, LS means of original data are presented, combined with  $P$ -values of the transformed data. All statements of significance are based on testing at  $P \leq 0.05$ . Comparisons between treatments, presented in the tables, are based on the factorial analysis. The slope ( $\beta$ ) of linear effects of dietary energy-to-protein ratio, also within GC, are presented in the results section.

## RESULTS

### Feed Allocation and Nutrient Intake

Daily feed allocations within a treatment were the same for all pens. In all phases feed allocation was on average higher for EGC breeders than for SGC breeders ( $P < 0.001$ ; Table 2). To achieve pair-gaining, feed allocation in all phases decreased with an increasing energy-to-protein ratio. However, this decrease was not the same for both GC (GC x diet (linear)  $P \leq 0.001$ ; Table 2).

To achieve pair-gaining within SGC feed allocation of the different diets was adjusted with  $-0.6$  g/% AME<sub>n</sub>,  $-1.4$  g/% AME<sub>n</sub>, and  $-1.8$  g/% AME<sub>n</sub> for the rearing phase, first phase of lay, and second phase of lay, respectively. To achieve pair-gaining within EGC feed allocation of the different diets was adjusted with  $-0.7$  g/% AME<sub>n</sub>,  $-2.1$  g/% AME<sub>n</sub>, and  $-2.3$  g/% AME<sub>n</sub> for the rearing phase, first phase of lay, and second phase of lay, respectively (GC x diet (linear)  $P \leq 0.001$ ; Table 2). On average daily feed allocation in EGC breeders was 10.9 g, 27.5 g, and 15.6 g higher than in SGC breeders for the rearing phase, first phase of lay, and second phase of lay, respectively ( $P < 0.001$ ; Table 2).

Interactions between GC and diet (linear) were also observed for daily energy and CP intake in all phases ( $P \leq 0.002$ ), with exception of daily energy intake during the rearing phase ( $P = 0.43$ ). During the rearing phase, energy intake increased with  $0.3$  kcal/d/% AME<sub>n</sub> ( $P < 0.001$ ), in both GC. In the first phase of lay, energy intake did not differ between the diets within SGC breeders, whereas energy intake decreased with  $-1.3$  kcal/d/% AME<sub>n</sub> ( $P = 0.001$ ) within EGC breeders. In the second phase of lay, energy intake decreased in both GC with increasing dietary energy-to-protein intake, but this effect was more profound within EGC breeders ( $\beta = -2.1$  kcal/d/% AME<sub>n</sub>) than within SGC breeders ( $\beta = -1.0$  kcal/d/% AME<sub>n</sub>;  $P = 0.002$ ). Within the SGC, daily CP intake decreased with  $-0.1$  g/% AME<sub>n</sub>,  $-0.2$  g/% AME<sub>n</sub>, and  $-0.3$  g/% AME<sub>n</sub> for the rearing phase, first phase of lay, and second phase of lay, respectively. Within the EGC, daily CP intake decreased with  $-0.1$  g/% AME<sub>n</sub>,  $-0.3$  g/% AME<sub>n</sub>, and  $-0.3$  g/% AME<sub>n</sub> for the rearing phase, first phase of lay, and second phase of lay, respectively (GC x diet (linear)  $P \leq 0.001$ ; Table 2).

Although significant differences were observed in energy intake, differences were rather small when expressed as relative difference to the 100% AME<sub>n</sub> diet (Figure 1). Relative differences in CP intake, expressed as percentage to the 100% AME<sub>n</sub> diet, were much larger (Figure 1). To achieve pair-gaining, relative differences in energy and CP intake did not significantly differ between GC for each diet in the rearing phase (Figure 1A and 1B). This means that the correction (percentage compared to the 100% AME<sub>n</sub> diet) needed for pair-gaining in feed allocation per diet was similar

Table 2. Average feed allocation (FA) and nutrient intake during rearing (0-21 wk), first phase of lay (22-40 wk), and second phase of lay (41-60 wk) of broiler breeders with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age.

Item	0-21 wk			22-40 wk			41-60 wk		
	FA (g/d)	Energy (kcal/d)	CP <sup>1</sup> (g/d)	FA (g/d)	Energy (kcal/d)	CP <sup>1</sup> (g/d)	FA (g/d)	Energy (kcal/d)	CP <sup>1</sup> (g/d)
Growth curve (n=12)									
SGC	66.6	182.2 <sup>b</sup>	9.0	142.4	412.0	19.5	147.1	426.6	19.8
EGC	77.5	212.0 <sup>a</sup>	10.5	169.9	491.3	23.3	162.7	471.6	21.9
SEM	0.1	0.1	0.1	0.3	0.8	0.0	0.19	0.51	0.03
Diet (n=6)									
96% AME <sub>n</sub>	75.7	195.3 <sup>d</sup>	10.2	168.4	459.3	23.1	169.1	462.6	22.9
100% AME <sub>n</sub>	73.2	196.6 <sup>c</sup>	9.9	157.8	448.5	21.6	156.4	445.8	21.1
104% AME <sub>n</sub>	70.8	197.8 <sup>b</sup>	9.6	151.5	447.9	20.7	150.4	445.8	20.2
108% AME <sub>n</sub>	68.4	198.5 <sup>a</sup>	9.2	146.9	451.0	20.0	143.6	442.3	19.3
SEM	0.1	0.2	0.1	0.4	1.2	0.1	0.27	0.75	0.04
Treatment (n=3)									
SGC 96% AME <sub>n</sub>	70.0 <sup>e</sup>	180.5	9.5 <sup>e</sup>	151.7 <sup>e</sup>	413.7 <sup>e</sup>	20.8 <sup>e</sup>	158.9 <sup>e</sup>	434.6 <sup>d</sup>	21.5 <sup>e</sup>
100% AME <sub>n</sub>	67.6 <sup>f</sup>	181.7	9.1 <sup>f</sup>	144.4 <sup>f</sup>	410.3 <sup>c</sup>	19.8 <sup>f</sup>	149.0 <sup>f</sup>	424.6 <sup>ef</sup>	20.1 <sup>f</sup>
104% AME <sub>n</sub>	65.4 <sup>g</sup>	182.9	8.9 <sup>g</sup>	138.7 <sup>g</sup>	410.2 <sup>c</sup>	19.0 <sup>g</sup>	143.6 <sup>g</sup>	425.8 <sup>e</sup>	19.3 <sup>g</sup>
108% AME <sub>n</sub>	63.2 <sup>h</sup>	183.5	8.5 <sup>h</sup>	134.8 <sup>h</sup>	413.9 <sup>c</sup>	18.4 <sup>h</sup>	136.8 <sup>h</sup>	421.3 <sup>f</sup>	18.4 <sup>h</sup>
EGC 96% AME <sub>n</sub>	81.5 <sup>a</sup>	210.1	11.0 <sup>a</sup>	185.1 <sup>a</sup>	505.0 <sup>a</sup>	25.4 <sup>a</sup>	179.3 <sup>a</sup>	490.5 <sup>a</sup>	24.3 <sup>a</sup>
100% AME <sub>n</sub>	78.7 <sup>b</sup>	211.6	10.6 <sup>b</sup>	171.2 <sup>b</sup>	486.6 <sup>b</sup>	23.5 <sup>b</sup>	163.8 <sup>b</sup>	467.0 <sup>b</sup>	22.1 <sup>b</sup>
104% AME <sub>n</sub>	76.1 <sup>c</sup>	212.7	10.3 <sup>c</sup>	164.3 <sup>c</sup>	485.7 <sup>b</sup>	22.5 <sup>c</sup>	157.1 <sup>d</sup>	465.8 <sup>bc</sup>	21.2 <sup>d</sup>
108% AME <sub>n</sub>	73.6 <sup>d</sup>	213.5	9.9 <sup>d</sup>	158.9 <sup>d</sup>	488.1 <sup>b</sup>	21.7 <sup>d</sup>	150.4 <sup>e</sup>	463.3 <sup>c</sup>	20.2 <sup>e</sup>
SEM	0.1	0.2	0.1	0.7	1.8	0.1	0.1	1.1	0.1
P-value									
Growth curve (GC)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Diet (factorial)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Diet (linear)	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Diet (quadratic)	0.17	0.08	0.28	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GC x Diet (factorial)	<0.001	0.87	<0.001	0.004	0.003	0.003	<0.001	<0.001	<0.001
GC x Diet (linear)	<0.001	0.43	<0.001	0.001	0.001	0.001	<0.001	0.002	<0.001
GC x Diet (quadratic)	0.85	0.96	0.88	0.06	0.06	0.06	0.01	0.02	0.01

<sup>a-h</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>Based on the calculated CP content

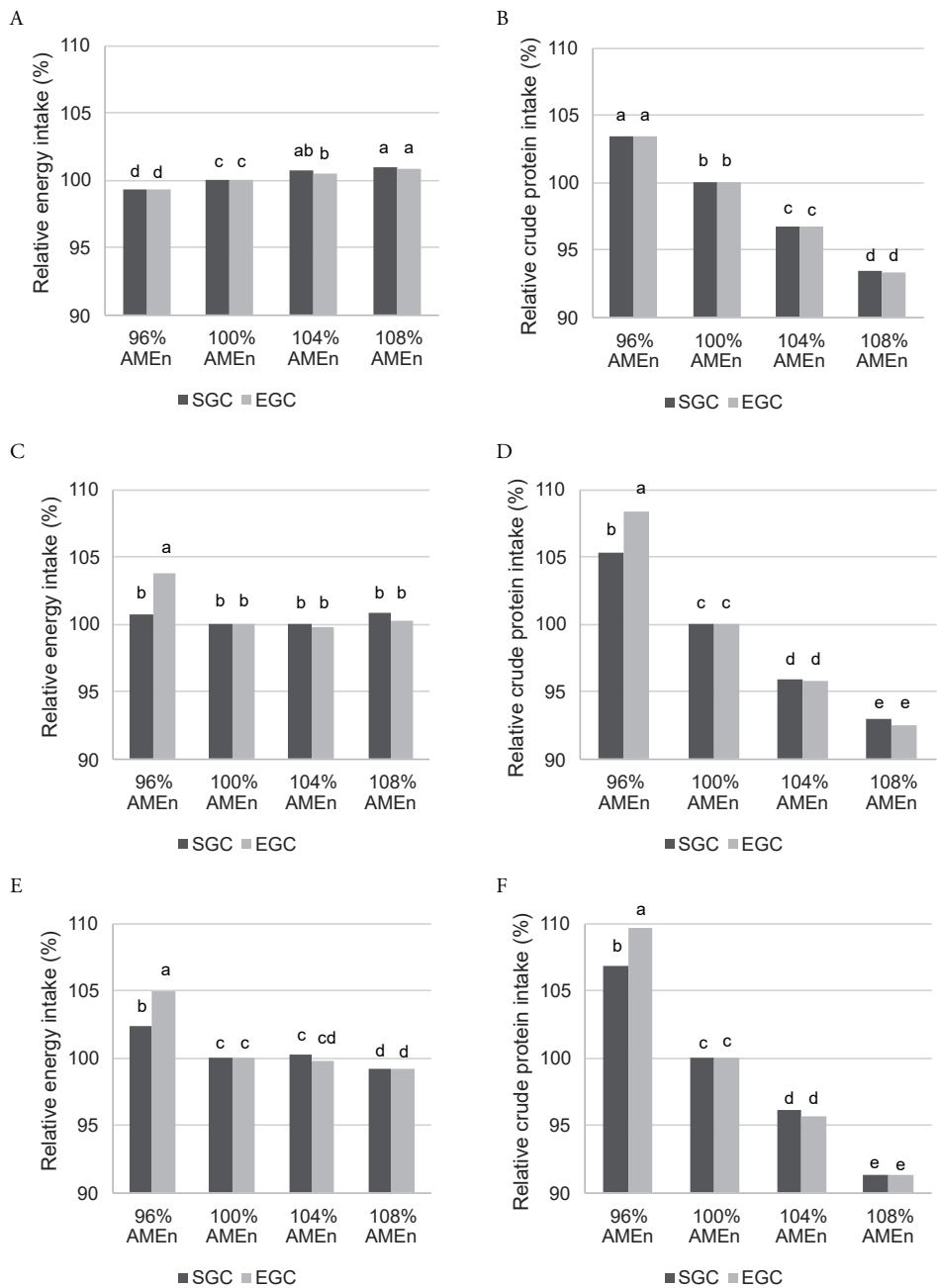


Figure 1. Energy and CP<sup>1</sup> intake during the rearing phase (0-21 wk; A, B), first phase of lay (22-40 wk; C, D), and second phase of lay (41-60 wk; E, F), expressed as percentage relative to the 100% AME<sub>n</sub> diet within growth curve, of broiler breeders with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age. <sup>a-c</sup>LSmeans lacking a common superscript differ (P ≤ 0.05). <sup>1</sup>Based on the calculated CP content.

for each GC. During the first and second laying phase, relative differences in energy and CP intake were similar for breeders in each GC on the 100%, 104% and 108% AME<sub>n</sub> diets (Figure 1C, 1D, 1E, and 1F). Again, this means the correction needed for pair-gaining in feed allocation was similar for each GC for these diets. However, this was not the case for the 96% AME<sub>n</sub> diet. EGC breeders fed the 96% AME<sub>n</sub> diet required a higher feed and thus nutrient intake for pair-gaining than SGC breeders fed the 96% AME<sub>n</sub> diet during the laying phase (Figure 1C, 1D, 1E, and 1F).

### Body Weight, Uniformity and Mortality

Breeders on the different diets closely followed their targeted GC (SGC or EGC; Figure 2). Although daily feed allocations were adjusted weekly for each diet to obtain pair-gaining within each GC, temporary differences in BW among diets occurred. After adjustment in daily feed allocation, differences in BW disappeared.

SD in BW was higher in the EGC than in the SGC up to 12 wk of age ( $P < 0.02$ ; Figure 3), whereas the CV did not differ between GC (data not presented). BW uniformity (SD and CV) showed an interaction between GC and diet (linear) at 31, 40, and from 50 to 58 wk of age ( $P < 0.05$ ). Within the EGC, a linear increase in dietary energy-to-protein ratio led to a linear increase in SD and CV ( $\beta = 6.1$  g SD/% AME<sub>n</sub> and  $\beta = 0.14$  % CV/% AME<sub>n</sub> on average, respectively). Within the SGC, a linear increase in dietary energy-to-protein ratio, led to a linear decrease in SD and CV ( $\beta = -9.5$  g SD/% AME<sub>n</sub> and  $\beta = -0.26$  % CV/% AME<sub>n</sub> on average, respectively).

The average mortality from 2 to 60 wk of age was 8.4%. No differences were observed in mortality between treatments (data not presented).

### Abdominal Fat Pad

No interaction between GC and dietary energy-to-protein ratio on abdominal fat pad percentage was observed (Table 3). From wk 16 until wk 46, with exception at 24 wk of age, EGC breeders had a higher abdominal fat pad percentage ( $\Delta = 0.77$  % on average;  $P < 0.05$ ) than SGC breeders (Table 3). At 12, 24, and 60 wk of age, no differences in abdominal fat pad percentage were observed between GC. At 12 wk of age and from 21 to 36 wk of age, a linear increase in dietary energy-to-protein ratio led to a linear increase in abdominal fat pad percentage ( $\beta = 0.06$  %/% AME<sub>n</sub> on average;  $P < 0.05$ ; Table 3). At wk 16, 46, and 60, no significant differences in abdominal fat pad percentage between diets were observed. Between 36 and 46 wk of age, abdominal fat pad percentage decreased for the 100%, 104%, and 108% AME<sub>n</sub> breeders ( $\Delta = -0.36$  % on average), whereas the 96% AME<sub>n</sub> breeders, showed an increase in abdominal fat pad percentage ( $\Delta = 0.62$  %).

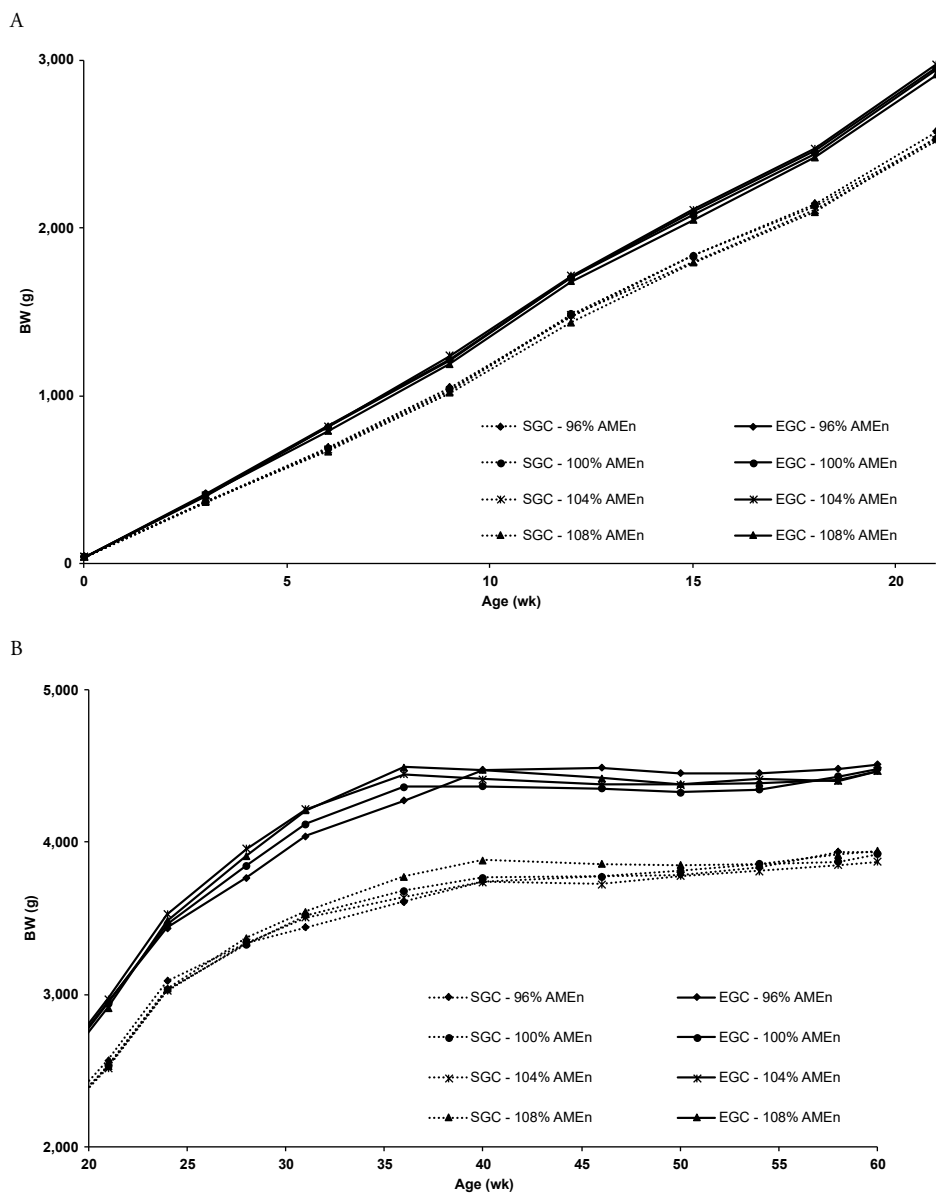


Figure 2. Body weight during the rearing phase (A; 0-21 wk) and production phase (B; 21-60 wk) of broiler breeders with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AMEn), fed from 0 to 60 wk of age.



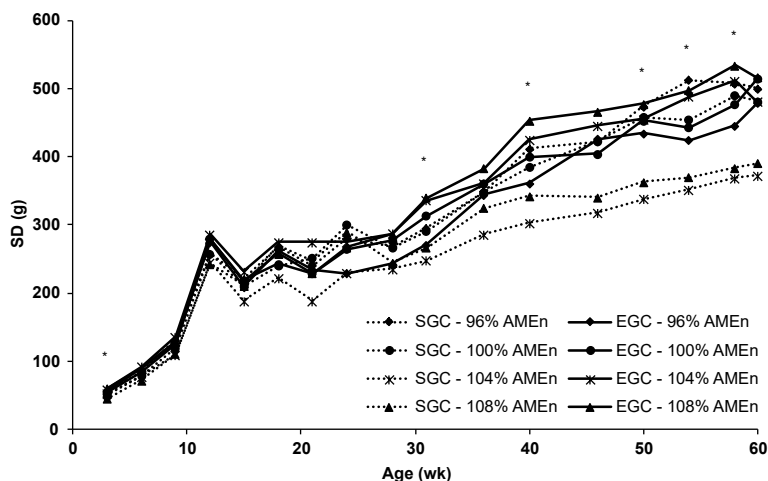


Figure 3. Uniformity, expressed as standard deviation (SD), of broiler breeders from 3 to 60 wk of age, with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age. \*LSmeans within age with asterisk show a significant linear interaction effect of energy-to-protein ratio within GC on SD ( $P \leq 0.05$ ).

## Egg Production Traits

No interaction between GC and dietary energy-to-protein ratio on sexual maturation was observed (Table 4). The EGC breeders reached sexual maturity earlier ( $\Delta = 4.1$  d;  $P < 0.001$ ) and had an earlier production of settable eggs ( $\Delta = 6.5$  d;  $P < 0.001$ ) than the SGC breeders. For both GC, a linear increase in dietary energy-to-protein ratio led to a later age at sexual maturity ( $\beta = 0.14$  d/% AME<sub>n</sub>;  $P = 0.007$ ) and later age at first settable egg ( $\beta = 0.39$  d/% AME<sub>n</sub>;  $P < 0.001$ ). An interaction between GC and dietary energy-to-protein ratio on peak egg production was observed ( $P = 0.03$ ; Table 4). Peak egg production of breeders slightly increased with an increase in dietary energy-to-protein ratio within the SGC ( $\beta = 0.1$  %/% AME<sub>n</sub>), but within the EGC, a decrease in peak egg production was observed with an increase in dietary energy-to-protein ratio ( $\beta = -0.4$  %/% AME<sub>n</sub>).

Throughout the laying phase (22–60 wk), egg weight was higher for EGC breeders ( $\Delta = 2.3$  g on average;  $P < 0.001$ ) than for SGC breeders (Table 4). The effect of dietary energy-to-protein ratio on egg weight was much smaller, differed between phase of lay and was dependent on GC. In the first phase of lay, a linear increase in dietary energy-to-protein ratio led to a linear decrease in egg weight ( $\beta = -0.06$  g/% AME<sub>n</sub>;  $P = 0.007$ ), for breeders in both GC. In the second phase of lay, an interaction between GC and diet (linear) was observed ( $P = 0.02$ ). Within EGC breeders, an increase in

Table 3. Abdominal fat pad, as percentage of live BW, of broiler breeders from 12 to 60 wk of age with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age.

Item		Age (wk)								
		12	16	21	24	28	31	36	46	60
Growth curve (n=12)										
SGC		0.4	0.3 <sup>b</sup>	1.1 <sup>b</sup>	1.5	1.6 <sup>b</sup>	1.5 <sup>b</sup>	1.9 <sup>b</sup>	1.5 <sup>b</sup>	1.7
EGC		0.4	0.6 <sup>a</sup>	1.5 <sup>a</sup>	1.7	2.3 <sup>a</sup>	2.5 <sup>a</sup>	2.8 <sup>a</sup>	2.9 <sup>a</sup>	2.3
SEM		0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2
Diet (n=6)										
96% AME <sub>n</sub>		0.3	0.4	1.0 <sup>b</sup>	1.1 <sup>b</sup>	1.5 <sup>b</sup>	1.5 <sup>c</sup>	1.9	2.5	2.4
100% AME <sub>n</sub>		0.4	0.4	1.1 <sup>b</sup>	1.4 <sup>b</sup>	1.9 <sup>ab</sup>	2.0 <sup>b</sup>	2.4	2.1	1.6
104% AME <sub>n</sub>		0.4	0.4	1.3 <sup>ab</sup>	1.9 <sup>a</sup>	2.0 <sup>a</sup>	2.1 <sup>b</sup>	2.5	2.2	2.0
108% AME <sub>n</sub>		0.5	0.6	1.7 <sup>a</sup>	2.0 <sup>a</sup>	2.3 <sup>a</sup>	2.4 <sup>a</sup>	2.7	2.3	2.1
SEM		0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2
Treatment (n=3)										
SGC	96% AME <sub>n</sub>	0.2	0.3	0.9	0.9	1.3	0.9	1.6	1.8	2.3
	100% AME <sub>n</sub>	0.3	0.3	0.9	1.1	1.6	1.5	2.1	1.1	1.3
	104% AME <sub>n</sub>	0.4	0.3	1.2	1.9	1.6	1.4	1.9	1.6	1.9
	108% AME <sub>n</sub>	0.4	0.5	1.4	1.9	1.7	2.2	2.1	1.7	1.5
EGC	96% AME <sub>n</sub>	0.3	0.6	1.2	1.2	1.8	2.0	2.1	3.2	2.5
	100% AME <sub>n</sub>	0.4	0.5	1.3	1.7	2.2	2.4	2.8	3.0	1.9
	104% AME <sub>n</sub>	0.4	0.4	1.4	1.8	2.5	2.7	3.0	2.7	2.2
	108% AME <sub>n</sub>	0.6	0.8	2.0	2.1	2.9	2.7	3.2	2.9	2.7
SEM		0.1	0.1	0.2	0.2	0.2	0.1	0.3	0.3	0.4
P-value <sup>1</sup>										
Growth curve (GC)		0.20	0.005	0.01	0.15	<0.001	<0.001	0.001	<0.001	0.10
Diet (factorial)		0.06	0.07	0.02	0.002	0.03	<0.001	0.10	0.51	0.45
Diet (linear)		0.009	0.06	0.001	<0.001	0.002	<0.001	0.02	0.56	0.74
GC x Diet (factorial)		0.31	0.94	0.69	0.44	0.40	0.06	0.67	0.60	0.72
GC x Diet (linear)		0.76	0.72	0.48	0.55	0.10	0.23	0.23	0.47	0.38

<sup>a-c</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>4</sup>Quadratic contrasts were not significant.

dietary energy-to-protein ratio led to a linear decrease in egg weight ( $\beta = -0.13$  g/% AME<sub>n</sub>), whereas within SGC breeders, an increase in dietary energy-to-protein ratio led to a linear increase in egg weight ( $\beta = 0.03$  g/% AME<sub>n</sub>).

During the first phase of lay, EGC breeders produced a higher total number of eggs per breeder ( $\Delta = 2.5$  eggs; P=0.02; Table 5) than SGC breeders. During the second phase of lay, no difference between GC were observed on total number of eggs per

Table 4. Age at sexual maturity, age at first hatching egg, peak egg production, and egg weight during first phase of lay (22-40 wk), second phase of lay (41-60 wk), and total laying phase (22-60 wk) of broiler breeders with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age.

		Age at sexual maturity <sup>1</sup> (d)	Age first hatching egg <sup>2</sup> (d)	Peak egg production <sup>3</sup> (%)	Egg weight (g)		
Item					24-40 wk	41-60 wk	24-60 wk
Growth curve (n=12)							
SGC		174.5 <sup>a</sup>	177.3 <sup>a</sup>	91.4	57.2 <sup>b</sup>	67.3 <sup>b</sup>	62.6 <sup>b</sup>
EGC		170.4 <sup>b</sup>	170.8 <sup>b</sup>	89.2	59.7 <sup>a</sup>	69.4 <sup>a</sup>	64.9 <sup>a</sup>
SEM		0.3	0.5	0.6	0.1	0.2	0.2
Diet (n=6)							
96% AME <sub>n</sub>		171.7	172.1 <sup>c</sup>	91.0	58.9 <sup>a</sup>	68.7	64.2
100% AME <sub>n</sub>		171.9	173.0 <sup>bc</sup>	91.3	58.6 <sup>ab</sup>	68.3	63.8
104% AME <sub>n</sub>		172.7	174.5 <sup>b</sup>	89.7	58.2 <sup>b</sup>	68.2	63.6
108% AME <sub>n</sub>		173.3	176.7 <sup>a</sup>	89.2	58.2 <sup>b</sup>	68.1	63.5
SEM		0.4	0.7	0.8	0.2	0.3	0.2
Treatment (n=3)							
SGC	96% AME <sub>n</sub>	174.2	175.0	90.1 <sup>abc</sup>	57.4	67.1	62.6
	100% AME <sub>n</sub>	173.7	176.2	92.4 <sup>a</sup>	57.2	67.2	62.6
	104% AME <sub>n</sub>	174.6	178.4	92.4 <sup>a</sup>	57.1	67.4	62.7
	108% AME <sub>n</sub>	175.4	179.8	90.8 <sup>ab</sup>	57.1	67.4	62.6
EGC	96% AME <sub>n</sub>	169.2	169.2	91.9 <sup>a</sup>	60.2	70.4	65.7
	100% AME <sub>n</sub>	170.2	169.8	90.3 <sup>abc</sup>	60.1	69.3	65.1
	104% AME <sub>n</sub>	170.9	170.7	87.1 <sup>c</sup>	59.3	69.0	64.5
	108% AME <sub>n</sub>	171.1	173.7	87.7 <sup>bc</sup>	59.2	68.8	64.4
SEM		0.6	1.0	1.2	0.3	0.4	0.3
P-value <sup>4</sup>							
Growth curve (GC)		<0.001	<0.001	0.02	<0.001	<0.001	<0.001
Diet (factorial)		0.07	0.001	0.27	0.05	0.39	0.19
Diet (linear)		0.007	<0.001	0.08	0.007	0.11	0.03
GC x Diet (factorial)		0.61	0.76	0.05	0.41	0.11	0.16
GC x Diet (linear)		0.65	0.67	0.03	0.12	0.02	0.03

<sup>a-c</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>Defined as age at 50% production.

<sup>2</sup>Defined as age at egg weight 50 g.

<sup>3</sup>Determined as a 3-wk moving average of %/breeder/d.

<sup>4</sup>Quadratic contrasts were not significant.

Table 5. Egg production traits (n/breeder) during first phase of lay (22-40 wk), second phase of lay (41-60 wk), and total laying phase (22-60 wk) of broiler breeders with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age.

Item	22-40 wk			41-60 wk			22-60 wk		
	Total eggs	Settable eggs <sup>1</sup>	Unsettable eggs <sup>2</sup>	Total eggs	Settable eggs <sup>1</sup>	Unsettable eggs <sup>2</sup>	Total eggs	Settable eggs <sup>1</sup>	Unsettable eggs <sup>2</sup>
Growth curve (n=12)									
SGC	93.2 <sup>b</sup>	86.4	6.9 <sup>b</sup>	95.9	94.8	1.2 <sup>b</sup>	189.1	181.1	8.0 <sup>b</sup>
EGC	95.7 <sup>a</sup>	87.2	8.5 <sup>a</sup>	97.2	95.4	1.8 <sup>a</sup>	192.9	182.6	10.3 <sup>a</sup>
SEM	0.6	0.6	0.2	1.8	1.8	0.1	2.2	2.3	0.3
Diet (n=6)									
96% AME <sub>n</sub>	95.9	89.0 <sup>a</sup>	7.0 <sup>b</sup>	97.6	96.2	1.4	193.5	185.2	8.3
100% AME <sub>n</sub>	95.6	88.3 <sup>ab</sup>	7.3 <sup>b</sup>	95.5	93.9	1.6	191.1	182.3	8.9
104% AME <sub>n</sub>	94.1	86.0 <sup>bc</sup>	8.1 <sup>ab</sup>	96.7	95.2	1.5	190.7	181.2	9.5
108% AME <sub>n</sub>	92.3	83.8 <sup>c</sup>	8.5 <sup>a</sup>	96.3	95.0	1.4	188.6	178.7	9.8
SEM	0.9	0.9	0.3	2.6	2.7	0.2	3.4	3.4	0.4
Treatment (n=3)									
SGC	93.1	86.8	6.3	96.5	95.3	1.1	189.6	182.2	7.4
96% AME <sub>n</sub>	94.0	87.2	6.8	93.5	92.4	1.1	187.5	179.6	7.9
100% AME <sub>n</sub>	94.4	87.0	7.4	98.7	97.5	1.2	193.0	184.5	8.6
104% AME <sub>n</sub>	91.4	84.4	7.0	95.0	93.8	1.1	186.3	178.2	8.1
108% AME <sub>n</sub>	98.8	91.1	7.7	98.7	97.1	1.6	197.4	188.2	9.2
EGC	97.2	89.4	7.8	97.6	95.5	2.0	194.8	184.9	9.8
96% AME <sub>n</sub>	93.8	85.0	8.7	94.7	92.9	1.8	188.4	177.9	10.5
100% AME <sub>n</sub>	93.1	83.2	9.9	97.7	96.1	1.6	190.8	179.3	11.6
104% AME <sub>n</sub>	1.3	1.2	0.5	3.8	3.9	0.3	4.9	4.9	0.6
SEM									
P-value <sup>3</sup>									
Growth curve (GC)	0.02	0.36	<0.001	0.66	0.82	0.02	0.32	0.69	<0.001
Diet (factorial)	0.06	0.004	0.05	0.96	0.95	0.87	0.82	0.65	0.15
Diet (linear)	0.006	<0.001	0.004	0.83	0.84	0.99	0.35	0.21	0.02
GC x Diet (factorial)	0.16	0.08	0.27	0.73	0.75	0.88	0.60	0.59	0.56
GC x Diet (linear)	0.08	0.02	0.13	0.79	0.80	0.96	0.49	0.40	0.23

<sup>a-c</sup>LMeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>Settable egg = clean egg (≥50 g).

<sup>2</sup>Unsettable egg = small (<50 g), double yolk, abnormal shell, dirty or floor egg.

<sup>3</sup>Quadratic contrasts were not significant.

breeder produced (Table 5). The EGC breeders produced more unsettable eggs during the first ( $\Delta = 1.6$  eggs;  $P < 0.001$ ) and second phase of lay ( $\Delta = 0.6$  eggs;  $P = 0.02$ ) than the SGC breeders. Over the whole laying phase (wk 22 to 60), EGC breeders had a higher production of unsettable eggs ( $\Delta = 2.3$  eggs;  $P < 0.001$ ) than SGC breeders. The GC effect was observed in almost all categories of unsettable eggs. The EGC breeders produced more double yolked eggs (2.2 vs. 0.9 eggs;  $P < 0.001$ ), dirty eggs (1.5 vs. 0.9 eggs;  $P = 0.002$ ), abnormal shell eggs (0.7 vs. 0.4 eggs;  $P = 0.002$ ), and cracked shell eggs (1.0 vs. 0.7 eggs;  $P = 0.006$ ) than the SGC breeders. No differences between GC were observed in number of small eggs or floor eggs.

The effect of dietary energy-to-protein ratio on total and unsettable egg production differed per phase of lay (Table 5). During the first phase of lay, an increase in dietary energy-to-protein ratio linearly decreased total number of eggs ( $\beta = -0.31$  eggs/%  $AME_n$ ;  $P = 0.006$ ) and linearly increased number of unsettable eggs ( $\beta = 0.13$  eggs/%  $AME_n$ ;  $P = 0.004$ ; Table 5). This was mainly due to a linear increase in double yolked eggs ( $\beta = 0.05$  double yolked eggs/%  $AME_n$ ;  $P = 0.01$ ) and small eggs ( $\beta = 0.07$  small eggs/%  $AME_n$ ;  $P = 0.03$ ). In the second phase of lay, no significant effects of dietary energy-to-protein ratio were observed on total and unsettable egg production.

A linear interaction between GC and dietary energy-to-protein ratio was observed on settable egg production (Table 5). An increase in dietary energy-to-protein ratio linearly decreased settable egg production ( $P < 0.001$ ) in both GC, but the effect on settable egg production was more profound within EGC breeders ( $\beta = -0.70$  eggs/%  $AME_n$ ) than within SGC breeders ( $\beta = -0.19$  eggs/%  $AME_n$ ; GC x Diet (linear),  $P = 0.02$ ). In the second phase of lay, no significant effects of dietary energy-to-protein ratio were observed on settable egg production.

## Feather Development

A linear interaction between GC and dietary energy-to-protein ratio was observed on average feather cover score (Table 6; GC x Diet (linear),  $P = 0.04$ ). Within SGC breeders, a linear increase in dietary energy-to-protein ratio led to a linear increase in feather cover score ( $\beta = 0.02$  points/%  $AME_n$ ), whereas this linear effect was not observed within EGC breeders ( $\beta = 0.00$  points/%  $AME_n$ ). Feather cover score increased with breeder age ( $P < 0.001$ ). Feather weight did not differ between treatments (Table 6).

Table 6. Average feather cover score and feather weight from 5 to 60 wk of age, of broiler breeders with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age.

Item		Feather cover score <sup>1</sup>				Feather weight	
		Back	Tail	Thigh	Wing	Average	(% of BW)
Growth curve (n=12)							
	SGC	1.18	1.14	1.50 <sup>b</sup>	1.42	1.31	3.8
	EGC	1.25	1.16	1.61 <sup>a</sup>	1.45	1.37	3.7
	SEM	0.1	0.1	0.1	0.1	0.1	0.1
Diet (n=6)							
	96% AME <sub>n</sub>	1.19	1.11	1.56 <sup>ab</sup>	1.38	1.31 <sup>b</sup>	3.8
	100% AME <sub>n</sub>	1.18	1.11	1.51 <sup>b</sup>	1.43	1.31 <sup>b</sup>	3.7
	104% AME <sub>n</sub>	1.22	1.14	1.51 <sup>b</sup>	1.41	1.30 <sup>b</sup>	3.7
	108% AME <sub>n</sub>	1.28	1.24	1.65 <sup>a</sup>	1.51	1.42 <sup>a</sup>	3.8
	SEM	0.1	0.1	0.1	0.1	0.1	0.1
Treatment (n=3)							
SGC	96% AME <sub>n</sub>	1.06	1.05	1.44	1.28	1.21	3.8
	100% AME <sub>n</sub>	1.23	1.11	1.46	1.45	1.31	3.7
	104% AME <sub>n</sub>	1.18	1.13	1.46	1.41	1.20	3.8
	108% AME <sub>n</sub>	1.25	1.27	1.64	1.53	1.42	3.8
EGC	96% AME <sub>n</sub>	1.31	1.16	1.69	1.49	1.41	3.8
	100% AME <sub>n</sub>	1.13	1.10	1.55	1.40	1.30	3.7
	104% AME <sub>n</sub>	1.26	1.15	1.55	1.42	1.35	3.6
	108% AME <sub>n</sub>	1.31	1.21	1.66	1.49	1.42	3.7
	SEM	0.1	0.1	0.1	0.1	0.1	0.1
P-value <sup>2</sup>							
	Growth curve (GC)	0.43	0.91	0.003	0.43	0.33	0.51
	Diet (factorial)	0.26	0.06	0.03	0.23	0.002	0.71
	Diet (linear)	0.08	0.020	0.11	0.07	<0.001	0.62
	Diet (quadratic)	0.41	0.20	0.007	0.54	0.12	0.32
	Age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	GC x Diet (factorial)	0.08	0.37	0.18	0.14	0.07	0.81
	GC x Diet (linear)	0.47	0.10	0.04	0.08	0.04	0.96
	GC x Age	0.53	0.82	<0.001	0.26	0.11	0.19

<sup>ab</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>Feather cover score ranges from 0 (intact feathers) to 5 (completely denuded area). Each value is a mean of the replicates determined at a 5-wk interval during rearing (0-21 wk) and a 10-wk interval during production (21-60 wk).

<sup>2</sup>Interactions between GC and Diet (quadratic), between Diet and Age and between Diet, Growth curve, and Age were not significant.

## DISCUSSION

The objective of this study was to evaluate effects of GC and dietary energy-to-protein ratio during rearing and production on productive performance of broiler breeder hens. Interactions will be discussed within the section of dietary energy-to-protein ratio.

### Growth Curve

In the current study, pullets and breeders required on average a 15.1% higher feed allocation to obtain a 15% higher BW. A higher feed allocation to achieve a higher BW is in line with other studies (Renema et al., 2001b; Gous and Cherry, 2004; Ekmay et al., 2012; Van Emous et al., 2013; De Los Mozos et al., 2017; Van der Klein et al., 2018). In the current study, the extra feed allocation and thus higher BW resulted in a higher abdominal fat pad. This was according to expectations and in line with other studies (Renema et al., 2001b; Robinson et al., 2007; Van Emous et al., 2013; Van der Klein et al., 2018; Salas et al., 2019), as body composition is related to feed allocation (Robinson et al., 2007) and diet composition (Spratt and Leeson, 1987).

Sexual maturation occurred 4.1 d earlier in breeders of the EGC than in breeders of the SGC. Other studies reported similar findings, with a 2.8 d (24% heavier) to 15 d (74% heavier) earlier sexual maturity for heavier breeders (Renema et al., 2001b, 2007a; Gous and Cherry, 2004; Sun and Coon, 2005; Ekmay et al., 2012; Van der Klein et al., 2018). Sexual maturation depends on physiological cues, such as photorefractory and photosensitivity (Gous and Cherry, 2004; Van der Klein et al., 2018; Van Emous et al., 2018), but metabolic cues are suggested to play a role as well (Bédécarrats et al., 2016; Hanlon et al., 2020; Van der Klein et al., 2020). It has been suggested that broiler breeders require a certain protein threshold for sexual maturation (Sun et al., 2006; Eitan et al., 2014; Salas et al., 2019), whereas others emphasize the presence of a fat threshold for sexual maturation (Van der Klein et al., 2018; Zuidhof, 2018; Hadinia et al., 2020). In the current study, a 1.6% higher abdominal fat pad was observed at 21 wk of age for EGC breeders compared to SGC breeders, indicating an approximately 84 g higher body fat mass (Zuidhof, 2018). As BW difference between the GC was 406 g at 21 wk of age, the remaining difference in BW can probably be attributed to an increased lean and ash mass. A higher BW thus appears to be the result of both a higher body protein and a higher body fat mass and based on these, it is unclear whether a higher protein or higher fat mass is the main driver for sexual maturation in breeders.

Besides an earlier age at sexual maturity, EGC breeders laid on average 2.3 g heavier eggs than SGC breeders. Other studies observed no effect of a 13 to 20% higher BW

at 20 wk of age on egg weight (Gous and Cherry, 2004; Renema et al., 2007a; Ekmay et al., 2012; Van Emous et al., 2013; Van der Klein et al., 2018; Salas et al., 2019) or only a 0.9 g (Sun and Coon, 2005) to 1.9 g (Renema et al., 2001a) increase in egg weight. Some of these studies converged different GC up to 20 wk of age to a similar BW during production (Sun and Coon, 2005; Renema et al., 2007a; Van Emous et al., 2013), whereas other studies fixed either GC, and thus BW gain (Van der Klein et al., 2018), or feed allowance (Gous and Cherry, 2004; Ekmay et al., 2012; Salas et al., 2019) during the laying phase, irrespective of BW at 20 wk of age. The amount of feed available for growth and egg production depends on the amount of feed that is used for maintenance. The latter one being mainly dependent on BW (Caldas et al., 2018; Hadinia et al., 2018) and to a lesser extent on body composition (Gous, 2015). A fixed BW, GC, or feed allowance during the laying phase probably reduced the amount of feed available for growth and egg production for heavier breeders compared to lighter breeders. In this way, lighter breeders may benefit from this, as they have more nutrients available for egg production and consequently produce similar egg weights as heavier breeders (Gous and Cherry, 2004; Sun and Coon, 2005; Renema et al., 2007a; Ekmay et al., 2012; Van Emous et al., 2013; Van der Klein et al., 2018; Salas et al., 2019). In the current study and that of Renema et al. (2001a), a relative difference in GC throughout production was maintained by a higher feed allowance for the EGC breeders compared to SGC breeders. With this feeding strategy, EGC breeders receive the same relative amount of nutrients for maintenance, growth, and egg production as the SGC breeders. The results thus imply that a higher GC leads to heavier eggs, only when a difference in GC and thus feed allowance is maintained during production. A higher egg weight, in turn, might be beneficial for day-old chick quality (Ulmer-Franco et al., 2010; Nangsuay et al., 2011). Further research should investigate the impact of GC on offspring quality and performance.

In the first phase of lay, EGC breeders had a higher total egg production compared to SGC breeders. This was also observed by Ekmay et al. (2012) and can probably be explained by a longer laying phase, as a results of an earlier start of production. However, number of settable eggs did not differ between both GC, as the EGC breeders had a higher number of unsettable eggs. Similar findings were observed in other studies, where heavier breeders had a higher number of unsettable eggs (Renema et al., 2001a; Gous and Cherry, 2004; Sun and Coon, 2005; Sun et al., 2006). Van der Klein et al. (2020) extensively reviewed mechanisms associated with reproductive dysregulation. They suggested that metabolic status and feed allowance affect endocrine (dys)regulation of follicle selection and maturation. However, mechanisms are complex and not yet fully elucidated (Van der Klein et al., 2020).



Over the whole laying phase, no difference in total or settable number of eggs was observed between both GC, which is in line with others (Renema et al., 2001a; Gous and Cherry, 2004; Sun and Coon, 2005; Salas et al., 2019). These results implicate that a 15% higher GC is neither beneficial nor detrimental for total number of settable eggs. However, it is known that *ad libitum* feeding of breeders is detrimental for total number of eggs produced (Robinson et al., 1991; Bruggeman et al., 1999; Heck et al., 2004; Sun et al., 2006). This suggests there is an upper limit, which might be related to either BW, body protein, or body fat mass, until which egg production is unaffected. When this limit is exceeded, egg production may drop.

### Dietary Energy-to-Protein Ratio

Pullets and breeders fed a diet with a higher dietary energy-to-protein ratio required less feed to achieve a similar BW. This is in line with previous studies, where a higher dietary energy-to-protein ratio was achieved by a higher energy content (Moraes et al., 2014; Van Emous et al., 2015). However, in studies where a higher dietary energy-to-protein ratio was achieved by a lower protein content an opposite relation was observed. In that case, a higher dietary energy-to-protein ratio required a higher feed allocation to achieve a similar BW (Van Emous et al., 2013; Moraes et al., 2014; Lesuisse et al., 2017, 2018). These results indicate that dietary energy-to-protein ratio per se and feed allocation are not correlated to achieve a certain target BW. However, when diets have either a higher energy or a higher protein content, consequently changing the ratio, a lower feed allocation is required to achieve a similar BW. These studies indicate that absolute intake of energy or protein determines growth in breeders, rather than the ratio between them.

The current study indicated that an absolute intake of energy determined growth in pullets and not protein intake. During the rearing phase, it was observed that pair-gaining of pullets required a similar energy intake between the different diets, while protein intake differed. Moraes et al. (2014) also observed a similar energy intake between diets different in energy-to-protein ratio, to achieve pair-gaining during the rearing phase. Feed restriction of pullets might play a role in this. In broilers, it is observed that, when feed restriction is applied, energy intake is limiting for growth of broilers (Boekholt et al., 1994; Leeson et al., 1996). Pullets cannot compensate for an energy limitation by increasing their feed intake, as is observed in *ad libitum* fed broilers (Leeson et al., 1996; Yang et al., 2009). Conversion of dietary protein and energy into body protein and body fat requires a minimum energy intake (Boekholt et al., 1994). Energy intake might therefore limit growth in pullets.

After peak production, SD within the SGC was lower with an increasing energy-to-protein ratio, whereas SD within the EGC, was higher with an increasing energy-to-

protein ratio. Management plays a major role in BW uniformity (Aviagen, 2018), where a similar feed intake among breeders within a flock is key in aiming for a high BW uniformity (Zuidhof, 2018). A larger amount of feed gives less dominant breeders an opportunity to compete for feed, which might lead to a higher BW uniformity (De Beer and Coon, 2009). This is in line with observations within the EGC, as a higher feed allocation corresponded with a lower SD and thus a higher BW uniformity during production. However, within the SGC, breeders fed the lowest amount of feed had the lowest SD and thus a better BW uniformity than breeders with a higher amount of feed. A more severe feed restriction leads to a higher eating rate (De Los Mozos et al., 2017). It can be speculated that feed allocation was so restricted for breeders on the 104% and 108% AME<sub>n</sub> diet, within the SGC, that the daily feed portion was consumed in 1 feeding bout in a short time, leading to a uniform feed intake for all breeders within a pen. For the 96% and 100% AME<sub>n</sub>, within the SGC, the daily feed portion might not have been consumed in 1 feeding bout, due to a limitation in physical eating capacity. In that case, breeders with a higher digestive capacity or more dominant breeders might have had the opportunity to consume a second feeding portion or a larger portion, leading to a decline in BW uniformity.

An increase in dietary energy-to-protein ratio led to a linear increase in abdominal fat pad and thus a higher body fat mass (Zuidhof, 2018). This is in line with other studies (Van Emous et al., 2013, 2015; Lesuisse et al., 2017, 2018; Salas et al., 2019). Growing animals always have a basic protein retention (Boekholt et al., 1994; Boekholt and Schreurs, 1997) and in case a surplus of energy is supplied, this is mostly retained as fat (Boekholt et al., 1994; Leeson et al., 1996). A higher dietary energy-to-protein ratio supplies pullets and breeders with a surplus of energy, which is deposited as fat. Interestingly, all diets had a decreased abdominal fat pad percentage between 36 and 60 wk of age, with exception of 96% AME<sub>n</sub> diet. A reduction in fat mass after peak production was also observed by Salas et al. (2019). Fat in egg yolk is highly depended on body fat mobilization during late production (Salas et al., 2017), suggesting that body fat mobilization after 36 wk of age supports egg production. Breeders fed the 96% AME<sub>n</sub> diet had a further increase in abdominal fat pad between 36 and 46 wk of age, prior to a decrease in abdominal fat pad between 46 and 60 wk of age. These breeders required a relative high feed allocation, compared to the other diets, between 36 and 46 wk of age to achieve pair-gaining. This higher feed allocation and nutrient intake probably resulted in deposition of fat in the body. These results might indicate that these breeders were inefficient with their nutrients in this period, potentially due to an imbalance between energy and protein in the diet.

A change in body composition might have influenced sexual maturation (Bédécarrats et al., 2016; Hanlon et al., 2020; Van der Klein et al., 2020). Breeders fed a higher

dietary energy-to-protein ratio had a later age at sexual maturity. However, others did not observe an effect of dietary energy-to-protein ratio on sexual maturation (Joseph et al., 2002; Van Emous et al., 2013, 2015, 2018; England et al., 2014; Lesuisse et al., 2017, 2018; Salas et al., 2019). Contradictory to the current study, all these studies altered dietary energy-to-protein ratio by adjusting protein content of the diet, with exemption of the study of Salas et al. (2019). Additionally, the current study design allowed linear analysis of dietary energy-to-protein ratio, whereas all other studies had a factorial design. Linear analysis of our data showed a significant effect of dietary energy-to-protein ratio on sexual maturation, whereas the factorial analysis did not show this. Salas et al. (2019) only started their dietary treatments at 20 wk of age, which indicates that energy content during the rearing phase might play a role in sexual maturation. Broiler breeders require a protein or fat threshold for sexual maturation (Zuidhof, 2018; Salas et al., 2019; Hadinia et al., 2020). Breeders with a higher abdominal fat pad percentage, and thus a higher body fat mass (Zuidhof, 2018), at 21 wk of age had a later age at sexual maturation compared to breeders with a lower fat pad percentage, at a similar BW. These results suggest that a fat threshold did not play an important role in sexual maturation. Future studies should consider analysis of body protein and body fat content at different ages during rearing to determine their importance for sexual maturation.

An increase in energy-to-protein ratio led to a decrease in average egg weight. On average a 0.7 g lower egg weight was observed for the 108% AME<sub>n</sub> diet than for the 96% AME<sub>n</sub> diet. This is comparable with Van Emous et al. (2015), who observed a 0.4 g lower egg weight, with 7.1% higher energy content in the diet. Spratt and Leeson (1987) and Sun and Coon (2005) observed a 1.8 g and 0.85 g higher egg weight with 38% and 5.4% higher energy content in the diet, respectively. However, those studies were based on pair-feeding instead of pair-gaining, meaning the higher energy content group also had a higher BW and consequently a higher egg weight. Other studies did not observe an effect of a 8.2% (Moraes, 2013) to 45% (Salas et al., 2019) higher energy content on egg weight. The latter one might be explained by a similar protein intake, as protein content and intake seems to have a more profound effect on egg weight than energy intake. Dietary protein is an important source of egg protein for egg formation (Ekmay et al., 2014) and consequently a reduced protein intake during lay might influence egg weight. A reduction in protein content in the diet (12.5 % to 25%) resulted in a 0.7 g to 4.7 g lower egg weight (Spratt and Leeson, 1987; Joseph et al., 2000; England et al., 2014; Lesuisse et al., 2017, 2018). These effects were only observed when fed during the laying phase, as a lower crude protein content during the rearing phase alone did not affect egg weight in the laying phase (Moraes, 2013; Van Emous et al., 2013, 2015). Further research should investigate whether or not the ratio between egg yolk and albumen is influenced as well by changing the dietary

energy-to-protein ratio, as this might eventually affect chick quality (Finkler et al., 1998; Willems et al., 2015).

An earlier start of production and an earlier age at first settable egg, due to a higher egg weight, almost entirely explains the higher settable egg production during the first phase of lay for breeders fed a lower dietary energy-to-protein ratio. This is in line with observations from other studies (Joseph et al., 2000; Lesuisse et al., 2017, 2018), who reduced crude protein content by 25% in the diet. However, studies that reduced crude protein or energy content by 5% to 20% did not observe an effect on egg production during the first phase of lay (Joseph et al., 2002; Sun and Coon, 2005; Van Emous et al., 2013, 2015, 2018; England et al., 2014). Maybe more important, these studies only fed a diet lower in protein during either the rearing (Van Emous et al., 2013, 2015) or the laying phase (Joseph et al., 2002; Sun and Coon, 2005; England et al., 2014; Van Emous et al., 2018). This suggests that a lower dietary energy-to-protein ratio might be beneficial for egg production during the first phase of lay, but only when diets are fed both during rearing and first phase of lay.

In line with Sun and Coon (2005) and Salas et al. (2019), in the second phase of lay and over the total laying phase, no differences were observed in total and settable egg production. Contradictory, in the study of Van Emous et al. (2015), a higher dietary energy-to-protein ratio during rearing improved persistency between wk 45 and 60 of age. They speculated that a higher fat mass and a lower lean mass at 20 wk of age was beneficial for laying persistency. Body fat mass needs relative low maintenance (Gous, 2015), which might increase amount of energy available for egg production for fatter breeders than for leaner breeders. The current study did not observe a difference between diets in abdominal fat pad percentage at 46 and 60 wk of age, which might explain the lack of differences in egg production during the second phase of lay.

## CONCLUSIONS

It can be concluded that a higher GC of breeders during rearing and production led to an earlier age at sexual maturation and a higher egg weight, but did not affect total number of settable eggs produced. Feeding breeders a lower dietary energy-to-protein ratio, while maintaining a similar GC, led to a lower abdominal fat pad and an earlier age at sexual maturation. In the first phase of lay, feeding a lower dietary energy-to-protein ratio led to a higher egg weight and a higher number of settable eggs produced. This dietary effect was more profound when breeders were on a higher GC. In the second phase of lay, feeding a lower dietary energy-to-protein ratio led to a higher egg weight when breeders were on a higher GC, but not when breeders were

on a standard GC. Dietary energy-to-protein ratio did not affect number of settable eggs produced in the second phase of lay. A higher body fat mass, within a similar BW, thus does not have beneficial effects on productive performance. We suggest further research to investigate the impact of GC and dietary energy-to-protein ratio on egg composition and offspring performance.

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# Chapter 3

## Impact of growth curve and dietary energy-to-protein ratio of broiler breeders on egg quality and egg composition

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## ABSTRACT

Egg characteristics have an impact on embryonic development and post-hatch performance of broilers. The impact of growth curve (GC) and dietary energy-to-protein ratio of broiler breeder hens on egg characteristics was investigated. At hatch, 1,536 pullets were randomly allotted to 24 pens in a 2 x 4 factorial dose-response design with 2 GC (standard growth curve = SGC or elevated growth curve = EGC (+ 15%)) and 4 diets, differing in energy-to-protein ratio (defined as 96%, 100%, 104% and 108% AME<sub>n</sub> diet). Feed allocation per treatment was adapted weekly to achieve the targeted GC and to achieve pair-gain of breeders within each GC. Breeders on an EGC produced larger eggs ( $\Delta = 2.3$  g;  $P < 0.001$ ) compared to breeders on a SGC. An exponential regression curve, with age (wk) of the breeders, was fitted to describe the impact of GC and dietary energy-to-protein ratio on egg composition. Yolk weight was 0.8 g higher for eggs from EGC breeders than from SGC breeders ( $a - 108.1 * 0.907^{Age}$ ), where  $a$  was 22.1 and 22.9 for SGC and EGC, respectively;  $R^2 = 0.97$ ;  $P < 0.001$ ). An interaction between GC and dietary energy-to-protein ratio on albumen weight was observed ( $P = 0.04$ ). Dietary energy-to-protein ratio did not affect albumen weight in SGC breeders ( $42.7 - 56.2 * 0.934^{Age}$ ;  $R^2 = 0.89$ ), but for EGC breeders, a higher dietary energy-to-protein ratio resulted in a 0.9 g lower albumen weight from 96% AME<sub>n</sub> to 108% AME<sub>n</sub> ( $a - 62.9 * 0.926^{Age}$ , where  $a$  was 43.4, 43.2, 42.8, and 42.5 for 96% AME<sub>n</sub>, 100% AME<sub>n</sub>, 104% AME<sub>n</sub>, and 108% AME<sub>n</sub>, respectively;  $R^2 = 0.86$ ). Albumen DM content decreased linearly with an increased dietary energy-to-protein ratio, but this was more profound in EGC breeders ( $\beta = -0.03$  %/% AME<sub>n</sub>) than in SGC breeders ( $\beta = -0.01$  %/% AME<sub>n</sub>;  $P = 0.03$ ). Overall, it can be concluded that an EGC for breeders led to larger eggs with a more yolk and albumen, whereas dietary energy-to-protein ratio had minor effects on egg composition.

**Key words:** broiler breeder, feed strategy, modelling, egg components

## INTRODUCTION

The hatch of healthy viable day-old chicks is crucial for health, welfare and performance of broilers (Tona et al., 2005; Van de Ven et al., 2012). Day-old chick quality depends, among others, on the amount and quality of nutrients stored within the egg (Koppenol et al., 2015; Willems et al., 2015b; Iqbal et al., 2017), the ability of the embryo to use these nutrients (Yalçin et al., 2008), on albumen (Benton and Brake, 1996) and shell quality (Maina, 2017), and epigenetic factors (Lesuisse et al., 2018).

A fresh hatching egg contains approximately 50% protein, 40-43% lipids and 6% carbohydrates on a DM basis (Nangsuay et al., 2013, 2015). These egg nutrients are used by the embryo to develop. The yolk is a major energy source and both the yolk and the albumen are major protein sources for tissue synthesis in the developing embryo (Noble and Cocchi, 1990; Willems et al., 2014a). The shell controls the exchange of water and gasses through the pores in the shell and serves as a calcium source (Nys et al., 1999; Hincke et al., 2012). Variation in nutrient density, in the total amount of nutrients deposited in either of these components, or shell properties might therefore influence day-old chick quality (Lourens et al., 2006; Nangsuay et al., 2011, 2015).

Nutrients deposited within the egg are fixed at the moment of oviposition and should therefore contain all nutrients for the embryo to develop. Nutrients deposited in the egg originate either from mobilized body reserves of the breeder or from her diet (Ekmay et al., 2014; Salas et al., 2017). A change in breeder body reserves or diet composition might therefore influence nutrient deposition in the egg.

Total body reserves of the breeder hen can be changed by altering the growth curve during rearing and production (Van der Klein et al., 2018; Heijmans et al., 2021). A 15 to 22 % higher growth curve from 0-60 wk of age resulted in approximately 200 to 230 g more body fat at 55 to 60 wk of age (Van der Klein et al., 2018; Zuidhof, 2018; Heijmans et al., 2021) and approximately 65 g more breast filet, as an indicator for more body protein (Van der Klein et al., 2018). It can be hypothesized that more body reserves of the breeder hen is beneficial for egg production and egg composition (Ekmay et al., 2014; Salas et al., 2017). In breeders, no effect on egg composition was observed when breeders were 7.5% heavier during rearing alone, but had a similar BW and body composition during production (Van Emous et al., 2013, 2015a). It remains unclear whether a higher BW during the production phase affects egg composition. In layers, it was observed that 8% heavier layers produced 1.2 g heavier eggs with a 0.6 g heavier yolk and 0.6 g heavier albumen compared to lighter layers (Pérez-Bonilla et al., 2012). It is hypothesized that heavier broiler breeders will

produced larger eggs with a larger yolk, which eventually will be beneficial for chick quality (Nangsuay et al., 2015).

Another strategy to change breeder body reserves, while maintaining a similar BW, is by altering the dietary energy-to-protein ratio. In broiler breeders, feeding diets with 25 % lower dietary CP or 8 % higher dietary energy concentration from 0-60 wk of age resulted in 5 to 11 % more body fat at the same BW (Lesuisse et al., 2017; Zuidhof, 2018; Heijmans et al., 2021). Body fat is mobilized for yolk production (Salas et al., 2017) and consequently, it can be hypothesized that more body fat will be beneficial for yolk production and eventually chick quality (Nangsuay et al., 2015). However, it was observed in breeders that a higher dietary energy-to-protein ratio, by a reduction of 22-25% dietary CP concentration, did not affect yolk weight, albumen height or shell thickness (Lesuisse et al., 2017), but led to a 1.3 to 4.8 g lower albumen weight (Joseph et al., 2000; Lesuisse et al., 2017) and 3.4 to 4.0 g lower day-old chick weight (Lesuisse et al., 2017). This suggests that a reduction in dietary CP might not be beneficial for egg composition and chick quality. It remains unclear whether a higher dietary energy-to-protein ratio, by an increased dietary energy content, while maintaining a similar CP content, might affect yolk weight or density without penalizing albumen weight and egg quality.

The aim of the current study was to investigate the impact of growth curve and dietary energy-to-protein ratio of broiler breeders on egg quality and egg composition.

## MATERIALS AND METHODS

### Experimental Design

The experiment consisted of a 2 x 4 factorial dose-response design with 2 growth curves (**GC**) (standard growth curve = **SGC** or elevated growth curve = **EGC**) and 4 diets, differing in energy-to-protein ratio, by step-wise increase in energy content from 96 to 108% at a similar CP content (defined as 96%, 100%, 104% and 108% AME<sub>n</sub> diet). A dose-response design was applied in order to estimate potential linear and quadratic contrasts for dietary energy-to-protein ratio over a larger range of dietary energy content. At the start of the experiment (d 0), 1,536 Ross 308 female broiler breeder pullets, originating from a 37 wk old grandparent flock (Aviagen-EPI, Roermond, The Netherlands) were randomly placed in 24 pens (64 pullets per pen). Treatments were randomly assigned within 3 blocks of 8 pens (n = 3 per treatment) and continued up to 60 wk of age. Feed allocation per diet was adapted weekly to achieve pair-gain of breeders within each GC. All experimental protocols were ap-

proved by the Central Commission on Animal Experimentation (The Hague, the Netherlands), approval number 2018.W-0023.001.

### Breeders, Housing and Management

A detailed description of this experiment was reported by Heijmans et al. (2021). In brief, each pen consisted of a floor area (4.9 m<sup>2</sup>) with wood shavings as bedding and an elevated slatted floor (5.1 m<sup>2</sup>). On the elevated slatted area, a track feeding system was placed with a grill preventing rooster access. Feed was provided once per day. Drinking nipples were also placed on the elevated slatted floor and water was supplied *ad libitum*. Pullets were reared on a 8L:16D (10 lux) photoperiod and instantly photo-stimulated at 21 wk of age (11L:13D), with a gradual increase up to 23 wk of age (13L:11D). Laying nests were available to the breeders from 20 wk of age onward. At 20 wk of age, all pens were standardized to 45 breeders per pen (4.5 breeders per m<sup>2</sup>), closest to the average pen weight and 4 20-wk old Ross 308 roosters were placed per pen. Roosters were fed a commercially available diet once a day in a rooster feeding pan. Height of the feeding pan was adjusted to prevent female access.

### Experimental Diets and Feed Allocation

Experimental diets were formulated isonitrogenous. Dietary AME<sub>n</sub> levels were step-wise increased from 96% to 108% (96%, 100%, 104%, and 108%), where the 100% AME<sub>n</sub> treatment was the AME<sub>n</sub> recommended by the breeding company (Aviagen-EPI, 2016b). Dietary AME<sub>n</sub> was increased by a higher inclusion of crude fat (soy oil and lard) and starch (maize starch), while decreasing inclusion of crude fiber (cellulose and finely ground oat hulls). The 96% and 108% AME<sub>n</sub> diets were produced first. The intermediate diets (100% and 104% AME<sub>n</sub>) were produced by homogeneous mixing 96% and 108% AME<sub>n</sub> diets in a 2:1 (100% AME<sub>n</sub>) or 1:2 (104% AME<sub>n</sub>) ratio. A detailed description of the diets was reported by Heijmans et al. (2021). Dietary ingredients, and calculated and analyzed nutrient content of the experimental diets is presented in Table 1. The weekly growth target of the SGC was according to the breeder recommendation (Aviagen, 2016b), whereas the EGC targeted a 15% higher weekly growth relative to the SGC throughout rearing and production. Daily feed allocation was calculated and adjusted weekly based on the desired GC. As starting point to achieve pair-gain of breeders, feed allocation of the SGC was according to breeder recommendation (Aviagen, 2016b) and feed allocation of the EGC was 15% higher, compared to the SGC. Hereafter, growth and egg production in the week prior were the directives for calculating the daily feed allocation. Within each GC, daily feed allocation was adjusted weekly based on dietary energy-to-protein ratio to achieve pair-gaining. As starting point to achieve pair-gain of breeders, feed allocation of the 100% AME<sub>n</sub> was according to breeder recommendation (Aviagen, 2016b). Feed allocation of the other treatments (96%, 104% and 108% AME<sub>n</sub>) were adjusted

Table 1. Dietary ingredients, and calculated and analyzed nutrients of diets (g/kg, as-fed basis)

Item	Starter 1 (0-21 days)			Starter 2 (22-42 days)			Grower (43-112 days)			Pre-breeder (113-160 days)			Breeder 1 (161-280 days)			Breeder 2 (281-420 days)		
	96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>	
Ingredient																		
Maize	450.0	450.0		500.0	500.0		400.0	400.0		500.0	500.0		440.0	440.0		460.0	460.0	
Wheat	100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0	
Soybean meal	240.9	245.1		141.3	146.3		76.1	80.7		48.9	52.8		149.8	152.5		130.5	133.4	
Sunflower meal	50.0	50.0		90.0	90.0		150.0	150.0		165.0	165.0		80.0	80.0		90.0	90.0	
Wheat middlings	-	-		-	-		100.0	100.0		25.0	25.0		-	-		-	-	
Oat hulls (fine)	50.0	1.0		56.0	5.1		65.0	19.3		50.0	1.0		48.0	1.0		46.6	1.0	
Cellulose	44.1	1.0		47.9	5.0		50.0	5.0		46.8	1.0		44.5	1.0		45.2	1.0	
Soya oil	11.1	17.8		9.5	14.3		8.0	12.0		5.0	7.0		4.8	10.8		11.9	14.9	
Lard	3.0	4.2		4.2	6.8		3.3	6.7		5.0	10.2		29.5	34.9		23.5	32.1	
Maize starch	14.0	94.5		14.3	96.2		19.9	99.2		11.7	96.1		14.7	91.6		1.0	76.9	
Chalk	13.9	14.1		13.8	13.9		13.3	13.4		-	-		-	-		-	-	
Limestone (coarse)	-	-		-	-		-	-		24.5	24.6		71.0	71.1		73.4	73.5	
Monocalcium phosphate	9.8	9.2		10.5	9.9		5.4	4.9		5.8	5.2		6.0	5.5		6.5	5.9	
Sodium bicarbonate	3.3	3.3		3.3	3.3		2.5	2.5		3.3	3.3		2.7	2.7		3.0	2.9	
Salt	1.8	1.8		1.7	1.7		2.2	2.2		1.5	1.5		2.1	2.1		2.0	2.0	
L-Lysine	1.73	1.69		1.88	1.80		0.23	0.15		1.63	1.58		0.44	0.42		0.36	0.34	
L-Threonine	0.68	0.68		0.54	0.54		-	-		0.49	0.48		0.57	0.58		0.54	0.55	
DL-Methionine	2.34	2.34		1.71	1.71		0.65	0.65		1.13	1.13		1.73	1.77		1.59	1.62	
Choline Chloride-50%	0.8	0.8		0.8	0.8		0.8	0.8		1.5	1.4		1.4	1.3		1.5	1.4	
Xylanase	0.1	0.1		0.1	0.1		0.1	0.1		0.1	0.1		0.1	0.1		0.1	0.1	
Phytase	0.05	0.05		0.05	0.05		0.05	0.05		0.05	0.05		0.05	0.05		0.05	0.05	
Premix rearing <sup>1</sup>	2.5	2.5		2.5	2.5		2.5	2.5		-	-		-	-		-	-	
Premix laying <sup>2</sup>	-	-		-	-		-	-		2.5	2.5		2.5	2.5		2.5	2.5	



Table 1. Continued

Item	Starter 1 (0-21 days)			Starter 2 (22-42 days)			Grower (43-112 days)			Pre-breeder (113-160 days)			Breeder 1 (161-280 days)			Breeder 2 (281-420 days)		
	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>	96% AME <sub>n</sub>	108% AME <sub>n</sub>	AME <sub>n</sub>
Calculated content <sup>3</sup>																		
AME <sub>n</sub> (kcal/kg)	2,570	2,890		2,570	2,890		2,545	2,865		2,640	2,970		2,735	3,080		2,735	3,080	
Crude protein	175.1	175.0		143.7	143.6		136.5	136.5		123.0	122.5		138.5	137.7		135.2	134.3	
Crude fat	41.5	49.0		42.0	49.0		40.0	47.0		38.8	45.7		60.0	71.1		61.6	72.8	
Crude fibre	77.1	37.7		88.0	48.3		111.5	71.5		105.6	64.3		81.4	42.0		85.2	43.9	
Starch	379.5	446.9		408.6	477.5		371.5	438.5		407.5	480.4		368.2	434.4		373.8	436.0	
Starch:fat	9.1	9.1		9.7	9.7		9.3	9.3		10.5	10.5		6.1	6.1		6.1	6.0	
Linoleic acid	18.0	21.0		18.0	20.3		17.0	19.0		16.3	17.4		16.8	20.0		20.0	22.0	
Digestible lysine	9.0	9.0		7.0	7.0		4.8	4.8		5.1	5.1		5.9	5.9		5.5	5.5	
Calcium	9.8	9.8		9.8	9.8		8.9	8.9		13.1	13.1		31.0	31.0		31.0	31.0	
Retainable phosphorus	4.1	4.1		4.1	4.1		3.3	3.3		3.2	3.2		3.2	3.2		3.2	3.2	
Analyzed content																		
Crude protein <sup>4</sup>	170.2	172.9		145.1	148.0		133.0	135.1		129.6	127.4		145.2	142.2		139.9	135.1	
Crude fat <sup>4</sup>	37.0	43.2		38.3	44.3		39.0	42.4		33.1	41.1		57.6	66.8		58.2	67.3	
Starch	401.0	463.0		408.0	472.0		377.0	431.0		415.6	486.3		376.4	436.8		371.7	432.5	

<sup>1</sup>Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 3.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 6.0 mg; Vitamin B<sub>6</sub> 4.0 mg; Vitamin B<sub>12</sub> 20 µg; Niacinamide 35 mg; D-pantothenic acid 15 mg; Folic acid 1.5 mg; Biotin 0.20 mg; Iron 40 mg; Copper 16 mg; Manganese 120 mg; Zinc 90 mg; Iodine 1.25 mg; Selenium 0.3 mg.

<sup>2</sup>Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 5.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 12.0 mg; Vitamin B<sub>6</sub> 5.0 mg; Vitamin B<sub>12</sub> 40 µg; Niacinamide 55 mg; D-pantothenic acid 15 mg; Folic acid 2.0 mg; Biotin 0.40 mg; Iron 50 mg; Copper 10 mg; Manganese 120 mg; Zinc 90 mg; Iodine 2.0 mg; Selenium 0.3 mg.

<sup>3</sup>Calculated according to CVB (2012).

<sup>4</sup>Analyzed values were within boundaries of the analytical error.

relatively to the 100% AME<sub>n</sub> treatment to achieve a similar daily AME<sub>n</sub> intake. Hereafter, growth and egg production in the week prior were the directives for calculating the daily feed allocation.

## Measurements

**Egg Weight and Laying Rate.** Eggs were collected and weighed daily per pen. Average egg weight of all eggs produced, excluding double yolked eggs, was calculated per pen per week. Laying rate was calculated as the total number of eggs produced divided by the number of breeders per pen per week, corrected for mortality.

**Egg Quality.** Egg quality was determined weekly from 25 to 28 wk of age. Hereafter, egg quality was determined every other week until 60 wk of age, with exception from 42 and 48 wk of age. At each age, 10 settable eggs per pen were randomly selected for analysis. Eggshell breaking strength was measured at the equator of each egg, using an eggshell tester (Futura, Löhne, Germany). Albumen height was measured at approximately 1 cm distance from the yolk, using an albumen height gauge (TSS, York, UK). Eggshell thickness without membranes was measured at three regions of the egg (blunt end, equator, and pointed end) of three eggs per pen, using an electronic micrometer (Helios Preisser, Gammertingen, Germany). Albumen height, breaking strength, and shell thickness were averaged per pen per age.

**Fresh Egg Composition.** Fresh egg composition was measured from the same eggs as used for egg quality analysis. Eggs were weighed individually and thereafter the yolk was separated from the albumen and weighed. Eggshells, including shell membranes were tissue cleaned, dried at 180 °C for twenty minutes, and weighed. Albumen weight was calculated as the difference between egg weight and the sum of yolk weight and eggshell weight. Yolk weight, shell weight, and albumen weight were averaged per pen per age.

**DM Analysis.** At 26, 28, 33, 36, and 60 wk of age, yolk samples, used for fresh egg composition, were pooled in three samples per age per pen. At the same ages, including 46 wk of age, albumen samples, used for fresh egg composition, were pooled in three samples per age per pen. The yolk and albumen samples were stored at -20 °C for further analysis. Samples were freeze dried and DM determined by the proximate method (AOAC, 1990). Yolk and albumen dry matter percentage were averaged per pen per age.

## Statistical Analysis

All data were analyzed, using the Restricted Maximum Likelihood variance component analysis procedure within a linear mixed model (Genstat 19<sup>th</sup> Edition, 2019).

Pen was used as the experimental unit for all analyses. Means and model residuals were checked on homogeneity of variance prior to analyses. The model used was:

$$Y_{ijkl} = \mu + GC_i + Diet_j + GC_i \times Diet_j + Age_k + GC_i \times Age_k + Diet_j \times Age_k + GC_i \times Diet_j \times Age_k + Block_l + e_{ijkl}$$

Where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the overall mean,  $GC_i$  is the growth curve ( $i$  = SGC or EGC),  $Diet_j$  is the energy-to-protein ratio in the diet ( $j$  = 96%, 100%, 104% or 108% AME<sub>n</sub>),  $GC_i \times Diet_j$  is the interaction between growth curve and diet,  $Age_k$  is age of the breeder flock ( $k$  = 22 to 60 wk of age),  $Block_l$  is the block ( $k$  = 1, 2 or 3), and  $e_{ijkl}$  is the residual error. Pre-liminary analysis showed that interactions between GC and Age, Diet and Age, and between GC, Diet and Age were not significant for any of the variables and consequently they were excluded from the model. Age was excluded from the model for egg weight and laying rate analysis, as these variables were analyzed per week. Fisher adjustments were used for multiple comparisons of factorial analysis.

Additionally, effects of Diet and Diet  $\times$  GC interaction were analyzed as linear or quadratic contrasts. If linear effects of dietary energy-to-protein ratio were observed, also within GC, the slope ( $\beta$ ) is presented in the result section. If quadratic effects of dietary energy-to-protein ratio, also within GC, were observed, the estimated AME<sub>n</sub> percentage at which the dependent variable was at the maximum (concave quadratic relation) or minimum (convex quadratic relation) was calculated and presented in the result section. Data are presented as LS means  $\pm$  SEM.

Additionally, to describe differences in egg composition over time, weight of the yolk, albumen and shell for each GC, diet and diet  $\times$  GC interaction in relation to breeder age were fitted, using the non-linear regression procedure in Genstat, analogue to Nonis and Gous (2013), based on the following exponential regression curve:

$$Y = a + b * c^{Age}$$

where  $Y$  is either yolk, albumen or shell weight and  $a$ ,  $b$  and  $c$  are the fitted coefficients for the exponential regression curve and  $Age$  is the age of the breeder hen in wk. First, the model was fitted as a single curve with the same coefficients for each GC or diet (model I). Next, the model was step-wise expanded with a separate constant coefficient ( $a$ ; model II) for parallel lines, with a separate constant ( $a$ ) plus linear ( $b$ ; model III) coefficients for separate lines, or with all coefficients separate (model IV), for each GC  $\times$  diet interaction. After each model fit, it was evaluated whether or not the model significantly improved, compared to the previous model. A significantly lower residual mean square error, a lower Bayesian Information Criterion (BIC), and

a higher  $R^2$  indicated a better fit, compared to the previous model. The final model used (I to IV), was the model that significantly improved the fit compared to the previous model and no significant improvement of the fit was observed of the next model. Estimated coefficients and  $R^2$  of fitted models are presented. All statements of significance are based on testing at  $P \leq 0.05$ .

## RESULTS

Results on nutrient intake, BW development, and productive performance, including settable egg production are presented elsewhere (Heijmans et al., 2021). No differences between treatments were observed on total settable egg production. On average, settable egg production was 181.9 eggs per breeder from 22 to 60 wk of age.

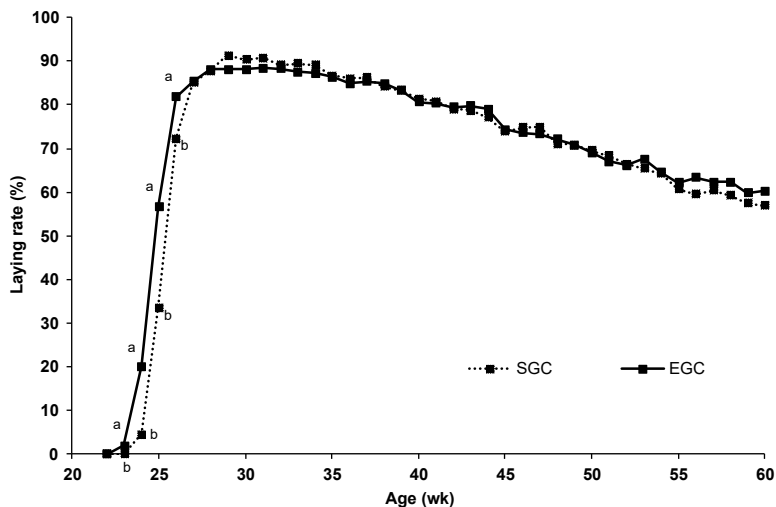
### Laying Rate

An interaction between GC and dietary energy-to-protein ratio on laying rate at 28, 29 and 41 wk of age was observed (data not presented). At these ages, laying rate decreased linearly with an increasing dietary energy-to-protein ratio within EGC breeders ( $\beta = -0.5 \text{ \%}/\% \text{ AME}_n$  on average), whereas laying rate increased linearly with an increasing dietary energy-to-protein ratio within SGC breeders ( $\beta = 0.3 \text{ \%}/\% \text{ AME}_n$  on average). EGC breeders had a higher laying rate from 23 to 26 wk of age than SGC breeders ( $\Delta = 12.5 \text{ \%}$  on average; Figure 1). From 30 to 60 wk of age, with exception of the interaction at 41 wk of age, no differences in laying rate between EGC and SGC breeders were observed (Figure 1).

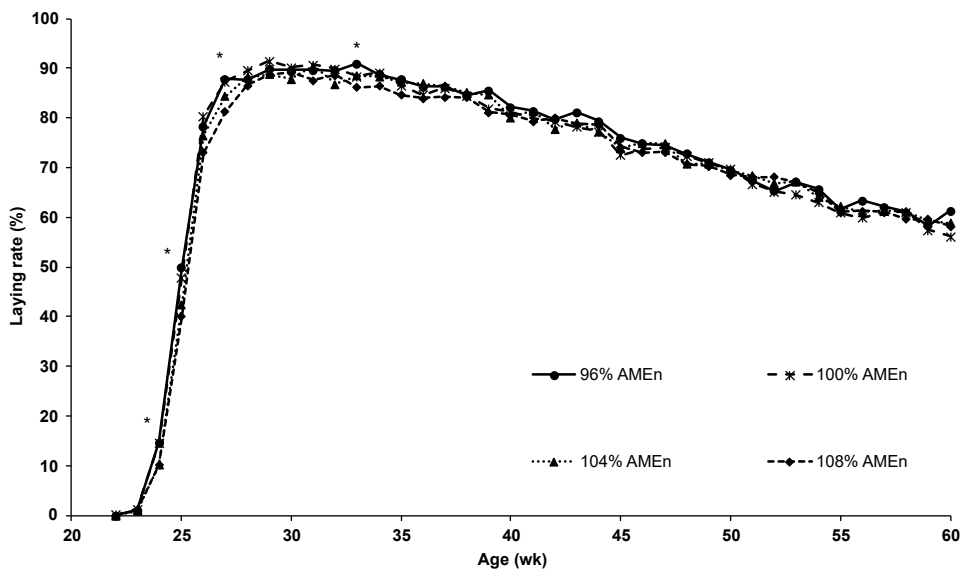
Laying rate decreased linearly with an increasing dietary energy-to-protein ratio at 24, 25, 27 and 33 wk of age ( $\beta = -0.6 \text{ \%}/\% \text{ AME}_n$  on average; Figure 2). No other difference in laying rate between different dietary energy-to-protein ratio was observed from 34 to 60 wk of age, with exception of the interaction at 41 wk of age (Figure 2).

### Egg Weight

Egg weight was affected linearly by dietary energy-to-protein ratio. Therefore, only egg weights of the following treatments are presented; 96%  $\text{AME}_n$  SGC, 108%  $\text{AME}_n$  SGC, 96%  $\text{AME}_n$  EGC, and 108%  $\text{AME}_n$  EGC (Figure 3). An interaction between GC and dietary energy-to-protein ratio (linear) on egg weight was observed at 28, 35, 41, 42, 44 to 51, 59, and 60 wk of age ( $P \leq 0.05$ ; Figure 3). At all these ages, with exception of 28 wk of age, egg weight decreased linearly with an increasing dietary energy-to-protein ratio for EGC breeders ( $\beta = -0.13 \text{ g}/\% \text{ AME}_n$  on average), whereas egg weight increased linearly with an increasing dietary energy-to-protein ratio for SGC breeders ( $\beta = 0.04 \text{ g}/\% \text{ AME}_n$  on average). At 28 wk of age, in both GC, egg weight decreased with an increasing dietary energy-to-protein ratio, but this was more



**Figure 1.** Laying rate of broiler breeders fed on 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) from 0 to 60 wk of age. <sup>a,b</sup>LSmeans within age lacking a common superscript differ ( $P \leq 0.05$ ).



**Figure 2.** Laying rate of broiler breeders fed 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AMEn), fed from 0 to 60 wk of age. LSmeans within age with asterisk show a significant linear effect of energy-to-protein ratio ( $P \leq 0.05$ ).

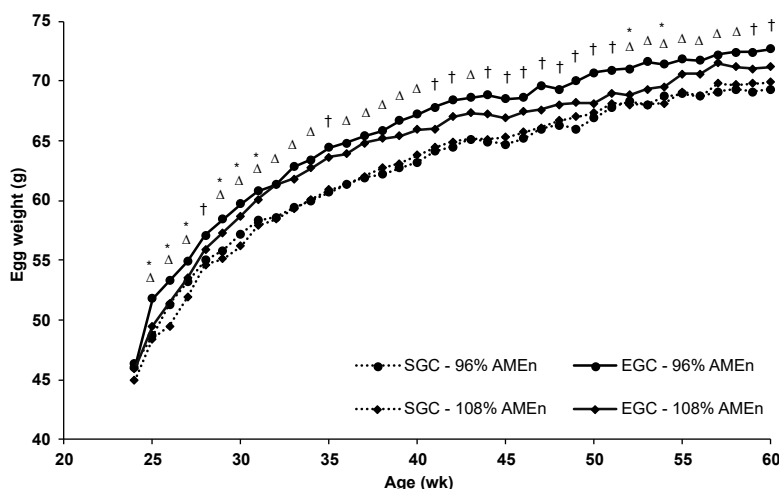


Figure 3. Egg weight of broiler breeders fed on 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 2 diets, differing in energy-to-protein ratio (96 or 108% AME<sub>n</sub>), from 0 to 60 wk of age. <sup>†</sup>ΔLSmeans within age with a dagger (GC x diet (linear)), with a triangle (GC) or with an asterisk (diet (linear)) showed a significant effect ( $P \leq 0.05$ ). Data on the 2 intermediate diets (100% or 104% AME<sub>n</sub>) is not presented, as effects of dietary energy-to-protein ratio were linearly.

profound in EGC breeders ( $\beta = -0.12$  g/% AME<sub>n</sub>) than in SGC breeders ( $\beta = -0.04$  g/% AME<sub>n</sub>). Regardless of the interactions indicated above, at all ages EGC breeders produced heavier eggs than SGC breeders ( $\Delta = 2.3$  g on average;  $P < 0.001$ ). At 25 to 31, 52, and 54 wk of age, a linear effect of dietary energy-to-protein ratio was observed ( $P < 0.05$ ). Breeders with a higher dietary energy-to-protein ratio produced lighter eggs ( $\beta = -0.10$  g/% AME<sub>n</sub>).

### Egg Quality

In total, egg quality of 4,320 eggs was determined over a period of 25 to 60 wk of age. In the first phase of lay (24-40 wk of age), no interaction between GC and dietary energy-to-protein ratio and neither an effect of dietary energy-to-protein ratio on albumen height was observed (Table 2). Eggs from EGC breeders had a lower albumen height than eggs from SGC breeders in this phase ( $\Delta = 0.1$  mm;  $P = 0.03$ ). In the second phase of lay (41-60 wk of age) and over the total laying period, albumen height was 0.5 mm and 0.3 mm higher, respectively, in SGC breeders than in EGC fed at 96% AME<sub>n</sub>. This difference disappeared with a higher dietary energy-to-protein ratio in a quadratic way ( $P < 0.05$ ).

In the first phase of lay (24-40 wk of age), a quadratic interaction between GC and dietary energy-to-protein ratio on breaking strength was observed (Table 2). Within the SGC, the highest breaking strength was estimated at 101% AME<sub>n</sub> ( $\Delta_{\max} = 1.5$  N), whereas within the EGC, the lowest breaking strength was estimated at 101% AME<sub>n</sub> ( $\Delta_{\max} = -0.8$  N). In the second phase of lay (41-60 wk of age) and over the total laying period (24-60 wk of age), no interaction between GC and dietary energy-to-protein ratio nor a dietary energy-to-protein ratio effect on breaking strength was observed. In the second phase of lay (41-60 wk of age;  $\Delta = 0.7$  N;  $P = 0.05$ ) and over the total laying period (24-60 wk of age;  $\Delta = 0.7$  N;  $P < 0.001$ ), breaking strength was higher in eggs of SGC breeders than in eggs of EGC breeders. After correction for egg weight differences, differences in breaking strength were still significant between eggs from SGC and EGC breeders. No effect of GC, dietary energy-to-protein ratio, or the interaction between them, on shell thickness was observed (Table 2).

### Egg Composition

In total, egg composition of 4,320 eggs was determined over a period of 25 to 60 wk of age. Egg composition of the treatments during the first phase of lay (24-40 wk of age), second phase of lay (41-60 wk of age) and over the total laying period (24-60 wk of age) can be found in supplementary Table S1.

The exponential regression curves were fitted to describe the impact of GC and dietary energy-to-protein ratio on albumen, yolk and shell weight throughout the laying phase. An interaction between GC and dietary energy-to-protein ratio was observed on predicted albumen weight. In SGC breeders, dietary energy-to-protein ratio did not affect predicted albumen weight. A common line (model I) had the best fit (predicted albumen weight SGC =  $42.7 - 56.2 * 0.934^{Age}$  ( $R^2 = 0.89$ ;  $P < 0.001$ )). However, in EGC breeders, the predicted albumen weight decreased in step-wise manner with 0.9 g when dietary energy-to-protein ratio increased from 96% AME<sub>n</sub> to 108% AME<sub>n</sub> (Figure 4;  $P < 0.001$ ) Predicted albumen weight for EGC breeders could be expressed as  $a - 62.9 * 0.926^{Age}$  ( $R^2 = 0.86$ ;  $P < 0.001$ ), where  $a$  was 43.4, 43.2, 42.8, and 42.5 for 96% AME<sub>n</sub>, 100% AME<sub>n</sub>, 104% AME<sub>n</sub>, and 108% AME<sub>n</sub>, respectively ( $P < 0.001$ ). Regardless of the interaction indicated above, predicted albumen weight was always lower in SGC breeders than in EGC breeders (Figure 4).

No interaction between GC and dietary energy-to-protein ratio nor a dietary energy-to-protein ratio effect was observed on predicted yolk or shell weight (data not presented). Predicted yolk weight was 0.8 g higher for eggs from EGC breeders than from SGC breeders throughout the laying phase: predicted yolk weight =  $a - 108.1 * 0.907^{Age}$  ( $R^2 = 0.97$ ;  $P < 0.001$ ), where  $a$  was 22.1 and 22.9 for SGC and EGC breeders, respectively (Figure 5). Predicted shell weight was 0.1 g higher for eggs from EGC breeders than from SGC breeders throughout the laying phase:

Table 2. Average albumen height, breaking strength and shell thickness during first phase of lay (22-40 wk), second phase of lay (41-60 wk) and the whole laying phase (22-60 wk) of eggs produced by broiler breeders fed on 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from 0 to 60 wk of age.

Item	22-40 wk			41-60 wk			22-60 wk		
	Albumen height (mm)	Breaking strength (N)	Shell thickness (μm)	Albumen height (mm)	Breaking strength (N)	Shell thickness (μm)	Albumen height (mm)	Breaking strength (N)	Shell thickness (μm)
Growth curve (n=12)									
SGC	7.7 <sup>a</sup>	38.1	363	6.8	38.2 <sup>a</sup>	369	7.3	38.1 <sup>a</sup>	366
EGC	7.6 <sup>b</sup>	37.3	363	6.7	37.5 <sup>b</sup>	367	7.2	37.4 <sup>b</sup>	365
SEM	0.0	0.2	1	0.0	0.2	1	0.0	0.1	1
Diet (n=6)									
96% AME <sub>n</sub>	7.7	37.6	363	6.8	37.7	366	7.3	37.6	364
100% AME <sub>n</sub>	7.6	37.9	361	6.8	38.0	371	7.2	38.0	365
104% AME <sub>n</sub>	7.7	37.7	364	6.7	37.6	367	7.2	37.6	365
108% AME <sub>n</sub>	7.7	37.6	364	6.7	38.2	368	7.2	37.8	366
SEM	0.0	0.3	2	0.1	0.3	2	0.0	0.2	1
Treatment (n=3)									
SGC	7.7	37.9 <sup>ab</sup>	364	7.0 <sup>a</sup>	38.2	369	7.4 <sup>a</sup>	38.0	366
96% AME <sub>n</sub>	7.6	38.7 <sup>a</sup>	363	6.7 <sup>bcd</sup>	37.8	369	7.2 <sup>b</sup>	38.3	366
100% AME <sub>n</sub>	7.8	38.5 <sup>a</sup>	363	6.8 <sup>abc</sup>	38.0	368	7.3 <sup>a</sup>	38.3	365
104% AME <sub>n</sub>	7.8	37.3 <sup>b</sup>	362	6.8 <sup>abc</sup>	38.9	368	7.3 <sup>a</sup>	38.0	365
108% AME <sub>n</sub>	7.6	37.2 <sup>b</sup>	361	6.5 <sup>d</sup>	37.3	364	7.1 <sup>b</sup>	37.3	362
EGC	7.6	37.1 <sup>b</sup>	359	6.9 <sup>ab</sup>	38.3	372	7.3 <sup>ab</sup>	37.6	365
96% AME <sub>n</sub>	7.6	37.0 <sup>b</sup>	365	6.6 <sup>cd</sup>	37.1	365	7.2 <sup>b</sup>	37.0	365
100% AME <sub>n</sub>	7.6	37.8 <sup>ab</sup>	366	6.6 <sup>cd</sup>	37.5	367	7.2 <sup>b</sup>	37.7	367
104% AME <sub>n</sub>	0.1	0.4	3	0.1	0.5	3	0.1	0.3	2
108% AME <sub>n</sub>									
SEM									
P-value									
Growth curve (GC)	0.03	0.003	0.90	0.003	0.05	0.53	<0.001	<0.001	0.71
Diet (factorial)	0.45	0.74	0.74	0.76	0.55	0.47	0.80	0.64	0.87
Diet (linear)	0.80	0.89	0.40	0.39	0.55	0.95	0.67	0.75	0.48
Diet (quadratic)	0.31	0.33	0.65	0.93	0.60	0.51	0.43	0.75	0.94
GC x Diet (factorial)	0.39	0.02	0.47	0.001	0.21	0.49	0.004	0.49	0.61
GC x Diet (linear)	0.29	0.11	0.16	0.26	0.29	0.78	0.96	0.71	0.21
GC x Diet (quadratic)	0.55	0.006	0.71	0.006	0.16	0.42	0.02	0.35	0.82
Age	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001

<sup>a-d</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).



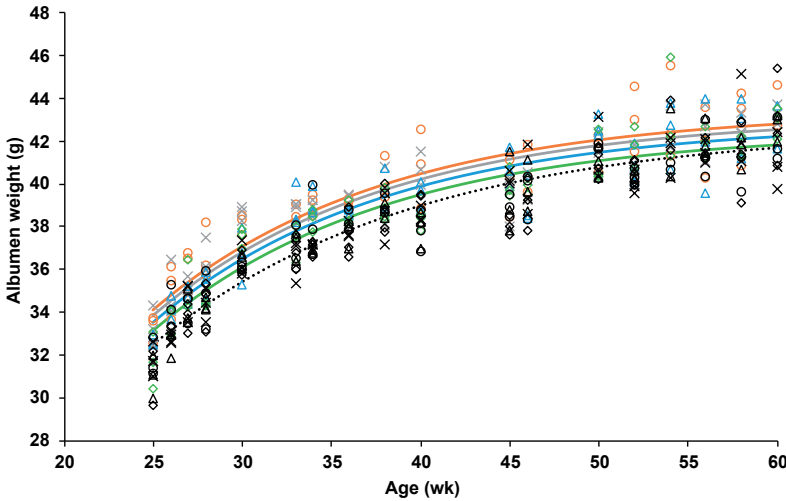


Figure 4. Observed (symbols) and predicted (lines) albumen weight of broiler breeders fed on a standard growth curve (black symbols, dashed line) and 4 diets, differing in energy-to-protein ratio; 96% AME<sub>n</sub> (○), 100% AME<sub>n</sub> (×), 104% AME<sub>n</sub> (Δ), or 108% AME<sub>n</sub> (◇) or fed on an elevated growth curve (+15% compared to standard, solid lines) and 4 diets, differing in energy-to-protein ratio; 96% AME<sub>n</sub> (red ○), 100% AME<sub>n</sub> (grey ×), 104% AME<sub>n</sub> (blue Δ), or 108% AME<sub>n</sub> (green ◇), from 0 to 60 wk of age. Each symbol represents 1 replicate at each time point.

predicted shell weight =  $a - 4.9 * 0.967^{Age}$  ( $R^2 = 0.88$ ;  $P < 0.001$ ), where  $a$  was 7.1 and 7.2 for SGC and EGC breeders, respectively (Figure 5).

No effect of GC, dietary energy-to-protein ratio or the interaction between them was observed on DM content of the yolk (Table 3). A linear interaction between GC and dietary energy-to-protein ratio was observed on DM content of the albumen (Table 3). In both GC, a linear increase in dietary energy-to-protein ratio resulted in a linear decrease in DM content of the albumen, but this was more profound in EGC breeders ( $\beta = -0.03 \text{ \%}/\% \text{ AME}_n$ ) than in SGC breeders ( $\beta = -0.01 \text{ \%}/\% \text{ AME}_n$ ;  $P = 0.03$ ).

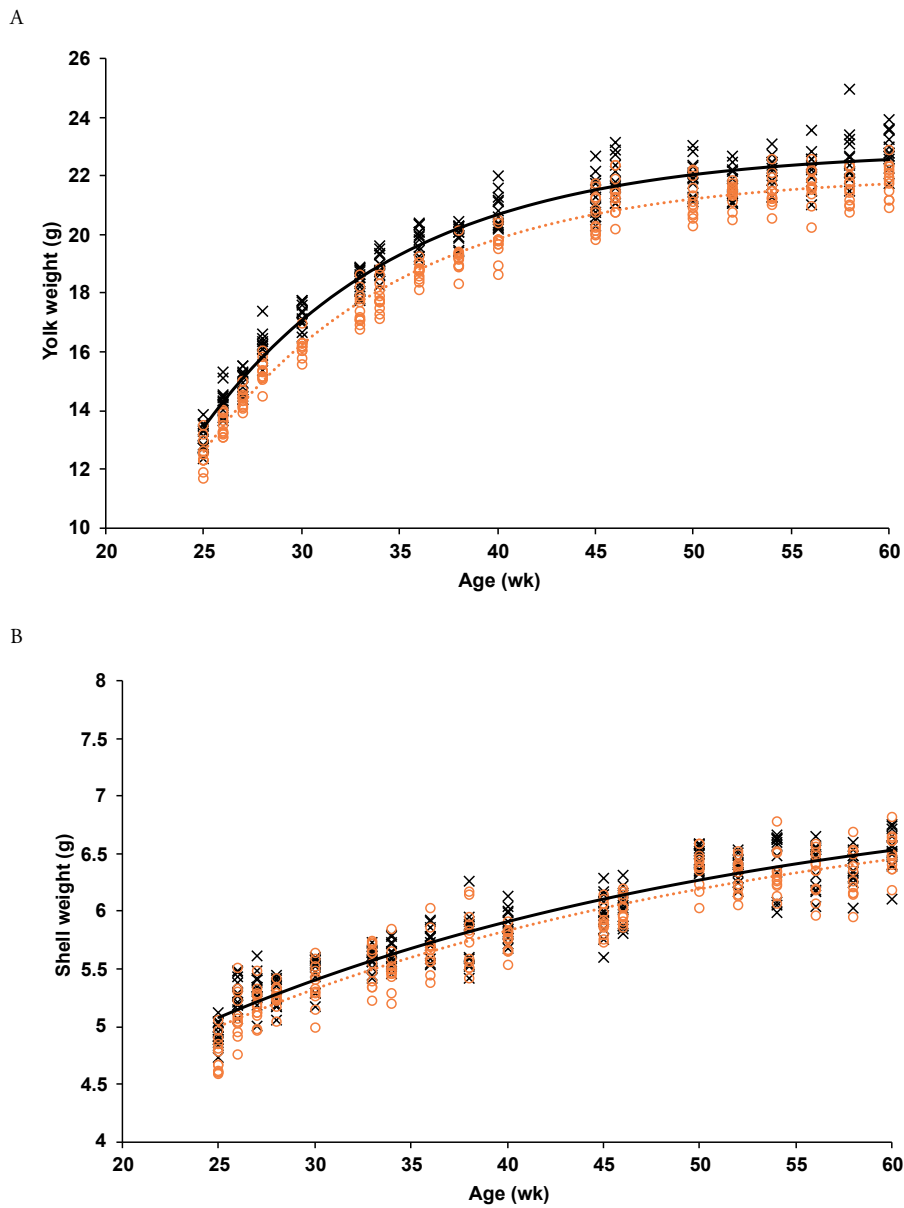


Figure 5. Observed (symbols) and predicted (lines) yolk weight (A) and shell weight (B) of broiler breeders fed on 2 different growth curves; standard growth curve (red  $\circ$ , dashed line) or elevated growth curve (+15%; black  $\times$ , solid line) from 0 to 60 wk of age. Each symbol represents 1 replicate at each time point.

Table 3. Average egg yolk and albumen dry matter content of eggs produced by broiler breeders fed on 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from 0 to 60 wk of age.

Item		DM Yolk <sup>1</sup> (%)	DM Albumen <sup>2</sup> (%)
Growth curve (n=12)			
SGC		50.9	13.9
EGC		51.0	13.9
SEM		0.1	0.0
Diet (n=6)			
96% AME <sub>n</sub>		50.9	14.0
100% AME <sub>n</sub>		50.9	13.9
104% AME <sub>n</sub>		50.9	13.9
108% AME <sub>n</sub>		51.0	13.7
SEM		0.1	0.1
Treatment (n=3)			
SGC	96% AME <sub>n</sub>	50.9	13.9 <sup>bcd</sup>
	100% AME <sub>n</sub>	50.9	13.9 <sup>abc</sup>
	104% AME <sub>n</sub>	50.9	14.0 <sup>ab</sup>
	108% AME <sub>n</sub>	51.0	13.7 <sup>cd</sup>
EGC	96% AME <sub>n</sub>	50.9	14.1 <sup>a</sup>
	100% AME <sub>n</sub>	51.0	14.0 <sup>ab</sup>
	104% AME <sub>n</sub>	51.0	13.8 <sup>bcd</sup>
	108% AME <sub>n</sub>	50.9	13.7 <sup>d</sup>
	SEM	0.1	0.1
P-value			
Growth curve (GC)		0.68	0.61
Diet (factorial)		0.87	0.001
Diet (linear)		0.40	<0.001
Diet (quadratic)		0.93	0.08
GC x Diet (factorial)		0.78	0.05
GC x Diet (linear)		0.65	0.03
GC x Diet (quadratic)		0.37	0.15
Age		<0.001	<0.001

<sup>a-d</sup>LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Determined at 26, 28, 33, 36, and 60 wk of age.

<sup>2</sup>Determined at 26, 28, 33, 36, 46 and 60 wk of age.

## DISCUSSION

The objective of this study was to evaluate effects of growth curve and dietary energy-to-protein ratio of broiler breeder hens on egg characteristics. Results will be discussed on main effects. Interactions will be discussed within the discussion of dietary energy-to-protein ratio.

### Growth Curve

In the current study, EGC breeders cycle had on average a 12.5% higher laying rate in the first 4 weeks of lay than SGC breeders. Sun and Coon (2005) and Van der Klein et al. (2018) also observed a 7.1 to 17.3% higher laying rate in the first 4 to 6 weeks of lay for breeders that were 22 to 37% heavier at the end of rearing compared to standard breeders. The higher laying rate in the first weeks of the laying cycle can be explained by an earlier sexual maturation of heavier breeders (Sun and Coon, 2005; Renema et al., 2007; Van der Klein et al., 2018; Heijmans et al., 2021). From 30 wk of age onwards, no differences between GC in laying rate were observed, which is in line with Van der Klein et al. (2018). Currently, breeders are fed restrictedly to control their BW development in order to ensure good health and reproductive performance (Robinson et al., 1991; Bruggeman et al., 1999; Sun et al., 2006). In the current study and other studies, breeders with a 7.2 to 22.5% higher BW than standard (Van der Klein et al., 2018; Zukiwsky et al., 2021) or even *ad libitum* fed breeders (Zukiwsky et al., 2021) realized a similar rate of lay as breeders with a standard BW. All these results suggest that relaxation in feed restriction level might be possible, leading to an improved welfare of breeders, without negative effects on rate of lay. However, it remains unclear whether or not a higher than standard BW deteriorates fertility of breeders, which is another important factor for reproduction. Future studies should consider the impact of growth curve on fertility in current broiler breeders.

To our knowledge, only a limited number of studies is available on the impact of GC or BW of the broiler breeder hen (Van Emous et al., 2015a) or layer hen (Pérez-Bonilla et al., 2012) on egg quality parameters. Over the total laying period, some minor effects of GC on egg quality were observed, but it can be questioned whether or not these differences are relevant in perspective to offspring quality. Eggshell breaking strength was 0.8 N lower for eggs from EGC breeders than from SGC breeders. This was also observed after correction for differences in egg weight. Eggshell strength has been found to be positively related to the proportional eggshell weight and eggshell thickness, as reviewed by Roberts (2004). It was observed that eggshell thickness was similar between eggs from both GC, but as a proportion of egg weight, eggshells were smaller from EGC breeders than eggshells from SGC breeders, which might explain the lower eggshell breaking strength. A lower eggshell breaking strength in

eggs obtained from EGC breeders, compared to SGC breeders might have negative effects on embryonic development, as (hairline) cracks lead to dehydration of the egg (Narushin and Romanov, 2002) during storage and incubation.

Albumen height, as a measure for albumen viscosity, was 0.1 mm lower in eggs from EGC breeders than from SGC breeders. Other studies in breeders (Van Emous et al., 2015a) and layers (Pérez-Bonilla et al., 2012) did not observe an effect of GC or BW on albumen height. Ovomucin is the main albumen protein responsible for albumen height (Silversides and Budgell, 2004; Wang et al., 2019), which might indicate a slightly lower deposition of albumen ovomucin in eggs from EGC breeders. A lower albumen viscosity might enhance oxygen transport to the embryo (Benton and Brake, 1996), leading to a higher hatchability and chick quality (Tona et al., 2003) for offspring from EGC breeders.

Eggs from EGC breeders were larger throughout the laying phase than eggs from SGC breeders. This has been previously discussed in Heijmans et al. (2021). These eggs from EGC breeders had a larger yolk, albumen and shell, than eggs from SGC breeders. Predicted yolk weight showed parallel lines for GC in relation to breeder age. This means that the absolute difference in yolk weight between the GC remained similar throughout the laying phase, where the eggs from EGC breeders consistently had a 0.8 g larger predicted yolk. After correction for differences in egg weight between the GC, yolk was still relatively larger in eggs from EGC breeders. In layers, it was also observed that heavier layers produced larger eggs with a larger yolk compared to lighter layers (Pérez-Bonilla et al., 2012). We hypothesize that EGC breeders produce larger yolks due to their higher feed intake, more specifically due to their higher energy intake. Sun et al. (2006) observed higher plasma levels of insulin and triiodothyronine ( $T_3$ ) and a lower plasma level of glucagon with a higher feed intake. Higher plasma levels of insulin and  $T_3$  and lower glucagon levels stimulate *de novo* lipogenesis (Richards et al., 2003; Nguyen et al., 2008; Buyse and Decuypere, 2015). *De novo* lipogenesis synthesizes yolk precursors in the liver, like yolk directed very low density lipoproteins (VLDL<sub>y</sub>) (Walzem et al., 1999; Buyse and Decuypere, 2015). These VLDL<sub>y</sub> are transported to the ovary, where they are endocytosed in the yolk. The higher energy intake of EGC breeders might thus lead to a higher production of VLDL<sub>y</sub>, which results in larger yolks. In turn, it is expected that a larger yolk will be beneficial for chick quality (Nangsuay et al., 2015). Dry matter percentage of the yolk did not differ between the GC, indicating a similar total nutrient density for the yolk from both GC.

On average, a 0.9 g larger albumen and 0.1 g larger shell of eggs from EGC breeders seems consequential to a larger yolk. After ovulation, the yolk passes through the

magnum, where the albumen is secreted around the yolk. A larger yolk might result in more distension of the lumen, which in combination with alterations in hormonal levels, induces signals to the storage granules of the albumen proteins to start secretion (Hiramoto et al., 1990; Johnson, 2015), finally resulting in a higher secretion of albumen proteins. Dry matter of the albumen did not differ between eggs from EGC or SGC breeders. A larger egg, due to a larger yolk and albumen, is expected to be beneficial for day-old chick quality (Ulmer-Franco et al., 2010; Nangsuay et al., 2011; Willems et al., 2015a).

### Dietary Energy-to-Protein Ratio

In the first 4 weeks of the laying cycle, each percent decrease in dietary AME<sub>n</sub> increased laying rate with 0.6% in both GC. The higher laying rate was probably due to a maximum 14.1 % difference in CP intake in this phase, as energy intake was comparable for breeders on the different diets (Heijmans et al., 2021). Other authors also observed a 1.5 to 10% higher laying rate in the first 4 to 5 weeks of the laying cycle for breeders with a 4 to 22.6% higher CP intake, compared to a control (Joseph et al., 2000; Van Emous et al., 2015b; Lesuisse et al., 2017), whereas differences in dietary energy intake did not affect laying rate up to 60 wk of age (Van Emous et al., 2015b). A higher laying rate is due to an earlier sexual maturation of breeders fed a lower dietary energy-to-protein ratio ( $\beta = 0.14$  d/% AME<sub>n</sub>; Heijmans et al., 2021), which in turn is related to breeder body composition (Zuidhof, 2018; Salas et al., 2019; Hadinia et al., 2020). At 28 and 29 wk of age (peak production), a higher dietary energy-to-protein ratio increased laying rate in SGC breeders ( $\beta = 0.3$  %/% AME<sub>n</sub>), whereas it decreased laying rate in EGC breeders ( $\beta = -0.5$  %/% AME<sub>n</sub>). It can be speculated that total energy intake limited laying rate in SGC breeders. Another explanation might be that EGC breeders suffered from the relative high energy intake, leading to a fatty liver hemorrhagic syndrome, although in the current study incidence of fatty liver hemorrhagic syndrome was not determined. From 30 up to 60 wk of age, similar laying rates were observed regardless the dietary energy-to-protein ratio, which is comparable to results from Van Emous et al. (2015b). Other authors observed a 12% lower laying rate between 30 and 40 wk of age (Lesuisse et al., 2017) and 2.8% lower laying rate after 46 wk of age (Van Emous et al., 2018), when breeders were fed a diet with 12 to 25% lower CP compared to a control diet. For the period in between, 35 to 46 wk of age, they did not observe a difference in laying rate from 35 to 46 wk of age, when breeders were fed a 12 to 25% lower CP diet (Lesuisse et al., 2018; Van Emous et al., 2018). Combining results from all these studies suggests that laying rate is driven by dietary CP content rather than by dietary energy content, where a higher dietary CP content is beneficial for laying rate from start of production up to approximately 35 wk of age and after 45 wk of age. Between approximately 35 and 45 wk of age breeders mainly use body protein instead of dietary CP to support

egg production (Ekmay et al., 2014; Vignale et al., 2017, 2018) and consequently dietary CP content is of less importance.

Over the whole laying period, dietary energy-to-protein ratio did not affect shell breaking strength and shell thickness. This is in line with results from Van Emous et al. (2015a) and Lesuisse et al. (2017). Some minor effects of dietary energy-to-protein ratio on albumen height were observed. However, differences were maximum 0.2 mm in albumen height and again, it can be questioned whether or not differences are relevant in perspective to offspring quality.

Egg weight was affected by dietary energy-to-protein ratio. From 25 to 31 wk of age, lowering dietary energy from 108% to 96% AME<sub>n</sub> resulted in a linear increase of maximum 1.1 g in egg weight in both GC. The higher egg weight was probably due to a maximum 14.1 % difference in CP intake, as energy intake was comparable for breeders on the different diets (Heijmans et al., 2021). This is in line with other authors, who observed a 0.8 to 5.8 g higher egg weight at comparable breeder ages, when CP intake was increased with 12.5 to 25% (Joseph et al., 2000; England et al., 2014; Lesuisse et al., 2017). At start of production, dietary CP is an important source for egg formation (Ekmay et al., 2014) and therefore an increase in CP intake might thus be beneficial for egg weight.

Later during production, from approximately 41 to 51 wk of age, a lower dietary energy-to-protein ratio was beneficial for egg weight for EGC breeders ( $\beta = -0.13$  g/% AME<sub>n</sub>), whereas this was not observed in SGC breeders ( $\beta = 0.04$  g/% AME<sub>n</sub>). The higher egg weight on a lower dietary energy-to-protein ratio for EGC breeders was almost entirely explained by a larger (+0.9 g) albumen. Other authors also observed a 1.4 to 5.0 g higher egg weight, due to a 1.3 to 4.8 g larger albumen, when breeders had a 22.6 to 25% higher CP intake (Joseph et al., 2000; Lesuisse et al., 2017). This might be explained by differences in CP availability during albumen synthesis. Albumen is synthesized and deposited in the magnum during a 3 to 4h period when the yolk passes through the magnum (Hiramoto et al., 1990). A higher dietary CP availability in this period, when the yolk is in the magnum, increases synthesis of the albumen (Penz and Jensen, 1991). Although eating time was not determined for each treatment, visually it was observed that breeders with the lowest feed allocation (SGC 108% AME<sub>n</sub>) finished their daily portion around 4 to 6h after feeding, whereas breeders with the highest feed allocation (EGC 96% AME<sub>n</sub>) finished their daily portion around 10 to 12h after feeding. It can be speculated that EGC breeders still had feed (and thus dietary CP) available when the yolk passes through the magnum due to a higher feed allocation (Heijmans et al., 2021), whereas SGC breeders did not. A lower dietary energy-to-protein ratio thus led to a higher CP availability in EGC breeders,

at the time the yolk is in the magnum, which in turn led to an increased deposition of albumen. In SGC breeders, dietary CP might not have been available any more at the moment the yolk is in the magnum, due to a lower feed allocation. Therefore, no effect of dietary energy-to-protein ratio on albumen weight was observed.

A lower dietary energy-to-protein ratio resulted in a higher DM percentage of the albumen ( $\beta = -0.02 \text{ \%}/\% \text{ AME}_n$ ), although differences in DM percentages were maximal 0.4%. Albumen almost completely consists out of water and protein (Nangsuay et al., 2013). A maximum 14.1% higher CP intake for breeders on a lower dietary energy-to-protein ratio (Heijmans et al., 2021) might lead to a higher protein content of the albumen. Albumen is an important source of water and protein for tissue synthesis of the developing embryo (Willems et al., 2014a, 2015a; b; Da Silva et al., 2019). It has been observed that partial (3 mL) removal of albumen reduces prenatal protein availability and might have long term negative consequences on performance and physiology of the offspring (Willems et al., 2014a; b, 2015a; b). It can thus be speculated that a 0.9 g higher albumen weight and a 0.4% higher DM in eggs from EGC breeders on a lower dietary energy-to-protein ratio (96%  $\text{AME}_n$ ), compared to a higher dietary energy-to-protein ratio (108%  $\text{AME}_n$ ), leads to a better offspring quality and performance.

Dietary energy-to-protein ratio had no effect on predicted yolk weight or yolk DM percentage. This was also observed by Peebles et al. (2000). Breeders on the different diets had a similar energy intake, but a linear decrease in CP intake with an increasing dietary energy-to-protein ratio (Heijmans et al., 2021). As discussed previously, energy intake might be the determinant for production of VLDL, and ultimately yolk weight. Although no effects of dietary energy-to-protein ratio on albumen weight for SGC breeders or yolk weight for both GC were observed, it can be suggested that dietary energy-to-protein ratio might affect offspring quality and performance via potential epigenetic pathways (Lesuisse et al., 2017).

## CONCLUSIONS

It can be concluded that an elevated growth curve of broiler breeders or feeding a lower dietary energy-to-protein ratio led to a higher laying rate at start of production, potentially due to a higher CP intake or more CP in the body of the breeder hen. Growth curve or dietary energy-to-protein ratio had minor effects on egg quality. Breeders on an elevated growth curve produced larger eggs, with a more yolk, albumen and shell, compared to breeders on a standard growth curve, most probably due to a higher total nutrient intake. Dietary energy-to-protein ratio had minor effects on



egg composition. Total energy intake of breeders might be the determinant for yolk weight. It is expected that a larger yolk and/or albumen will be beneficial for offspring performance. Future studies should consider the impact of growth curve and dietary energy-to-protein ratio on offspring quality and performance.

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**Conflict of Interest Statement:** J. Heijmans and M. Duijster are employed by company De Heus Animal Nutrition B.V.. All authors declare that the research was conducted in absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY INFORMATION

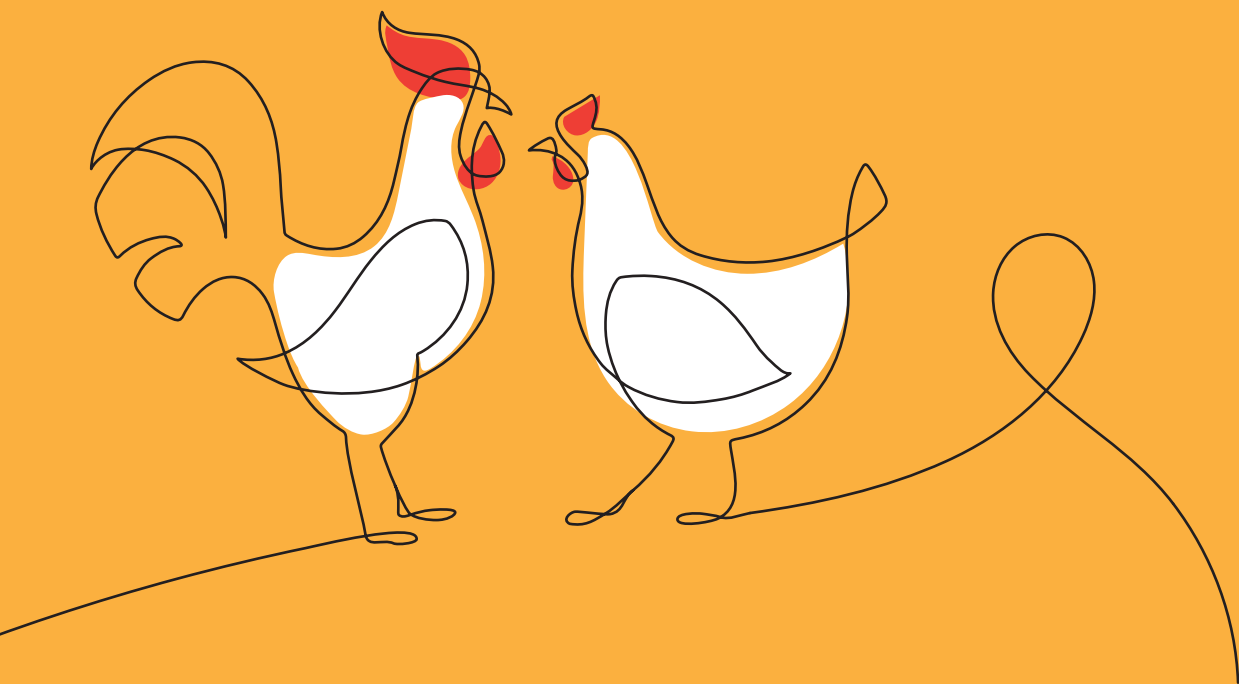
Table S1. Average individual egg weight (EW), yolk, albumen, and shell expressed as percentage of the EW during first phase of lay (24-40 wk), second phase of lay (41-60 wk) and the total laying period (24-60 wk) of broiler breeders fed on 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+1.5%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from 0 to 60 wk of age.

Item	24-40 wk				41-60 wk				24-60 wk			
	EW (g)	Yolk (%)	Albumen (%)	Shell (%)	EW (g)	Yolk (%)	Albumen (%)	Shell (%)	EW (g)	Yolk (%)	Albumen (%)	Shell (%)
Growth curve (n= 12)												
SGC	57.8	28.5	62.2	9.4	68.6 <sup>b</sup>	31.2 <sup>b</sup>	59.6	9.2 <sup>a</sup>	62.6	29.7 <sup>b</sup>	61.0	9.3
EGC	60.1	28.9	62.0	9.2	70.2 <sup>a</sup>	31.6 <sup>a</sup>	59.5	9.0 <sup>b</sup>	64.6	30.1 <sup>a</sup>	60.9	9.1
SEM	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1
Diet (n=6)												
96% AME <sub>n</sub>	59.3	28.5	62.3	9.2	69.6	31.5	59.6	9.0	63.9	29.8	61.1	9.1
100% AME <sub>n</sub>	59.2	28.7	62.1	9.2	69.4	31.4	59.5	9.1	63.7	29.9	61.0	9.2
104% AME <sub>n</sub>	58.7	28.7	62.0	9.3	69.4	31.4	59.5	9.1	63.4	29.9	60.9	9.2
108% AME <sub>n</sub>	58.5	28.8	61.8	9.3	69.2	31.3	59.6	9.1	63.2	30.0	60.8	9.2
SEM	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.1	0.0	0.1	0.0
Treatment (n=3)												
SGC 96% AME <sub>n</sub>	57.9 <sup>c</sup>	28.3 <sup>c</sup>	62.5 <sup>a</sup>	9.3 <sup>b</sup>	68.4	31.2	59.7	9.1	62.6 <sup>d</sup>	29.6	61.2 <sup>a</sup>	9.2 <sup>b</sup>
100% AME <sub>n</sub>	57.9 <sup>c</sup>	28.6 <sup>b</sup>	62.0 <sup>bc</sup>	9.3 <sup>b</sup>	68.7	31.3	59.6	9.2	62.7 <sup>d</sup>	29.8	61.0 <sup>b</sup>	9.3 <sup>b</sup>
104% AME <sub>n</sub>	57.6 <sup>c</sup>	28.4 <sup>bc</sup>	62.1 <sup>bc</sup>	9.5 <sup>a</sup>	68.8	31.3	59.5	9.2	62.6 <sup>d</sup>	29.7	61.0 <sup>ab</sup>	9.3 <sup>a</sup>
108% AME <sub>n</sub>	57.6 <sup>c</sup>	28.5 <sup>bc</sup>	62.2 <sup>abc</sup>	9.4 <sup>b</sup>	68.5	31.2	59.7	9.1	62.5 <sup>d</sup>	29.7	61.0 <sup>ab</sup>	9.3 <sup>b</sup>
EGC 96% AME <sub>n</sub>	60.7 <sup>a</sup>	28.7 <sup>b</sup>	62.2 <sup>abc</sup>	9.2 <sup>c</sup>	70.8	31.7	59.5	8.9	65.2 <sup>a</sup>	30.0	61.0 <sup>ab</sup>	9.1 <sup>c</sup>
100% AME <sub>n</sub>	60.5 <sup>a</sup>	28.7 <sup>b</sup>	62.2 <sup>abc</sup>	9.1 <sup>c</sup>	70.1	31.6	59.4	9.0	64.8 <sup>ab</sup>	30.0	61.0 <sup>ab</sup>	9.1 <sup>c</sup>
104% AME <sub>n</sub>	59.8 <sup>b</sup>	29.0 <sup>a</sup>	61.9 <sup>c</sup>	9.1 <sup>c</sup>	70.0	31.5	59.5	9.0	64.3 <sup>bc</sup>	30.1	60.8 <sup>bc</sup>	9.1 <sup>c</sup>
108% AME <sub>n</sub>	59.3 <sup>b</sup>	29.2 <sup>a</sup>	61.5 <sup>d</sup>	9.4 <sup>b</sup>	69.8	31.5	59.5	9.1	64.0 <sup>c</sup>	30.2	60.6 <sup>c</sup>	9.2 <sup>b</sup>
SEM	0.2	0.1	0.1	0.0	0.3	0.1	0.1	0.0	0.3	0.1	0.1	0.0

Table S1. Continued

Item	24-40 wk				41-60 wk				24-60 wk			
	EW (g)	Yolk (%)	Albumen (%)	Shell (%)	EW (g)	Yolk (%)	Albumen (%)	Shell (%)	EW (g)	Yolk (%)	Albumen (%)	Shell (%)
P-value												
Growth curve (GC)	<0.001	<0.001	0.003	<0.001	<0.001	0.001	0.20	<0.001	<0.001	<0.001	0.002	<0.001
Diet (factorial)	<0.001	0.010	<0.001	0.02	0.54	0.90	0.99	0.25	<0.001	0.37	0.03	0.02
Diet (linear)	<0.001	<0.001	<0.001	0.006	0.19	0.47	0.91	0.11	<0.001	0.08	0.002	0.002
Diet (quadratic)	0.84	0.87	1.00	0.54	0.86	0.91	0.73	0.39	1.00	0.84	0.81	0.92
GC x Diet (factorial)	0.04	0.007	0.002	0.02	0.13	0.78	0.93	0.29	0.009	0.10	0.04	0.008
GC x Diet (linear)	0.004	0.04	0.04	0.51	0.06	0.33	0.86	0.16	0.002	0.39	0.17	0.16
GC x Diet (quadratic)	0.75	0.08	0.003	0.005	0.18	0.69	0.74	0.72	0.21	0.09	0.02	0.02
Age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001

<sup>a-d</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).





# Chapter 4

## Changes in body composition and energetic efficiency in response to growth curve and dietary energy-to-protein ratio in broiler breeders

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## ABSTRACT

Body composition plays an important role in reproduction in broiler breeders. The aim of this study was to evaluate the dynamics in body composition and energetic efficiency in broiler breeders, using different dietary strategies. 1,536 Day-old pullets were randomly allotted to 24 pens in a 2 x 4 factorial design with 2 growth curves (standard or elevated (+15%)) and 4 diets, with a step-wise increment in energy (96, 100, 104 and 108% AME<sub>n</sub>) fed on a pair-gain basis. Body composition was determined at 10 time points from 0 to 60 wk of age. Body protein mass was linearly related to body weight (BW) in growing breeders, which can be expressed as  $-6.4 + 0.184 * BW$  ( $R^2 = 0.99$ ;  $P < 0.001$ ). Body fat mass was exponentially related to BW in growing breeders, which can be expressed as  $-42.2 + 50.8 * 1.0006^{BW}$  ( $R^2 = 0.98$ ;  $P < 0.001$ ). A higher energy-to-protein ratio resulted in higher body fat mass at the same BW ( $P < 0.001$ ). Sexual maturation was related to body protein mass at 21 wk of age, where each 100 g of body protein mass extra advanced sexual maturation by 5.4 days ( $R^2 = 0.83$ ). Estimates of energetic efficiency for growth ( $k_g$ ) and egg production ( $k_e$ ) appeared not constant, but varied with age in a quadratic manner between 0.27 – 0.54 for  $k_g$  and between 0.28 – 0.56 for  $k_e$ . The quadratic relationship could be expressed as  $k_g = 0.408 - 0.0319 * Age + 0.00181 * Age^2$  ( $R^2 = 0.72$ ;  $P < 0.001$ ) and  $k_e = -0.211 + 0.034 * Age - 0.00042 * Age^2$  ( $R^2 = 0.46$ ;  $P < 0.001$ ). Body protein mass in broiler breeders is tightly regulated and mainly depended on BW and seems to be the main determinant for sexual maturation. Body fat mass is exponentially related to BW, where an increase in dietary energy-to-protein ratio results in a higher body fat mass. Treatments had minimal effects on estimated energetic efficiencies in breeders.

**Key words:** body protein, body fat, lean tissue, adipose tissue, broiler breeder

## INTRODUCTION

Modern commercial broiler chickens are effective meat producers. They have been selected for decades for increased growth rate and high feed efficiency, leading to a high meat yield (Zuidhof et al., 2014). Broiler breeder hens, the mothers of broilers, also possess the genetics for a high growth rate. Growth rate and reproduction are negatively correlated and therefore broiler breeders are commonly fed restricted quantities of feed according to a targeted growth curve to prevent excessive weight gain and ensure reproductive success (Decuyper et al., 2010). This genetic selection has changed the body composition of broiler breeders over the last decades towards a higher lean mass and a lower fat mass (Eitan et al., 2014; Zuidhof et al., 2014).

Body reserves of a broiler breeder hen play an important role in reproduction. Several studies have emphasized the importance of the breeders' metabolic status for sexual maturation (Bédécarrats et al., 2016; Hanlon et al., 2020; Van der Klein et al., 2020). Furthermore, it is suggested that body fat plays an important role in egg production (Van der Klein et al., 2018a), yolk synthesis (Salas et al., 2017), and laying persistency (Van Emous et al., 2015) and that body protein is an important source for albumen and yolk synthesis (Ekmay et al., 2014). Recently, concerns were raised that a biological limit in too low body fat mass for reproductive success may be approached or even reached in modern broiler breeder hens (Van der Klein et al., 2018a; Zuidhof, 2018; Hadinia et al., 2020). Changes in body composition might therefore influence reproductive success in broiler breeder hens. Development in body composition over different ages in broiler breeder hens has not been rigorously investigated before. Other studies have only considered body composition during the rearing period (Sakomura et al., 2003; De Los Mozos et al., 2017), during sexual maturation (Rabello et al., 2006; Hadinia et al., 2020), or during the production period (Caldas et al., 2018; Salas et al., 2019), only measured representatives of body composition, like abdominal fat pad and breast muscle weight (Van Emous et al., 2013; Lesuisse et al., 2017; Zuidhof, 2018), or only determined it at one specific age (Sun and Coon, 2005; Van der Klein et al., 2018a). The current study therefore aims to investigate body composition both during the rearing and production period in response to an altered growth curve and dietary energy-to-protein ratio. These two factors, have been shown to impact body composition in broiler breeder hens (Sun and Coon, 2005; Van Emous et al., 2013; Lesuisse et al., 2017; De Los Mozos et al., 2017; Van der Klein et al., 2018a; Salas et al., 2019).

Gaining insight in body composition development is also of importance for modelling energy partitioning in broiler breeders (Gous, 2015). In energy partitioning models, it is assumed that all dietary energy can be accounted for (Zuidhof, 2019). In

a factorial approach, energy is partitioned into maintenance, growth of body protein, growth of body fat, and egg production (Sakomura, 2004; Zuidhof, 2019), the latter three are also referred to as retained energy. Body composition models in relation to dietary factors can help to determine the quantity of retained energy in breeder hens. The challenge in practice is to maximize energetic retention and minimize energy losses, which is also referred to as energetic efficiency. There have been attempts to quantify energetic efficiency in broiler breeders and the role of environmental factors in this energetic efficiency (Sakomura et al., 2003; Rabello et al., 2006; Reyes et al., 2011, 2012). It remains unclear whether or not dietary factors might affect energetic efficiency in broiler breeders. Quantifying dietary factors that contribute to energetic efficiency can help to design diets and feeding strategies to maximize energy retention. Furthermore, there are indications that energetic efficiency for BW gain changes with age of the breeders (Sakomura et al., 2003), whereas most studies report a fixed value for energetic efficiency for BW gain or egg production, irrespective of age of the breeder (Rabello et al., 2006; Reyes et al., 2011, 2012).

The objective of the current study was to evaluate the development in body composition from pullet to mature broiler breeder hen, using different dietary strategies. Furthermore, we aimed to evaluate dynamics in energetic efficiency related to changes in body composition.

## MATERIALS AND METHODS

### Experimental Design

This experiment with female Ross 308 broiler breeders consisted of a 2 x 4 factorial arrangement with 2 growth curves (**GC**) (standard growth curve = **SGC** or elevated growth curve = **EGC**) and 4 diets with different energy-to-protein ratio, created by a stepwise increase in apparent metabolizable energy nitrogen corrected (**AME<sub>n</sub>**; defined as 96%, 100%, 104% and 108% **AME<sub>n</sub>** diet) at a similar CP content. Broiler breeders were allocated to the different treatments from hatch to 60 wk of age. Within each GC, feed allocation per diet was adapted weekly according to a paired-gain strategy. All experimental protocols were approved by the Central Commission on Animal Experimentation (The Hague, the Netherlands), approval number 2018.W-0023.001.

### Breeders, Housing and Management

Heijmans et al. (2021) reported a detailed description of this experiment. In short, at the start of the experiment (day 0), a total of 1,536 Ross 308 female broiler breeder day-old pullets were randomly assigned to 24 pens (64 pullets per pen) in 3 blocks of 8 pens ( $n = 3$  per treatment). Each pen consisted of two areas: a floor area with wood

shaving as bedding (4.9 m<sup>2</sup>) and an elevated slatted floor area (6.1 m<sup>2</sup>) with a track feeding system (9 m feeding length) with a grill to prevent rooster access to the feed, drinking nipples, perches (7.2 m) and laying nests. Until 20 wk of age, laying nests were covered with plastic to prevent access or sight to the laying nests. Breeders had *ad libitum* access to water. Pullets were kept at a photoperiod of 8L:16D (10 lux) until 21 wk of age. At 21 wk of age, pullets were photo-stimulated by an instant increase of the photoperiod to 11L:13D (20 lux), followed by a gradual increase to 13L:11D (40 lux) at 23 wk of age. At 20 wk of age, each pen was standardized to 45 breeders per pen closest to the average pen weight. At the same moment, 4 20-wk old Ross 308 roosters were placed per pen. A commercially available rooster diet (2,725 kcal of AME<sub>n</sub>/kg, 134 g of CP/kg, 5 g digestible lysine/kg) was provided to the roosters once per day in a rooster feeding pan. By adjusting the height of the feeding pan, female access to the rooster diet was prevented.

### Experimental Diets and Feed Allocation

Experimental diets were formulated with step-wise increment in dietary AME<sub>n</sub> level from 96% to 108% AME<sub>n</sub>, where the 100% AME<sub>n</sub> diet was according to breeder recommendations (Aviagen, 2016a). Diet were formulated isonitrogenous. A higher dietary AME<sub>n</sub> level was realized by exchanging fibrous ingredients (cellulose and finely ground oat hulls) for energy rich ingredients (soy oil, lard and maize starch), while maintaining a similar ratio between crude fat and starch. Table 1 presents the calculated and analyzed nutrient content of the 96% AME<sub>n</sub> and 108% AME<sub>n</sub> diets. The 100% AME<sub>n</sub> and 104% AME<sub>n</sub> diets were produced by mixing of the 96% and 108% AME<sub>n</sub> diets in a 2:1 and 1:2 ratio, respectively. The experimental diets were provided *ad libitum* from day of placement until 2 wk of age. Hereafter, pens assigned to the SGC followed the breeder recommendation for BW (Aviagen, 2016b), whereas the EGC pens were fed to obtain a 15% higher BW throughout rearing and production. Daily feed allocation was calculated and adjusted weekly based on realized and desired growth per GC. Growth and egg production in the week prior were the directives for calculations of the daily feed allocation. Within each GC, daily feed allocation for each dietary energy-to-protein ratio was adapted according to a paired-gain strategy.

### Measurements

**Body weight.** Body weight was measured weekly before feeding by individually weighing a minimum of 20 (rearing phase; 0 – 21 wk of age) or 15 (production phase; from 21 wk of age onwards) randomly selected female breeders per pen. Every 3 (rearing phase) or 4 (production phase) weeks all breeders within a pen were weighed.

**Egg production.** Eggs were collected, graded (single or double yolked) and weighed daily per pen. Average egg weight was calculated per pen per week as the total egg

Table 1. Dietary ingredients, and calculated and analyzed nutrients of the 96% AME<sub>n</sub> and 108% AME<sub>n</sub> diets (g/kg, as-fed basis) of broiler breeders. The intermediate diets (100% AME<sub>n</sub> and 104% AME<sub>n</sub>) were produced by mixing the 96% AME<sub>n</sub> and 108% AME<sub>n</sub> diets in a 2:1 (100% AME<sub>n</sub>) and 1:2 (104% AME<sub>n</sub>) ratio.

Item	Sarrer 1 (0-21 days)			Sarrer 2 (22-42 days)			Grower (43-112 days)			Pre-breeder (113-160 days)			Breeder 1 (161-280 days)			Breeder 2 (281-420 days)		
	96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>	
Ingredient																		
Maize	450.0	450.0		500.0	500.0		400.0	400.0		500.0	500.0		440.0	440.0		460.0	460.0	
Wheat	100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0		100.0	100.0	
Soybean meal	240.9	245.1		141.3	146.3		76.1	80.7		48.9	52.8		149.8	152.5		130.5	133.4	
Sunflower meal	50.0	50.0		90.0	90.0		150.0	150.0		165.0	165.0		80.0	80.0		90.0	90.0	
Wheat middlings	-	-		-	-		100.0	100.0		25.0	25.0		-	-		-	-	
Oat hulls (fine)	50.0	1.0		56.0	5.1		65.0	19.3		50.0	1.0		48.0	1.0		46.6	1.0	
Cellulose	44.1	1.0		47.9	5.0		50.0	5.0		46.8	1.0		44.5	1.0		45.2	1.0	
Soya oil	11.1	17.8		9.5	14.3		8.0	12.0		5.0	7.0		4.8	10.8		11.9	14.9	
Lard	3.0	4.2		4.2	6.8		3.3	6.7		5.0	10.2		29.5	34.9		23.5	32.1	
Maize starch	14.0	94.5		14.3	96.2		19.9	99.2		11.7	96.1		14.7	91.6		1.0	76.9	
Limestone (fine)	13.9	14.1		13.8	13.9		13.3	13.4		-	-		-	-		-	-	
Limestone (coarse)	-	-		-	-		-	-		24.5	24.6		71.0	71.1		73.4	73.5	
Monocalcium phosphate	9.8	9.2		10.5	9.9		5.4	4.9		5.8	5.2		6.0	5.5		6.5	5.9	
Sodium bicarbonate	3.3	3.3		3.3	3.3		2.5	2.5		3.3	3.3		2.7	2.7		3.0	2.9	
Salt	1.8	1.8		1.7	1.7		2.2	2.2		1.5	1.5		2.1	2.1		2.0	2.0	
L-Lysine	1.73	1.69		1.88	1.80		0.23	0.15		1.63	1.58		0.44	0.42		0.36	0.34	
L-Threonine	0.68	0.68		0.54	0.54		-	-		0.49	0.48		0.57	0.58		0.54	0.55	
DL-Methionine	2.34	2.34		1.71	1.71		0.65	0.65		1.13	1.13		1.73	1.77		1.59	1.62	
Choline Chloride-50%	0.8	0.8		0.8	0.8		0.8	0.8		1.5	1.4		1.4	1.3		1.5	1.4	
Xylanase	0.1	0.1		0.1	0.1		0.1	0.1		0.1	0.1		0.1	0.1		0.1	0.1	
Phytase	0.05	0.05		0.05	0.05		0.05	0.05		0.05	0.05		0.05	0.05		0.05	0.05	
Premix rearing <sup>1</sup>	2.5	2.5		2.5	2.5		2.5	2.5		-	-		-	-		-	-	
Premix laying <sup>2</sup>	-	-		-	-		-	-		2.5	2.5		2.5	2.5		2.5	2.5	

Table 1. Continued

Item	Starter 1 (0-21 days)			Starter 2 (22-42 days)			Grower (43-112 days)			Pre-breeder (113-160 days)			Breeder 1 (161-280 days)			Breeder 2 (281-420 days)		
	96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>		96% AME <sub>n</sub>	108% AME <sub>n</sub>	
Calculated content <sup>3</sup>																		
AME <sub>n</sub> (kcal/kg)	2,570	2,890		2,570	2,890		2,545	2,865		2,640	2,970		2,735	3,080		2,735	3,080	
Crude protein	175.1	175.0		143.7	143.6		136.5	136.5		123.0	122.5		138.5	137.7		135.2	134.3	
Crude fat	41.5	49.0		42.0	49.0		40.0	47.0		38.8	45.7		60.0	71.1		61.6	72.8	
Carbohydrates	535.6	569.0		558.1	592.1		546.4	580.5		557.4	593.6		507.3	538.1		502.9	535.8	
Crude fibre	77.1	37.7		88.0	48.3		111.5	71.5		105.6	64.3		81.4	42.0		85.2	43.9	
Starch	379.5	446.9		408.6	477.5		371.5	438.5		407.5	480.4		368.2	434.4		373.8	436.0	
Starch:fat	9.1	9.1		9.7	9.7		9.3	9.3		10.5	10.5		6.1	6.1		6.1	6.0	
Linoleic acid	18.0	21.0		18.0	20.3		17.0	19.0		16.3	17.4		16.8	20.0		20.0	22.0	
Digestible lysine	9.0	9.0		7.0	7.0		4.8	4.8		5.1	5.1		5.9	5.9		5.5	5.5	
Calcium	9.8	9.8		9.8	9.8		8.9	8.9		13.1	13.1		31.0	31.0		31.0	31.0	
Retainable phosphorus	4.1	4.1		4.1	4.1		3.3	3.3		3.2	3.2		3.2	3.2		3.2	3.2	
Analyzed content <sup>4</sup>																		
Crude protein	170.2	172.9		145.1	148.0		133.0	135.1		129.6	127.4		145.2	142.2		139.9	135.1	
Crude fat	37.0	43.2		38.3	44.3		39.0	42.4		33.1	41.1		57.6	66.8		58.3	68.7	
Starch (Ewers)	401.0	463.0		408.0	472.0		377.0	431.0		415.6	486.3		376.4	436.8		371.7	432.5	

<sup>1</sup>Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 3.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 6.0 mg; Vitamin B<sub>6</sub> 4.0 mg; Vitamin B<sub>12</sub> 20 µg; Nicotinamide 35 mg; D-pantothenic acid 15 mg; Folic acid 1.5 mg; Biotin 0.20 mg; Iron 40 mg; Copper 16 mg; Manganese 120 mg; Zinc 90 mg; Iodine 1.25 mg; Selenium 0.3 mg.

<sup>2</sup>Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 5.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 12.0 mg; Vitamin B<sub>6</sub> 5.0 mg; Vitamin B<sub>12</sub> 40 µg; Nicotinamide 55 mg; D-pantothenic acid 15 mg; Folic acid 2.0 mg; Biotin 0.40 mg; Iron 50 mg; Copper 10 mg; Manganese 120 mg; Zinc 90 mg; Iodine 2.0 mg; Selenium 0.3 mg.

<sup>3</sup>Calculated according to CVB (2012).

<sup>4</sup>Analysis according NEN-EN-ISO 16634-1 for crude protein, NEN-EN-ISO 6492-1999 for crude fat, and NEN-ISO 6493 for starch.

weight, excluding weight of the double yolked eggs, divided by the number of single yolked eggs. Laying rate was calculated as the total number of eggs divided by the number of breeders per pen per week, corrected for mortality. Age at sexual maturity (**ASM**) was defined as age at 50% laying rate and was determined per pen by linear interpolation of age in days at which breeders passed 50% laying rate.

**Body composition.** At day 0, 2 day-old pullets were selected for baseline measurement of body composition. Pullets were euthanized by a percussive blow to the head followed by cervical dislocation, weighed and pooled for body composition analysis. At 2, 6, 12, 16, 21, 28, 36, 46 and 60 wk of age, 2 female breeders per pen were selected before feeding within a range of approximately 2.5% of the average BW per treatment in that week. Selected breeders were euthanized by a percussive blow on the head followed by cervical dislocation and weighed (fresh BW). Breeders were then scalded for 30 seconds in water of approximately 65°C and defeathered by manual plucking. Breeders were then dissected and potential feed residues from the gastrointestinal tract were removed. From 12 wk of age onwards, the abdominal fat pad, including fat surrounding the gizzard and proventriculus was removed, weighed and reinserted into the abdominal cavity. In case the oviduct contained egg components, these were removed as well, as these were not considered as part of the body composition. Hereafter, the defeathered carcass was weighed (feather-free BW). The defeathered carcass was ground to a homogeneous mixture of which a sample was analyzed for moisture, crude protein and crude fat content. Moisture content was determined by drying a sample at 103°C for 16 h (NEN-ISO-6496). Crude protein content was analyzed by the Kjeldahl method (NEN-ISO-8968-1). Crude fat content was analyzed by acid hydrolysis, using gravimetry (NEN-ISO-1735). Total body protein and body fat mass (g) were calculated respectively as crude protein or crude fat content multiplied with the feather-free BW in grams. At 2 wk of age, only 16 randomly selected pullets from the 2 extreme dietary treatments (96% AME<sub>n</sub> and 108% AME<sub>n</sub>) were analyzed on body composition, because at that moment pullets were not yet feed restricted.

## Energy Efficiency Calculations

To calculate efficiency of energy utilization for BW gain (**k<sub>g</sub>**), data from the rearing phase was used in order to avoid bias in calculated values due to physiological processes involved in egg production. The following calculations were performed per pen per wk from 3 to 21 wk of age. Intake of AME<sub>n</sub> (**ME<sub>int</sub>**) was calculated by multiplying feed intake with the dietary AME<sub>n</sub> content. Metabolizable energy needed for maintenance (**ME<sub>m</sub>**) was calculated as  $389 \text{ kcal} * \text{BP}_m^{0.73} * \text{BP}_t / \text{BP}_m$  (Emmans, 1987), where BP<sub>m</sub> is the mature body protein weight of 0.982 kilogram (calculated as *ad libitum* BW of 5.37 kilogram (Heck et al., 2004) times the body protein formula presented in the current study) and BP<sub>t</sub> it the body protein weight in kilogram at timepoint *t*, which



represents the degree of maturity in body protein. Body protein and body fat mass were predicted based on the formulas presented in the current study in relationship to BW (Figure 1 and Figure 2). Body protein gain in grams (**BPG**) and body fat gain in grams (**BFG**) were calculated from initial ( $t$ ) and final mass ( $t+1$ ). The energy retained as BW gain (**ER<sub>g</sub>**) was estimated by multiplying BPG and BFG by 5.4 and 9.3 kcal (Reyes et al., 2011), respectively, and then adding up these values. Metabolizable energy needed for BW gain (**ME<sub>g</sub>**) was calculated by dividing ER<sub>g</sub> by  $k_g$ . For calculation of  $k_g$ , it was assumed that  $ME_{int} - ME_m - ME_g = 0$ . This leads to the following formula used for calculation of  $k_g$  per pen per week:

$$k_g = \frac{(5.4 * BPG + 9.3 * BFG)}{(ME_{int} - ME_m)}$$

A 3 wk rolling average of  $k_g$  was used for further analysis. To calculate efficiency of energy utilization for egg production (**k<sub>e</sub>**) data from 36 to 60 wk of age was used in order to avoid bias in calculated values due to physiological processes involved in BW gain, as growth was minimized in this period (1 g/d on average). Average BW gain was calculated per pen and used for further calculations. In case average BW gain was negative, zero growth was assumed (3 pens) as it remains unclear whether or not a negative BW gain yields energy or if there is a cost factor involved as well. Similar calculations were used for  $ME_{int}$ ,  $ME_m$ , BPG, BFG and ER<sub>g</sub> as described above. To calculate ME<sub>g</sub> during the production period, average calculated  $k_g$  at 21 wk of age was used. Daily egg yolk and albumen production were estimated based on the formulas presented by Heijmans et al. (2022) multiplied with the daily egg mass production. Egg protein in grams (**EP**) and egg fat in grams (**EF**) mass were estimated by multiplying daily egg yolk and albumen mass in grams with the average crude protein and crude fat content in the yolk and albumen of Ross 308 breeders eggs (Nangsuay et al., 2015). The energy retained as egg (**ER<sub>e</sub>**) was estimated by multiplying EP and EF by 5.4 and 9.3 kcal (Reyes et al., 2011), respectively and then adding up these values. Metabolizable energy for egg production (**ME<sub>e</sub>**) was calculated by dividing ER<sub>e</sub> by  $k_e$ . For calculation of  $k_e$ , it was assumed that  $ME_{int} - ME_m - ME_g - ME_e = 0$ . This leads to the following formula used for the calculation of  $k_e$  per pen per week:

$$k_e = \frac{(5.4 * EP + 9.3 * EF)}{(ME_{int} - ME_m - ME_g)}$$

A 3 wk rolling average of  $k_e$  was used for further analysis.

### Statistical Analysis

Data on body composition were analyzed per time point, where pen was used as the experimental unit for all analyses. Data were analyzed using the Restricted Maximum

Likelihood variance components analysis procedure with a linear mixed model (Genstat 19<sup>th</sup> Edition, 2019). The model used was:

$$Y_{ijk} = \mu + GC_i + Diet_j + GC_i \times Diet_j + Block_k + e_{ijk} [1],$$

where  $Y_{ijk}$  = the dependent variable,  $\mu$  was the overall mean,  $GC_i$  = the growth curve ( $i$  = SGC or EGC),  $Diet_j$  = the energy-to-protein ratio in the diet ( $j$  = 96%, 100%, 104%, or 108% AME<sub>n</sub>),  $GC_i \times Diet_j$  = the interaction between GC and Diet,  $Block_k$  = block within the room ( $k$  = 1, 2 or 3), and  $e_{ijk}$  = the residual error. Fisher adjustments were used for multiple comparisons of the factorial analysis. Additionally, effects of dietary energy-to-protein ratio were analyzed as linear or quadratic contrasts, also within GC. If linear effects were observed, the slope ( $\beta$ ) is presented in the results section. If quadratic effects of dietary energy-to-protein ratio, also within GC, were observed, the estimated AME<sub>n</sub> percentage at which the dependent variable was at the maximum (concave quadratic relation) or minimum (convex quadratic relation) was calculated and presented in the result section. Data are presented as LS means  $\pm$  SEM.

In addition, linear and exponential regression curves were fitted in Genstat to describe body composition development in broiler breeders in relation to BW. Preliminary analysis showed no interaction between GC and dietary energy-to-protein ratio on body composition at each time point and therefore the regression curves are only presented on the main effects. Furthermore, preliminary analysis showed a high correlation between defeathered BW and fresh BW ( $R^2 = 1.00$ ) and therefore, for practical applicability of the presented formulas, fresh BW was used for further modelling. Preliminary analysis also showed a similar relationship between fresh BW and body composition in growing breeder pullets (0-21 wk of age) as in growing laying breeders (21-36 wk of age) and therefore body composition data were split into growing breeders (0-36 wk of age) and non-growing, mature breeders (36-60 wk of age). For body protein mass in both growing and mature breeders and for body fat mass in mature breeders, preliminary analysis showed the highest  $R^2$  values and lowest Bayesian Information Criterion (BIC) for linear regression, compared to quadratic or exponential regression. A linear regression curve was therefore fitted, according to the following model:

$$Y = a + b * BW [2],$$

where  $Y$  is either body protein mass in growing or mature breeders or body fat mass in mature breeders,  $a$  and  $b$  are the fitted coefficients for the linear regression curve and  $BW$  is the fresh BW of the breeder hen in grams. First, the model was fitted as single regression curve with the same coefficients for each GC or dietary energy-to-protein ratio (model I). Next, the model was step-wise expanded with a separate constant coefficient ( $a$ ; model II) for parallel lines, or with a separate constant ( $a$ ) plus linear (

Table 2. Protein mass (g) in defeathered carcasses of broiler breeders from 6 to 60 wk of age fed at 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>) from 0 to 60 wk of age.

		Age (wk)							
Item		6	12	16	21	28	36	46	60
Growth curve (n = 12)									
SGC		126.5 <sup>b</sup>	235.4 <sup>b</sup>	342.5 <sup>b</sup>	445.7 <sup>b</sup>	598.9 <sup>b</sup>	671.2 <sup>b</sup>	680.9 <sup>b</sup>	708.9 <sup>b</sup>
EGC		146.2 <sup>a</sup>	267.7 <sup>a</sup>	389.2 <sup>a</sup>	516.9 <sup>a</sup>	683.6 <sup>a</sup>	779.2 <sup>a</sup>	758.2 <sup>a</sup>	805.0 <sup>a</sup>
SEM		1.3	2.5	2.7	3.7	5.6	4.5	5.7	7.8
Diet (n = 6)									
96% AME <sub>n</sub>		138.0	258.8	373.4 <sup>a</sup>	494.5 <sup>a</sup>	647.6	721.4	725.2	754.4
100% AME <sub>n</sub>		137.3	249.8	368.7 <sup>a</sup>	479.3 <sup>ab</sup>	641.2	726.2	732.4	764.2
104% AME <sub>n</sub>		135.8	250.7	364.0 <sup>ab</sup>	485.3 <sup>a</sup>	636.7	730.9	712.7	749.3
108% AME <sub>n</sub>		134.3	247.0	357.3 <sup>b</sup>	466.0 <sup>b</sup>	649.5	722.5	707.9	760.0
SEM		1.8	3.6	3.8	5.3	7.9	6.4	8.1	11.0
Treatment (n = 3)									
SGC	96% AME <sub>n</sub>	128.3	240.1	348.1	461.4	605.7	680.9	673.4	703.2
	100% AME <sub>n</sub>	128.3	233.4	345.8	435.3	596.7	663.9	699.6	707.3
	104% AME <sub>n</sub>	124.5	233.3	344.8	450.6	590.0	672.0	675.3	697.1
	108% AME <sub>n</sub>	124.9	234.9	331.2	435.4	603.4	668.0	675.3	728.3
EGC	96% AME <sub>n</sub>	147.7	277.5	398.7	527.7	689.5	761.9	777.1	805.7
	100% AME <sub>n</sub>	146.2	266.1	391.7	523.3	685.8	788.4	765.1	821.0
	104% AME <sub>n</sub>	147.1	268.1	383.1	520.0	683.3	789.7	750.1	801.6
	108% AME <sub>n</sub>	143.7	259.2	383.4	496.6	675.6	776.9	740.4	791.7
SEM		2.5	5.1	5.3	7.5	11.2	9.0	11.4	15.5
P-value									
Growth curve (GC)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Diet (factorial)		0.49	0.17	0.05	0.02	0.80	0.73	0.18	0.80
Diet (linear)		0.12	0.05	0.004	0.007	0.40	0.78	0.07	0.97
Diet (quadratic)		0.82	0.46	0.79	0.73	0.55	0.31	0.48	0.97
GC x Diet (factorial)		0.80	0.62	0.58	0.34	0.80	0.13	0.33	0.41
GC x Diet (linear)		0.86	0.26	0.93	0.53	0.66	0.19	0.17	0.21
GC x Diet (quadratic)		0.74	0.69	0.22	0.22	0.39	0.05	0.40	0.24

<sup>ab</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

$b$ ; model III) coefficients for separate lines, for each GC x dietary energy-to-protein ratio interaction. After each model fit, it was evaluated whether or not the model significantly improved, compared to the previous model. Improvement was based on a significantly lower residual mean square error, a lower BIC, or a higher  $R^2$ , compared to the previous model. The final model used (I to III), was the model that significantly improved the fit compared to the previous model, but no further significant improvement of the fit was observed of the next model.

For body fat mass in growing breeders only, preliminary analysis showed the highest  $R^2$  values and lowest BIC for exponential regression, compared to linear or quadratic regression. Therefore, an exponential regression curve was fitted for body fat mass in growing breeders:

$$Y = a + b * c^{BW} [3],$$

where  $Y$  is the body fat mass,  $a$ ,  $b$  and  $c$  are the fitted coefficients for the exponential regression curve and BW is the fresh BW of the breeder hen in grams. Similarly as model 2, a step-wise fitting and expansion was used as for each GC and/or dietary energy-to-protein ratio. The final model used, was the model that significantly improved the fit compared to the previous model, but no further significant improvement of the fit was observed of the next model.

Additionally, body protein mass and body fat mass were fitted against ASM in a multiple linear regression model:

$$ASM = \text{Body protein mass } (t) + \text{Body fat mass } (t) [4],$$

where ASM = age at sexual maturity (50% laying rate, in days),  $t$  represents the values at 6, 12, 16 or 21 wk of age. Body protein and body fat mass are expressed in grams.

Preliminary analysis showed the highest  $R^2$  values and lowest BIC for quadratic regression for dynamics of  $k_g$  and  $k_e$ , compared to linear, linear-plateau or exponential regression. Therefore, for analysis of the dynamics of  $k_g$  and  $k_e$  a quadratic regression curve was fitted for each GC, dietary energy-to-protein ratio and GC x dietary energy-to-protein ratio:

$$Y = a + b * \text{Age} + c * \text{Age}^2 [5],$$

where  $Y$  is the  $k_g$  or  $k_e$ ,  $a$ ,  $b$  and  $c$  are the fitted coefficients for the quadratic regression curve and Age is the age of the breeder hen in wk. Similarly as model 2, a step-wise fitting and expansion was used as for each GC and/or dietary energy-to-protein ratio. The final model used, was the model that significantly improved the fit compared to the previous model, but no further significant improvement of the fit was observed of

the next model. Data are presented as LSmeans  $\pm$  SEM. Estimated coefficients and  $R^2$  of fitted models are presented. Differences were reported where  $P \leq 0.05$ .

## RESULTS

Results on nutrient intake, BW development, uniformity, productive performance and egg composition are presented elsewhere (Heijmans et al., 2021, 2022).

### Body Composition

Defeathered BW of the selected breeders for body composition is presented in supplementary Table S1. Day-old breeder pullets had 5.9 g protein mass and 2.5 g fat mass in a body of 37.3 g. At 2 wk of age, pullets fed the 96% AME<sub>n</sub> diet had a lower body protein (38.1 g) and fat (21.9 g) mass, compared to pullets fed the 108% AME<sub>n</sub> diet (41.6 g and 29.4 g, respectively,  $P=0.005$  and  $P<0.001$ ). At none of the ages, an interaction was observed between breeder GC and dietary energy-to-protein ratio on body protein (Table 2) or fat mass (Table 3).

At all ages, EGC breeders had a higher body protein and fat mass, compared to SGC breeders ( $P \leq 0.02$ ). Increasing dietary energy-to-protein ratio decreased body protein mass linearly at 12 ( $\beta = -0.9$  g per % AME<sub>n</sub>), 16 ( $\beta = -1.3$  g per % AME<sub>n</sub>) and 21 ( $\beta = -2.0$  g per % AME<sub>n</sub>;  $P \leq 0.05$ ; Table 2) wk of age. At all other ages, no effect of dietary energy-to-protein was observed on body protein mass. Increasing dietary energy-to-protein ratio increased body fat mass linearly between 6 and 36 wk of age ( $\beta = 1.6, 2.8, 2.6, 5.1, 7.4$ , and  $10.2$  g per % AME<sub>n</sub> at 6, 12, 16, 21, 28, and 36 wk of age, respectively;  $P \leq 0.007$ ; Table 3). At 46 wk of age, a quadratic effect was observed of dietary energy-to-protein ratio on body fat mass, where the lowest body fat mass was estimated at 102% AME<sub>n</sub> ( $\Delta_{\max} = 97.5$  g;  $P = 0.04$ ; Table 3). At 60 wk of age, no effect of dietary energy-to-protein ratio was observed on body fat mass (Table 3).

A linear relationship was observed between BW and body protein mass in growing broiler breeders (0–36 wk of age; Figure 1;  $P < 0.001$ ). Separate lines had the best fit for each GC and each dietary energy-to-protein ratio. For SGC, the predicted body protein mass was expressed as  $-8.7 + 0.187 * BW$ , whereas for EGC the predicted body protein mass was expressed as  $-5.6 + 0.182 * BW$  (Figure 1A;  $R^2 = 0.99$ ;  $P < 0.001$ ). For dietary energy-to-protein ratio, the constant coefficients ( $a$ ) were estimated as -5.8, -5.9, -6.9, -7.3 and the linear coefficients ( $b$ ) were estimated as 0.187, 0.184, 0.184 and 0.181 for 96%, 100%, 104% and 108% AME<sub>n</sub> diet, respectively (Figure 1B;  $R^2 = 0.99$ ;  $P < 0.001$ ). Although separate regression lines significantly improved the model fit for each GC and each dietary energy-to-protein ratio, absolute differences in predicted body protein mass at each given BW were small. Consequently,

Table 3. Fat mass (g) in defeathered carcasses of broiler breeders from 6 to 60 wk of age fed at 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>) from 0 to 60 wk of age.

		Age (wk)							
Item		6	12	16	21	28	36	46	60
Growth curve (n = 12)									
SGC		31.7 <sup>b</sup>	85.9 <sup>b</sup>	106.2 <sup>b</sup>	210.4 <sup>b</sup>	321.3 <sup>b</sup>	485.2 <sup>b</sup>	415.8 <sup>b</sup>	531.9 <sup>b</sup>
EGC		41.4 <sup>a</sup>	108.2 <sup>a</sup>	153.5 <sup>a</sup>	272.1 <sup>a</sup>	498.3 <sup>a</sup>	741.5 <sup>a</sup>	706.4 <sup>a</sup>	670.8 <sup>a</sup>
SEM		2.5	5.8	5.3	9.1	10.3	17.4	12.8	35.8
Diet (n = 6)									
96% AME <sub>n</sub>		27.1 <sup>c</sup>	85.6 <sup>b</sup>	118.1 <sup>b</sup>	216.2 <sup>b</sup>	363.2 <sup>c</sup>	529.0 <sup>b</sup>	605.0 <sup>a</sup>	636.6
100% AME <sub>n</sub>		33.8 <sup>bc</sup>	87.5 <sup>b</sup>	123.6 <sup>b</sup>	225.5 <sup>b</sup>	404.0 <sup>bc</sup>	616.1 <sup>a</sup>	507.5 <sup>b</sup>	534.7
104% AME <sub>n</sub>		38.4 <sup>ab</sup>	95.3 <sup>ab</sup>	124.7 <sup>b</sup>	246.0 <sup>ab</sup>	414.0 <sup>ab</sup>	657.3 <sup>a</sup>	564.4 <sup>a</sup>	616.2
108% AME <sub>n</sub>		46.9 <sup>a</sup>	119.7 <sup>a</sup>	153.0 <sup>a</sup>	277.3 <sup>a</sup>	458.1 <sup>a</sup>	651.0 <sup>a</sup>	567.5 <sup>a</sup>	617.9
SEM		3.5	8.3	7.5	12.9	14.6	24.6	18.2	50.7
Treatment (n = 3)									
SGC	96% AME <sub>n</sub>	24.3	72.3	95.1	185.9	284.2	429.4	452.2	595.6
	100% AME <sub>n</sub>	29.8	75.4	89.0	197.5	330.8	484.7	348.8	453.3
	104% AME <sub>n</sub>	31.3	91.5	105.9	222.6	326.1	507.1	447.4	573.2
	108% AME <sub>n</sub>	41.2	104.5	134.8	235.6	344.3	519.4	414.9	505.3
EGC	96% AME <sub>n</sub>	29.8	98.9	141.1	246.6	442.2	628.6	757.8	677.6
	100% AME <sub>n</sub>	37.9	99.7	158.2	253.5	477.2	747.4	666.3	616.0
	104% AME <sub>n</sub>	45.5	99.2	143.6	269.3	501.8	807.5	681.5	659.1
	108% AME <sub>n</sub>	52.5	134.8	171.1	319.0	572.0	782.5	720.0	730.5
	SEM	4.9	11.7	10.6	18.3	20.7	34.8	25.7	71.7
P-value									
Growth curve (GC)		0.02	0.02	<0.001	<0.001	<0.001	<0.001	<0.001	0.02
Diet (factorial)		0.009	0.05	0.03	0.03	0.004	0.009	0.02	0.52
Diet (linear)		<0.001	0.007	0.007	0.002	<0.001	0.001	0.58	0.92
Diet (quadratic)		0.80	0.18	0.16	0.38	0.91	0.06	0.04	0.33
GC x Diet (factorial)		0.83	0.78	0.42	0.78	0.26	0.56	0.39	0.72
GC x Diet (linear)		0.44	0.94	0.39	0.60	0.08	0.29	0.67	0.45
GC x Diet (quadratic)		0.68	0.45	0.44	0.41	0.28	0.30	0.51	0.78

<sup>a-c</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

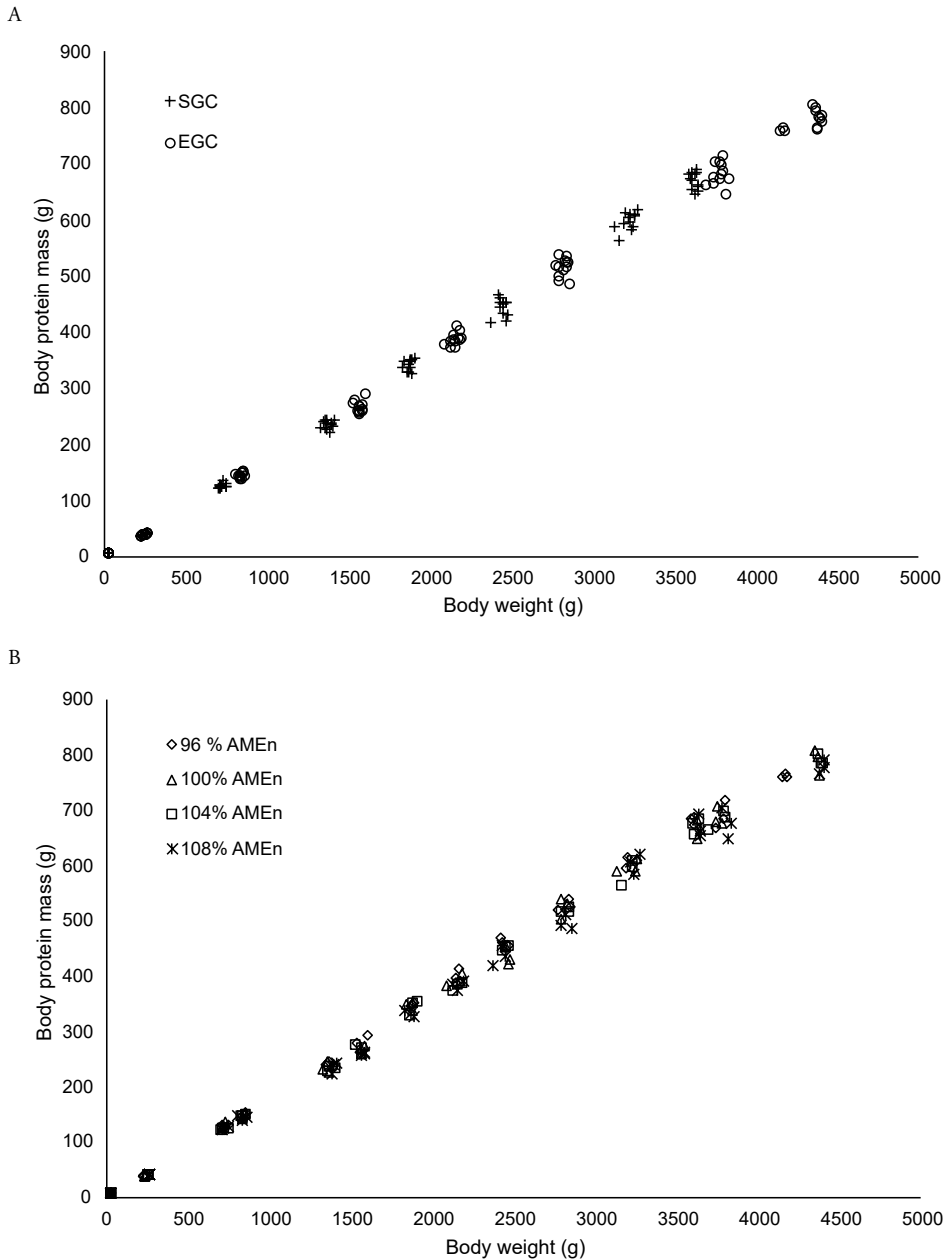


Figure 1. Relationship between body weight and body protein mass of broiler breeders between 0 and 36 wk of age fed at 2 different growth curves (A; SGC = standard growth curve or EGC = elevated growth curve (+15%);  $n = 12$ ) and 4 diets (B), differing in energy-to-protein ratio (96, 100, 104, or 108% AMEn;  $n = 6$ ) from day 0 onward. Each symbol represents 1 replicate (pen) at each body weight.

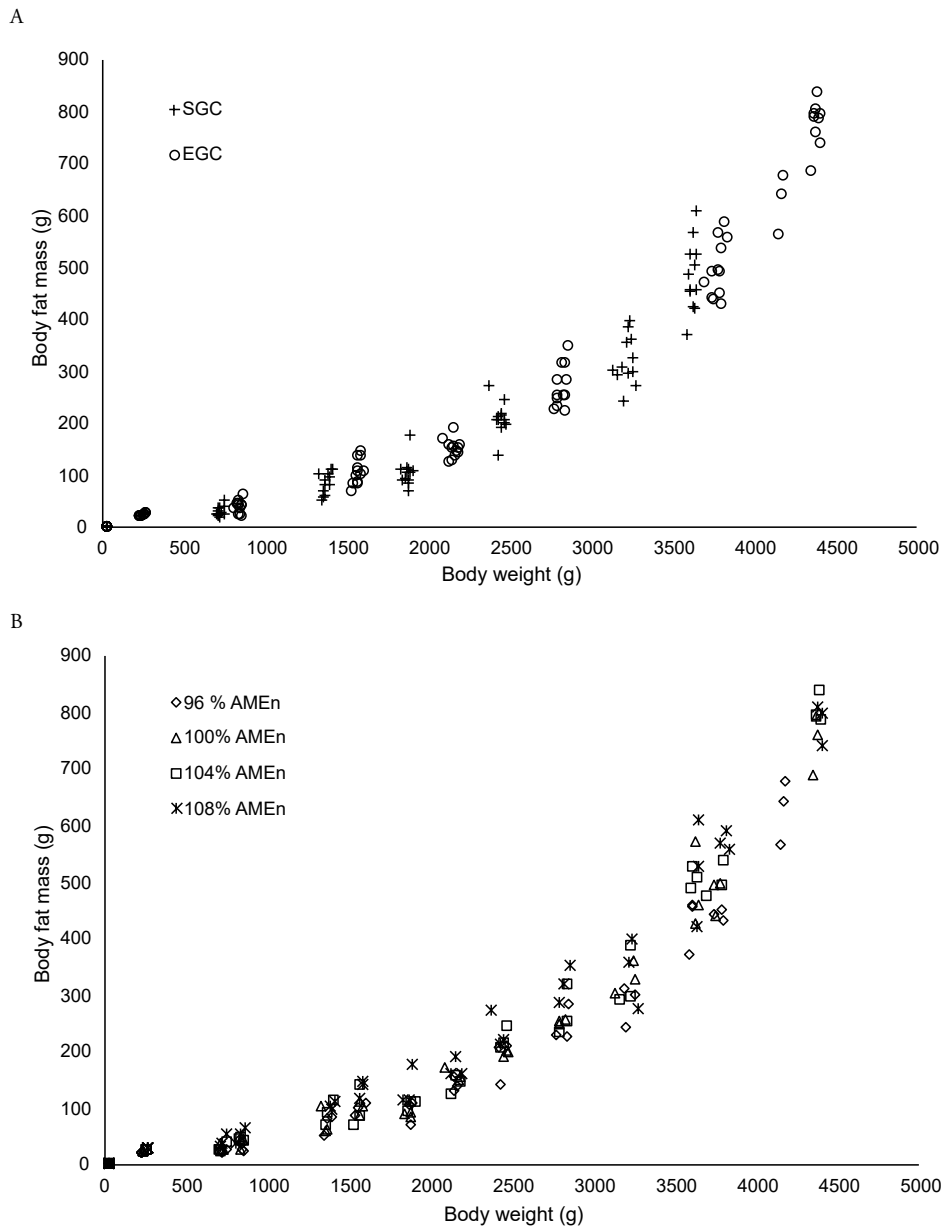


Figure 2. Relationship between body weight and body fat mass of a broiler breeder between 0 and 36 wk of age fed at 2 different growth curves (A; SGC = standard growth curve or EGC = elevated growth curve (+15%); n = 12) and 4 diets (B), differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>; n = 6) from day 0 onward. Each symbol represents 1 replicate (pen) at each body weight.



the common linear regression line is presented. A common linear regression line in growing breeders was expressed as  $-6.4 + 0.184 * BW$  ( $R^2 = 0.99$ ;  $P < 0.001$ ). In mature breeders (36 – 60 wk of age), a common line had the best fit for GC and dietary energy-to-protein ratio ( $126.4 + 0.15 * BW$ ;  $R^2 = 0.86$ ;  $P < 0.001$ ) to predict body protein mass.

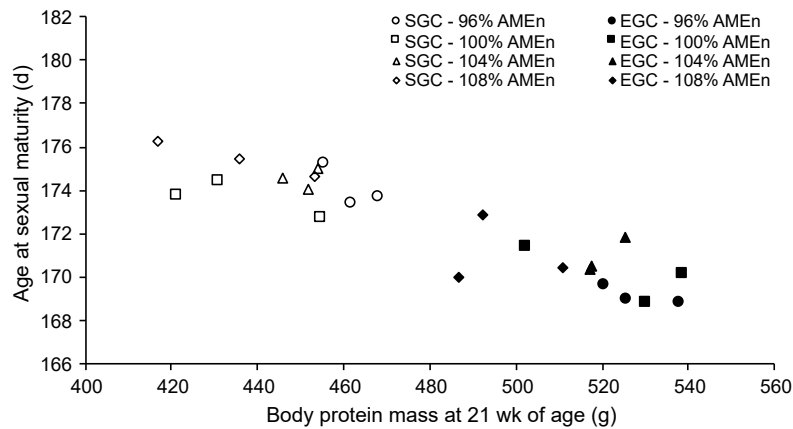
An exponential relationship was observed between BW and body fat mass in growing broiler breeders (0 – 36 wk of age; Figure 2;  $P < 0.001$ ). A common line for both GC had the best fit for predicted body fat mass, which can be expressed as  $-42.2 + 50.8 * 1.0006^{BW}$  (Figure 2A;  $R^2 = 0.98$ ;  $P < 0.001$ ). Separate lines had the best fit for each dietary energy-to-protein ratio, which was estimated with the following coefficients; the constant coefficients ( $a$ ) were estimated as -31.5, -43.5, 38.6, and -74.7, the linear coefficients ( $b$ ) were estimated as 39.6, 49.4, 47.5, and 82.5, and the exponential coefficients ( $c$ ) were estimated as 1.0007, 1.0006, 1.0007, and 1.0005 for 96%, 100%, 104% and 108% AME<sub>n</sub> diet, respectively (Figure 2B;  $R^2 = 0.98$ ;  $P = 0.03$ ). In mature breeders (36 – 60 wk of age), a linear common line had the best fit for each GC and dietary energy-to-protein ratio ( $-811 + 0.35 * BW$ ;  $R^2 = 0.61$ ;  $P < 0.001$ ) to predict body fat mass.

Age at sexual maturity was related to body protein mass at 21 wk of age (Figure 3A;  $R^2 = 0.83$ ;  $P < 0.001$ ). For each 100 g of body protein mass extra at 21 wk of age, ASM advanced with 5.4 d. The linear relationship was also observed at 6, 12 and 16 wk of age ( $R^2 = 0.78, 0.71$  and  $0.78$ , respectively, all  $P < 0.001$ ; data not shown). Body fat mass at 21 wk of age did not relate to ASM (Figure 3B;  $R^2 = 0.19$ ;  $P = 0.85$ ), neither at other ages during rearing ( $P = 0.57, 0.39$ , and  $0.69$  for 6, 12 and 16 wk of age, respectively; data not shown). Body protein percentage and body fat percentage at 21 wk of age did not relate to ASM ( $P = 0.19$  and  $0.25$ , respectively, data not shown).

## Energetic Efficiency

Figure 4 presents the average calculated values for  $k_g$  for each GC (Figure 4A) and each dietary energy-to-protein ratio (Figure 4B) from 0 to 21 wk of age. A quadratic relationship between  $k_g$  and age was observed ( $R^2 = 0.72$ ;  $P < 0.001$ ). Inclusion of GC and dietary energy-to-protein ratio further improved the model fit. Within SGC, parallel regression curves showed the best fit for each dietary energy-to-protein ratio, which can be expressed as  $a - 0.0304 * Age + 0.00173 * Age^2$ , where  $a$  were estimated as 0.418, 0.397, 0.386, and 0.381 for 96%, 100%, 104% and 108% AME<sub>n</sub> diet, respectively ( $R^2 = 0.74$ ;  $P < 0.001$ ). Within EGC, a common regression curve showed the best fit for all dietary energy-to-protein ratios, which can be expressed as  $0.420 - 0.0334 * Age + 0.00189 * Age^2$  ( $R^2 = 0.73$ ;  $P < 0.001$ ). At 21 wk of age,

A



B

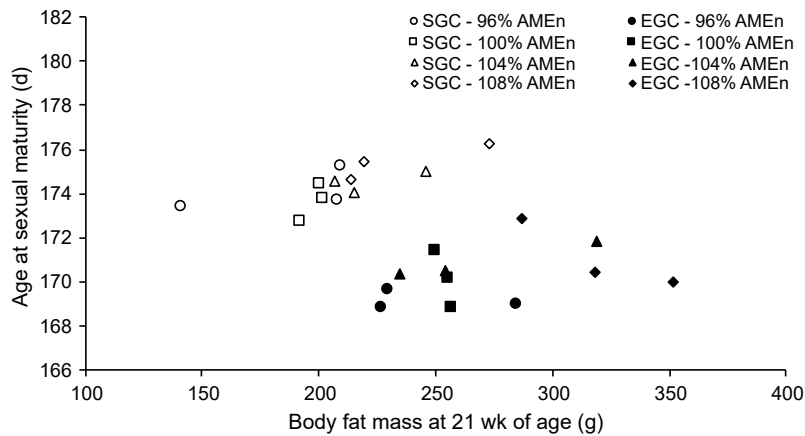


Figure 3. Relationship between body protein (A) and body fat (B) mass (g) at 21 wk of age and age at sexual maturity (age at 50 % egg production; d) of broiler breeders fed at 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AMEn) from day 0 onward.. Each symbol represents 1 replicate (n = 24 pens).

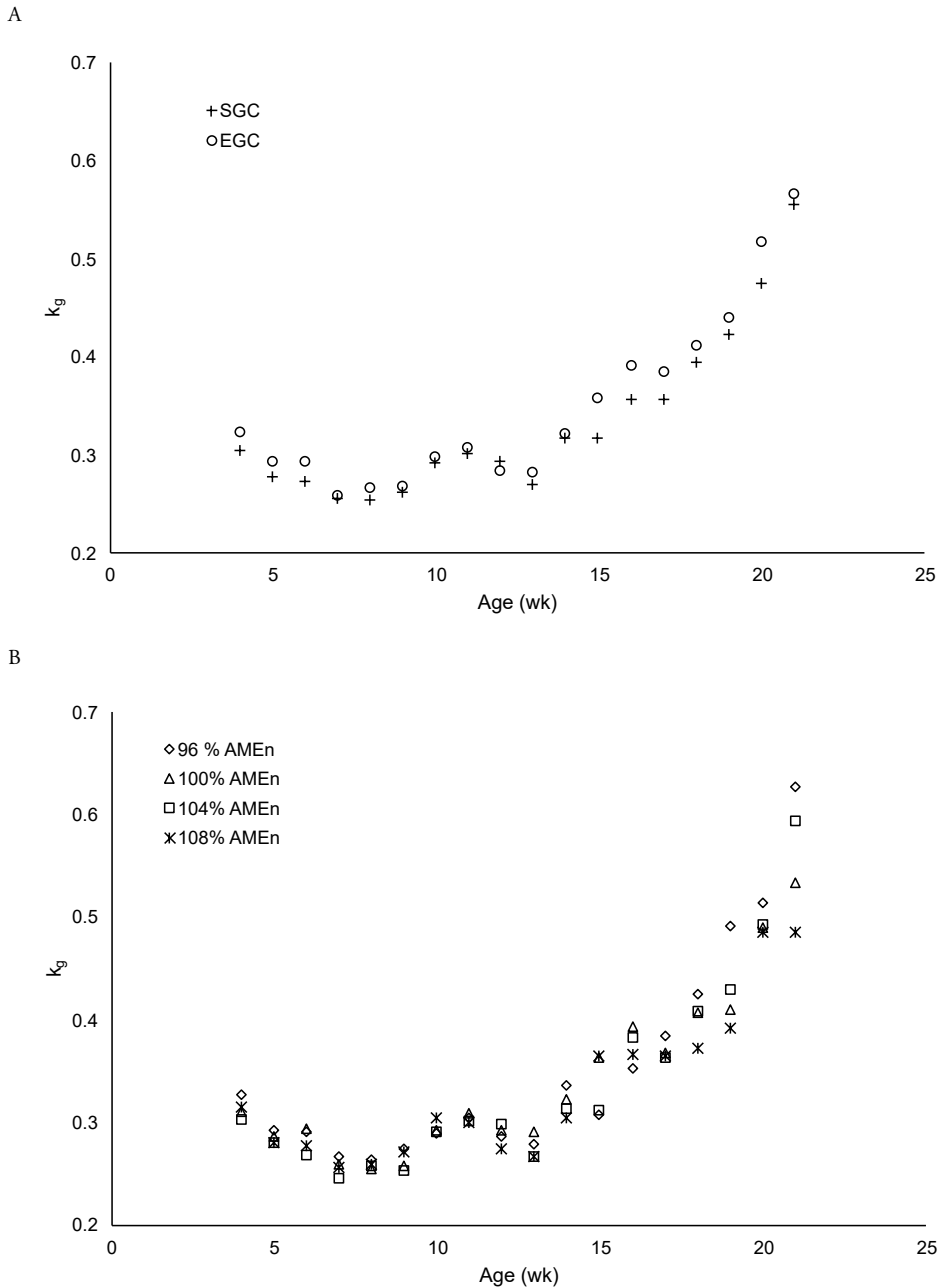


Figure 4. Relationship between broiler breeder age and calculated efficiency of energy utilization for body weight gain ( $k_g$ ) of broiler breeders between 0 and 21 wk of age fed at 2 different growth curves (A; SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets (B), differing in energy-to-protein ratio (96, 100, 104, or 108% AMEn) from day 0 onward. Each symbol represents the average calculated  $k_g$  per treatment at each time point.

average calculated  $k_g$  was 0.54, which was used for further calculations of  $k_e$  during the production period.

Figure 5 presents the average calculated values for  $k_e$  for each GC (Figure 5A) and each dietary energy-to-protein ratio (Figure 5B) from 36 to 60 wk of age. A quadratic relationship between  $k_e$  and age was observed ( $R^2 = 0.46$ ;  $P < 0.001$ ). Inclusion of GC and dietary energy-to-protein ratio further improved the model fit. Within SGC, parallel regression curves showed the best fit for each dietary energy-to-protein ratio, which can be expressed as  $a + 0.033 * Age - 0.00040 * Age^2$ , where  $a$  were estimated as -0.211, -0.186, -0.182, and -0.192 for 96%, 100%, 104% and 108% AME<sub>n</sub> diet, respectively ( $R^2 = 0.55$ ;  $P = 0.001$ ). Within EGC, separate lines had the best fit for each dietary energy-to-protein ratio, which was estimated with the following coefficients; the constant coefficients ( $a$ ) were estimated as -1.552, 0.142, 0.463, and 0.043, the linear coefficients ( $b$ ) were estimated as 0.081, 0.024, 0.010 and 0.026, and the quadratic coefficients ( $c$ ) were estimated as -0.00082, -0.00034, -0.00021 and -0.00036 for 96%, 100%, 104% and 108% AME<sub>n</sub> diet, respectively ( $R^2 = 0.81$ ;  $P < 0.001$ ).

## DISCUSSION

### Dynamics in Body Composition

To our knowledge, the dynamics in body composition in broiler breeder hens from hatch till the end of the production period has not been investigated before. Other studies have only considered body composition during the rearing period (Sakomura et al., 2003; De Los Mozos et al., 2017), during sexual maturation (Rabello et al., 2006; Hadinia et al., 2020), or during the production period (Caldas et al., 2018; Salas et al., 2019), or only measured representatives of body composition, like abdominal fat pad and breast muscle weight (Van Emous et al., 2013; Zuidhof, 2018). Measuring body composition both during the rearing and production period allowed to model relationships between BW of the breeders and body composition in both growing breeder pullets and mature breeders. The models provided a means of calculating body composition according to variations in BW. For the models, fresh BW was used instead of feather-free BW. Defeathering of the carcass is required to obtain a homogeneous mixture for BC analysis. For practical applicability of the BC models though fresh BW was used, because a high correlation ( $R^2 = 1.00$ ) was observed between fresh BW and feather-free BW. Additionally, fresh BW is easy to measure in practice, whereas feather-free BW requires euthanization of the breeder and no differences between treatments were observed in feather weight as percentage of fresh BW (Heijmans et al., 2021).

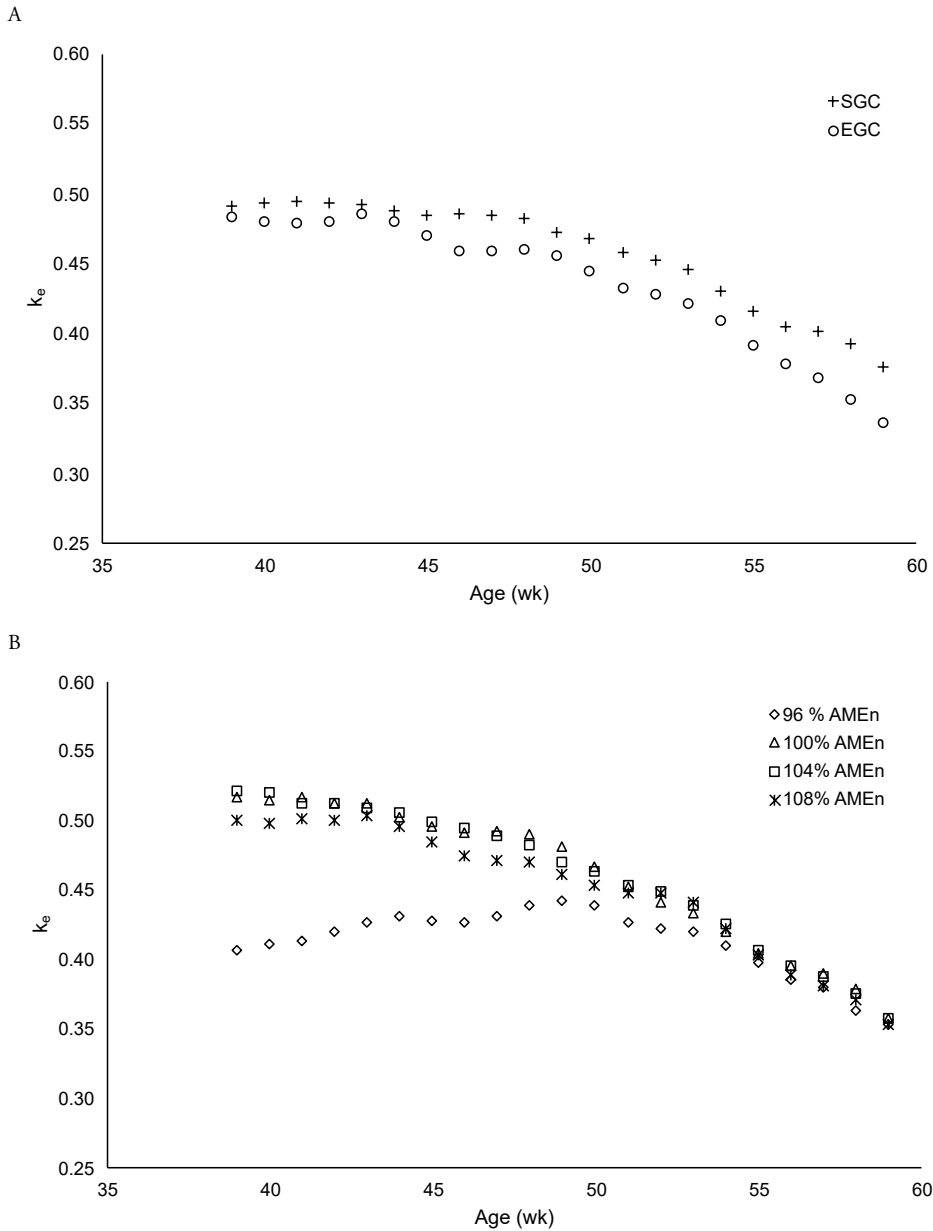


Figure 5. Relationship between broiler breeder age and calculated efficiency of energy utilization for egg production ( $k_e$ ) of broiler breeders between 36 and 60 wk of age fed at 2 different growth curves (A; SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets (B), differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>) from day 0 onward. Each symbol represents the average calculated  $k_e$  per treatments at each time point.

Body protein mass is tightly regulated and mainly depended on BW of the breeder hen and to a lower extent on GC or dietary energy-to-protein ratio. Growing animals always have a basic daily body protein retention, that they need to fulfil before additional body protein and fat can be retained (Boekholt et al., 1994; Boekholt and Schreurs, 1997). Sakomura et al. (2003) observed a comparable allometric relationship as presented in the current study between BW and body protein mass in growing breeder pullets of  $-9.1 + 0.171 * BW$ . Predicted body protein mass was lower in the study of Sakomura et al. (2003), most probably due to differences in genetics (Hubbard Hi-Yield vs. Ross 308 breeders). When looking at body protein content, instead of body protein mass, other studies also observed a lack of difference in body protein percentage or breast muscle percentage when breeders were 8 to 20% heavier, compared to a standard BW according to breeder guidelines (Renema et al., 2001; Van Emous et al., 2013; Salas et al., 2019). This again indicates a tight regulation of body protein content. In the current study, at the same BW, a breeder on the EGC had a lower body protein mass, compared to a breeder on the SGC. This indicates that slower growth results in a higher protein content, although predicted differences were small e.g.  $\Delta = 7$  g body protein mass at 2,000 g BW ( $\Delta = 0.4$  %). A lower dietary energy-to-protein ratio resulted in a higher body protein mass, at the same BW, although predicted differences were again small e.g.  $\Delta_{\max} = 14$  g body protein mass at 2,000 g BW ( $\Delta_{\max} = 0.7$  %). This is in line with other studies, who observed a higher breast muscle weight, as representative for total body protein mass, when breeders were fed a diet with a lower dietary energy-to-protein ratio (Van Emous et al., 2013; Lesuisse et al., 2017). Feeding breeders a lower dietary energy-to-protein ratio, while aiming for a similar BW, resulted in a 5.4 to 22.8 % higher dietary crude protein intake (Van Emous et al., 2013; Lesuisse et al., 2017; Heijmans et al., 2021). The surplus of dietary crude protein was thus only partly retained as (additional) body protein. In mature breeders, little further body protein growth occurred, due to a restriction in feed allowance and growth, as recommended by the breeder company (Aviagen, 2016b). This has also been observed by others (Nonis and Gous, 2016). It can be speculated that body protein growth will continue when breeders are allowed to grow further when feed allowance is further increased or when feed is provided *ad libitum*, as breeders have not reached their somatically mature weight yet (Gous, 2015; Zukiwsky et al., 2021).

Body fat mass showed an exponential relationship to BW. Sakomura et al. (2003) described a linear relationship between body fat mass and BW in growing breeders pullets of  $7.0 + 0.085 * BW$ . In that study, they only analyzed breeders up to approximately 2,000 g of BW, whereas the current study also included breeders up to 4,400 g of BW. When average weekly fat growth was calculated in growing breeders, based on Table 3, a fat growth spurt is observed after 16 wk of age (8.0 g/wk vs. 24.2

g/wk, 0 to 16 wk of age vs. 16 to 36 wk of age, respectively). This may explain why Sakomura et al. (2003) did not observe an exponential relationship, as the fat growth spurt occurred after approximately 2,000 g of BW. If we only analyzed data of breeders up to 2,000 g of BW, a linear regression curve showed a similar fit ( $R^2 = 0.85$  and  $BIC = 1135$ ) as an exponential regression curve ( $R^2 = 0.85$  and  $BIC = 1136$ ). A fat growth spurt towards the end of rearing was observed as well in layers (Kwakkel et al., 1993). It was speculated that the first fat growth is mainly deposited as intermuscular fat and the second fat growth spurt mainly as abdominal fat (Kwakkel et al., 1993). When calculating the proportion of abdominal fat to total fat, indeed we observe an increase from 6.5% at 16 wk of age to 13.2% at 36 wk of age. This indicates a faster accretion of abdominal fat at later ages, compared to non-abdominal fat in the body.

Body fat mass was higher in EGC breeders compared to SGC breeders at each age when body composition was determined. Other studies also observed a higher fat mass when breeders were 8 to 20% heavier, compared to a standard BW according to breeder guidelines (Renema et al., 2001; Sun and Coon, 2005; Van Emous et al., 2013; Salas et al., 2019). In the indicated studies, contrasts in GC were only maintained until 21 wk of age, resulting in breeders having a similar body fat mass during production, irrespective of initial BW and body fat mass differences at 21 wk of age (Renema et al., 2001; Sun and Coon, 2005; Van Emous et al., 2013; Salas et al., 2019). This is confirmed in the current study, as breeders had a similar predicted fat mass at the same BW, irrespective of GC. This indicates that body fat mass is related to BW rather than to growth rate.

Dietary treatments also had an effect on body fat mass. An increase in dietary energy-to-protein ratio resulted in a higher body fat mass at the same BW, i.e.  $\Delta_{\max} = 45$  g body fat mass at 2,000 g BW ( $\Delta_{\max} = 2.3\%$ ). This is in line with other studies (Van Emous et al., 2013, 2015; Lesuisse et al., 2017, 2018; Salas et al., 2019). If a surplus of energy is supplied, this is mostly retained as fat (Boekholt et al., 1994; Leeson et al., 1996; Boekholt and Schreurs, 1997). It remains unclear whether or not differences in fat mass persist when contrasts in dietary treatments disappear. Van Emous et al. (2013) showed that breeders had more abdominal fat and thus more fat mass at 20 wk of age, when dietary energy-to-protein ratio increased by decreasing the dietary protein content. When breeders were fed a standard diet hereafter, a similar body fat mass was observed at 40 wk of age. It can thus be suggested that differences in dietary treatments should be maintained to maintain differences in body fat mass.

In mature breeders, after 36 wk of age, body fat mass decreased for all dietary treatments, except for the 96% AME<sub>n</sub>. Salas et al (2019) also observed a decrease in fat mass after peak production. Two potential mechanisms might be involved in the decrease in

fat mass; 1) body fat is mobilized to support yolk fat (Salas et al., 2017) or egg (Nonis and Gous, 2012) production or 2) body fat is mobilized to fulfill energy requirements for basic daily protein retention (Boekholt and Schreurs, 1997) as breeders have not reached their somatically mature weight yet (Gous, 2015; Zukiwsky et al., 2021). Breeders fed the 96% AME<sub>n</sub> diet required a relative high feed intake to achieve pair-gaining (Heijmans et al., 2021), where the surplus of nutrients were deposited as fat. This indicates that these breeders were inefficient with their nutrients as mature breeders, which will be discussed further in the ‘energetic efficiency’ paragraph below.

### Age at Sexual Maturity

Sexual maturation of breeders pullets is a complex process which depends on multiple factors (Hanlon et al., 2020). Several authors emphasized the importance of metabolic status on sexual maturation (Bédécarrats et al., 2016; Hanlon et al., 2020; Van der Klein et al., 2020). Discrepancy exists whether a body protein (Sun et al., 2006; Eitan et al., 2014; Salas et al., 2019) or body fat (Van der Klein et al., 2018b; Zuidhof, 2018; Hadinia et al., 2020) threshold exists for sexual maturation, although none of the studies indicated above correlated body composition directly to sexual maturation. The current study shows a clear relationship between body protein mass at a given age during rearing and sexual maturation, where each 100 g extra body protein mass advanced sexual maturation with 5.4 d. In line with this, Lewis et al. (2007) observed that with each 100 g extra BW at 20 wk of age, sexual maturation advanced with 2 d. These results indicate that particularly body protein mass is important for sexual maturation. Two potential mechanisms might be involved. Firstly, protein is an important component of the oviduct and ovary (Ricklefs, 1976; Bowmaker and Gous, 1989; Kwakkel et al., 1993). An advanced development of the reproductive tract might have led to a higher total body protein mass. Future studies to sexual maturation should therefore include growth and composition of the reproductive tract in breeder pullets. Secondly, body protein is an important source for yolk protein (Ekmay et al., 2014) and yolk fat, via gluconeogenesis (Boonsinchai, 2015) and *de novo* lipogenesis (Salas et al., 2017) in young breeders. Around sexual maturation, an increase in body protein mobilization is observed (Vignale et al., 2017, 2018), indicating breeders use body protein reserves to support egg production. Body fat mass was not related to sexual maturation in the current study. This indicates either that body fat mass does not play a role in sexual maturation or that it was already beyond the threshold needed for sexual maturation. In the studies that hypothesized that body fat plays an important role in sexual maturation, results were either confounded with BW (Hadinia et al., 2020), and thus body protein, or body composition was measured in laying and non-laying breeders at 52 (Zuidhof, 2018) or 55 (Van der Klein et al., 2018b) wk of age and not around sexual maturation.



## Dynamics in Energetic Efficiency

To our knowledge, no other studies are available that attempt to model  $k_g$  and  $k_e$  in relation to age of the breeders. Quantifying factors that contribute to energy efficiency is challenging, but this can have profound economic and environmental consequences (Zuidhof, 2019). For the calculations of maintenance requirement only body protein mass was taken into account, as this was assumed as the metabolic active component of the body (Emmans, 1987; Gous, 2015; Nonis and Gous, 2018). Body fat is considered as inert and therefore does not require maintenance (Emmans, 1987; Gous, 2015; Nonis and Gous, 2018). One could argue that fatter breeders with a similar body protein mass as leaner breeders have a higher maintenance requirement as they have to carry more weight. Therefore, calculations were also performed using a  $ME_m$  formula which takes BW instead of body protein into account (Noblet et al., 2015). Absolute values for  $k_g$  were on average 0.11 higher and absolute values for  $k_e$  were on average 0.04 lower with that  $ME_m$  formula. The shape of the regression curves (quadratic relationship) and the treatment effects remained the same as with the body protein maintenance formula.

The current study shows a quadratic relationship between  $k_g$  and age of the pullets. Values for  $k_g$  ranged from 0.27 (8.8 wk of age) to 0.54 (21 wk of age). The calculated value of  $k_g$  at 21 wk of age (0.54) is comparable to reported  $k_g$  values of breeders during production (Rabello et al., 2006; Reyes et al., 2011, 2012). Rabello et al. (2006) calculated a  $k_g$  of 0.47 in Hubbard Hi-Yield breeders between 26 and 33 wk of age. Reyes et al. (2011, 2012) calculated a  $k_g$  of 0.59 in Cobb 500 breeders between 32 and 42 wk of age and 0.57 between 53 and 62 wk of age. The calculated values of  $k_g$  during rearing are lower compared to  $k_g$  values reported by Sakomura et al. (2003). They observed values for  $k_g$  of 0.79 (3 to 8 wk of age), 0.64 (9 to 14 wk of age), and 0.81 (15 to 20 wk of age) in Hubbard Hi-Yield breeders (Sakomura et al., 2003). The values presented in literature vary substantially due to differences in animal factors (e.g. age, genetic strain), environmental factors (e.g. ambient temperature), dietary factors (e.g. chemical composition of the diet) (Zuidhof, 2019), and methodologies used for determination of energetic efficiency (Sakomura et al., 2003).

Even though absolute values of  $k_g$  during rearing were higher in Sakomura et al. (2003), they also observed a quadratic shape for  $k_g$  during rearing. The shape of the quadratic regression line for  $k_g$  might be explained by feed restriction levels. Feed restriction is most severe between 7 and 16 wk of age (25 to 33% of *ad libitum*), whereas this is less severe during the production period (50 to 90% of *ad libitum*) (De Jong and Guémené, 2011). It can be hypothesized that a more severe feed restriction between 7 and 16 wk of age results in a lower energetic efficiency, compared to ages outside this range. Pullets might mobilize body fat during periods of severe feed restriction, result-

ing in a higher heat production and thus lower efficiency, in order to meet their energy requirements for basic daily body protein retention (Boekholt et al., 1994; Boekholt and Schreurs, 1997). In line with this hypothesis, within SGC pullets, a higher dietary energy-to-protein ratio resulted in a lower predicted  $k_g$ . An increase in dietary energy-to-protein ratio resulted in a lower feed allowance to obtain pair-gaining (Heijmans et al., 2021) and thus a more severe feed restriction, although differences in  $k_g$  between dietary treatments were relatively small ( $\Delta_{\max} = 0.04$ ). The dietary effect on  $k_g$  was not observed within EGC pullets. For EGC pullets, predicted values of  $k_g$  were even lower than predicted values of  $k_g$  for SGC pullets on the 96% AME<sub>n</sub> diet, whereas EGC pullets had a higher feed allowance (Heijmans et al., 2021). It remains unclear why dietary energy-to-protein ratio did not affect  $k_g$  in EGC pullets. Future studies should investigate energetic efficiency for breeder pullets in restricted and *ad libitum* fed pullets to confirm the impact of feed restriction level on energetic efficiency.

A quadratic relationship was also observed between  $k_e$  and age of the breeders. Predicted values of  $k_e$  ranged from 0.28 to 0.56 between 36 and 60 wk of age. For the calculations, in case average BW gain was negative, a growth of zero was assumed (3 pens; -1.1, -2.1 and -5.2 g/d average BW gain), as it remains unclear whether or not a negative BW gain yields energy or if there is a cost factor involved as well. If calculations were performed assuming a negative average BW gain only yields energy, average values of  $k_e$  were 0.001 lower. The shape of the regression curve and the treatment effects remained the same. The predicted values of  $k_e$  are lower compared to calculated  $k_e$  values in other studies with breeders. Rabello et al. (2006) calculated a  $k_e$  of 0.64 in Hubbard Hi-Yield breeders between 26 and 33 wk of age. Reyes et al. (2011, 2012) calculated a  $k_e$  of 0.73 in Cobb 500 breeders between 32 and 42 wk of age and 0.66 between 53 and 62 wk of age. Again, differences in  $k_e$  values might be due to differences in animal factors, environmental factors, dietary factors and methodologies used for calculations (Sakomura et al., 2003; Zuidhof, 2019), where the latter one potentially has the largest effect on differences in  $k_e$  values.

Predicted  $k_e$  decreased with 0.13 on average with age of the breeders. The decrease of  $k_e$  with age might partly be explained by a decrease in feather cover with increasing breeder age (Heijmans et al., 2021). Lower feather coverage will result in a higher maintenance requirement, as feathers provide insulation to the hen (Van Krimpen et al., 2014). Van Krimpen et al. (2014) calculated in laying hens that with each percent of feather coverage loss, this will require 0.23 kcal/d extra. In the current study, feather cover was 100% at 21 wk of age and decreased to approximately 68% at 59 wk of age ( $P < 0.001$ ; Heijmans, unpublished data). This corresponds to max 7.4 kcal/d extra to correct for feather coverage. If feather coverage was taken into account for  $k_e$  calculations, average  $k_e$  values were 0.01 higher from 36 to 46 wk of age and

0.02 higher from 46 wk of age onward, compared to  $k_e$  values when feather coverage was not taken into account. Hence, feather coverage did not explain the decrease of  $k_e$  with age. The decrease in  $k_e$  with age of the breeders is probably mostly attributed to a decrease in laying rate, as  $ME_{int}$ ,  $ME_m$ , and  $ME_g$  were quite constant from 36 to 60 wk of age and egg weight increased with age (Heijmans et al., 2022). It can thus be speculated that strategies aiming for a more persistent laying rate will also improve energetic efficiency.

For SGC breeders,  $k_e$  was 0.02 higher on average, compared to EGC breeders. This indicates that SGC breeders relatively retain more energy in eggs than EGC breeders. Both GC were fed a restricted amount of feed, but EGC breeders had a 15% higher feed allowance during production, compared to SGC breeders (Heijmans et al., 2021). It can be speculated that EGC breeders had less fasting time during the day, compared to SGC breeders. Fasting can improve digestibility of metabolizable energy in the diet with 1.8% compared to non-fasting (Wang et al., 2022). Assuming a 1.8% higher  $AME_n$  availability for SGC breeders (on average 7.7 kcal/d), would result in a 0.02 lower predicted  $k_e$  value on average for SGC breeders, which is then comparable to predicted  $k_e$  values for EGC breeders. Dietary energy-to-protein ratio had minimal effects on predicted  $k_e$  values, with exception of the 96%  $AME_n$  dietary treatment in EGC breeders. Up to approximately 50 wk of age, EGC breeder fed the 96%  $AME_n$  diet had a remarkably lower predicted  $k_e$  values (up to 0.27 lower), compared to the other dietary EGC treatments. These breeders required a high feed allowance for pair-gaining from approximately 32 to 50 wk of age (Heijmans et al., 2021), whereas this only resulted in a slight increase in egg weight and did not affect laying rate (Heijmans et al., 2022), compared to the other EGC dietary treatments. Although eating time was not determined in the current study, visually it was observed that these breeders were fed close to *ad libitum* (10 to 12h feed availability). Potential heat producing activities, related to high feed intake, were not taken into account in the calculations. It can be speculated that predicted  $k_e$  values of 96%  $AME_n$  EGC breeders will be closer to the predicted  $k_e$  values of other dietary treatments if the energy consuming activities, like longer eating and more digestive processes, were taken into account.

## CONCLUSIONS

It can be concluded that a linear relationship exists between body protein and BW of the breeder hen, with minimal effects of dietary treatments. Body protein is one of the factors determining sexual maturation in breeder pullets. Body fat mass showed an exponential relationship to BW, with a fat growth spurt towards the end of rearing and start of production. An increase in dietary energy-to-protein ratio results in a

higher body fat mass, at the same BW. Dietary treatments had minimal effects on estimated energetic efficiency in breeders, whereas age had a pronounced effect. Energetic efficiency for BW gain was lower in pullets from 7 to 16 wk of age, compared to younger or older breeder pullets. Energetic efficiency for egg production decreased with age of the breeders, which was mostly related to a lower laying rate.

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## SUPPLEMENTARY INFORMATION

Table S1. Defeathered body weight of broiler breeders selected for body composition analysis from 6 to 60 wk of age with 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), fed from 0 to 60 wk of age.

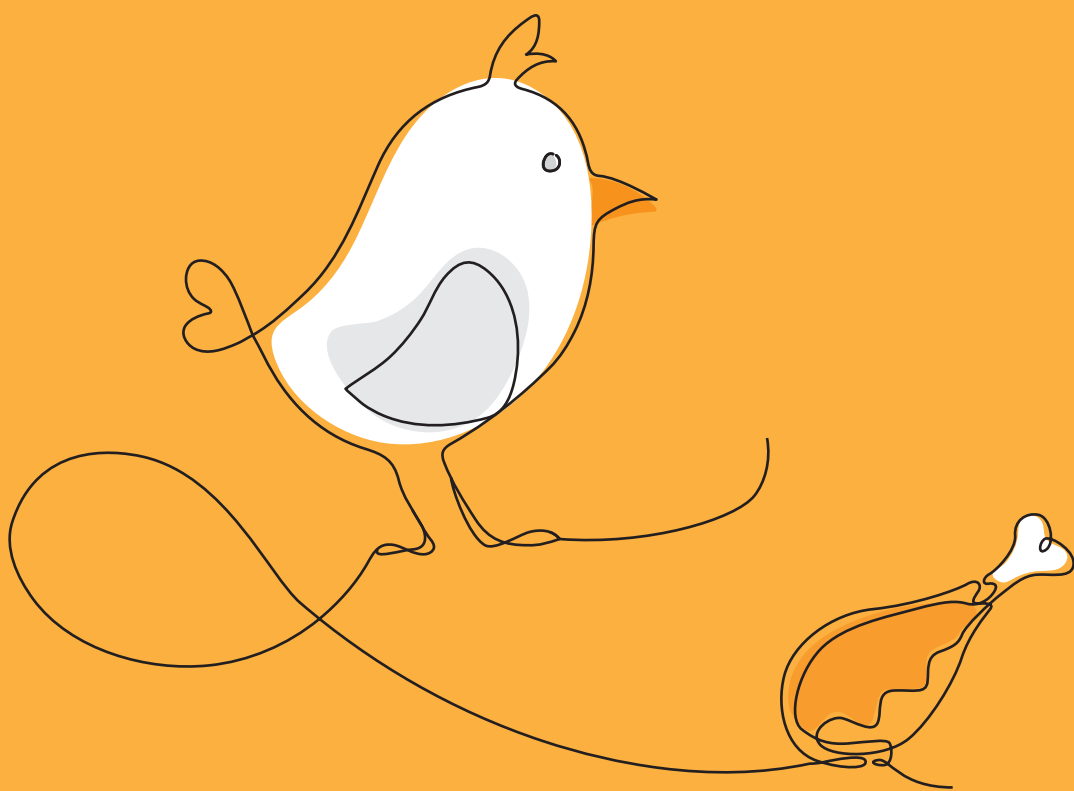
Item	Age (wk) <sup>1</sup>							
	6	12	16	21	28	36	46	60
Growth curve (n=12)								
SGC	686 <sup>b</sup>	1296 <sup>b</sup>	1771 <sup>b</sup>	2327 <sup>b</sup>	3072 <sup>b</sup>	3525	3569	3745 <sup>b</sup>
EGC	796 <sup>a</sup>	1485 <sup>a</sup>	2036 <sup>a</sup>	2680 <sup>a</sup>	3623 <sup>a</sup>	4225	4195	4343 <sup>a</sup>
SEM	5	8	13	7	11	6	15	16
Diet (n=6)								
96% AME <sub>n</sub>	743	1394	1912	2502	3333	3784	3943	4046
100% AME <sub>n</sub>	739	1383	1896	2516	3345	3895	3885	4030
104% AME <sub>n</sub>	741	1387	1902	2513	3334	3911	3872	4033
108% AME <sub>n</sub>	741	1399	1904	2483	3379	3910	3827	4066
SEM	7	12	18	11	15	8	21	23
Treatment (n=3)								
SGC 96% AME <sub>n</sub>	686	1299	1787	2328	3050	3510 <sup>d</sup>	3553 <sup>cd</sup>	3741
100% AME <sub>n</sub>	687	1291	1755	2356	3076	3532 <sup>d</sup>	3615 <sup>c</sup>	3707
104% AME <sub>n</sub>	683	1297	1777	2332	3063	3525 <sup>d</sup>	3589 <sup>cd</sup>	3749
108% AME <sub>n</sub>	690	1298	1766	2292	3099	3531 <sup>d</sup>	3520 <sup>d</sup>	3783
EGC 96% AME <sub>n</sub>	801	1489	2037	2676	3616	4057 <sup>c</sup>	4333 <sup>a</sup>	4351
100% AME <sub>n</sub>	791	1474	2036	2677	3613	4259 <sup>b</sup>	4156 <sup>b</sup>	4353
104% AME <sub>n</sub>	799	1476	2026	2694	3605	4296 <sup>a</sup>	4155 <sup>b</sup>	4316
108% AME <sub>n</sub>	792	1500	2043	2673	3659	4289 <sup>ab</sup>	4135 <sup>b</sup>	4350
SEM	10	16	25	15	21	11	30	33
P-value								
Growth curve (GC)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Diet (factorial)	0.99	0.76	0.94	0.15	0.15	<0.001	0.02	0.69
Diet (linear)	0.88	0.72	0.84	0.20	0.07	<0.001	0.001	0.54
Diet (quadratic)	0.75	0.30	0.59	0.05	0.28	<0.001	0.76	0.29
GC x Diet (factorial)	0.87	0.91	0.87	0.28	0.88	<0.001	0.005	0.59
GC x Diet (linear)	0.71	0.74	0.75	0.15	0.94	<0.001	0.03	0.32
GC x Diet (quadratic)	0.92	0.52	0.99	0.29	0.43	<0.001	0.004	0.69

<sup>a-d</sup>LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>At 2 wk of age, defeathered BW was 232 g of the 96% AME<sub>n</sub> diet and 264 g of the 108% AME<sub>n</sub> diet.







# Chapter 5

## Impact of growth curve and dietary energy-to-protein ratio of broiler breeders on offspring quality and performance

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## ABSTRACT

The impact of growth curve (GC) and dietary energy-to-protein ratio of broiler breeder hens on chick quality and broiler performance was investigated. Pullets ( $n = 1,536$ ) were randomly allotted to 24 pens and assigned to 1 of 8 treatments from hatch onwards, according to a  $2 \times 4$  factorial arrangement with 2 GC (standard growth curve = SGC or elevated growth curve = EGC, +15%) and 4 diets, differing in energy-to-protein ratio (96%, 100%, 104% and 108% AME<sub>n</sub> diet). At 28 and 36 wk of age, 60 hatching eggs per maternal pen were selected for incubation and 768 day-old broilers were assigned to 32 pens according to maternal treatment.

Broilers from EGC breeders were 1.9 g heavier at hatch ( $P < 0.001$ ) and 36 g heavier at slaughter ( $P = 0.001$ ) than broilers from SGC breeders due to an 1.0 g/d higher growth rate ( $P = 0.003$ ) and 1.5 g/d higher feed intake ( $P = 0.006$ ) from hatch to 32 d of age. An increase in breeder dietary energy-to-protein ratio resulted in a linear decrease in embryonic mortality in the first 3 days of incubation ( $\beta = -0.2$  % per % AME<sub>n</sub>;  $P = 0.05$ ). At hatch, broiler BW decreased with an increasing breeder dietary energy-to-protein ratio ( $\beta = -0.1$  g per % AME<sub>n</sub>;  $P = 0.001$ ), whereas at slaughter broiler BW increased with an increasing breeder dietary energy-to-protein ratio ( $\beta = 3.2$  g per % AME<sub>n</sub>;  $P = 0.02$ ). This was due to a linear increase in growth rate ( $\beta = 0.1$  g/d per % AME<sub>n</sub>;  $P = 0.004$ ) and feed intake ( $\beta = 0.1$  g/d per % AME<sub>n</sub>;  $P = 0.02$ ). Additionally, an increase in breeder dietary energy-to-protein ratio resulted in a linear decrease in body weight corrected feed conversion ratio ( $\beta = -0.002$  per % AME<sub>n</sub>;  $P = 0.002$ ). Overall, it can be concluded that a higher GC of breeders and an increase in breeder dietary energy-to-protein ratio enhances offspring performance.

**Key words:** broiler breeder, feed strategy, maternal nutrition, offspring, broiler

## INTRODUCTION

A good day-old chick quality is crucial for health, welfare and performance of broilers (Tona et al., 2005; Van de Ven et al., 2012). Most of the research on improving chick quality has focused on factors post oviposition and during incubation, for example egg handling, egg storage, incubation temperature and humidity (Molenaar et al., 2010b; Narinç and Aydemir, 2021). Recently, also potential effects of maternal nutrition on chick quality has gained more interest (Moraes et al., 2014; Lesuisse et al., 2017, 2018; Moraes et al., 2019; Zukiwsky et al., 2021a).

Breeder dietary energy-to-protein ratio might be an important factor for day-old chick quality (Spratt and Leeson, 1987) and offspring performance (Moraes et al., 2014). So far, results have been inconsistent. Lesuisse et al (2017, 2018) observed a 3.4 to 4 g lower day-old chick weight, but a 38 to 179 g higher BW at slaughter and a 0.03 lower feed conversion ratio in offspring from breeders that were fed 25% less dietary CP during rearing and production compared to breeders fed according to breeder recommendations. An 11 to 16% reduction in breeder dietary CP (compared to breeder recommendations), during the rearing phase alone did not affect day-old chick quality or offspring performance (Van Emous et al., 2015a; Moraes et al., 2019) or resulted in a 120 g lower BW at 36 d of age in female broilers (Moraes et al., 2014). In the studies of Moraes et al. (2014, 2019), however, dietary treatments were confounded with breeder BW, which has been shown to affect offspring performance as well (Bowling et al., 2018). These results may suggest that day-old chick quality and offspring performance benefit from a higher breeder dietary energy-to-protein ratio during both rearing and production. In all mentioned studies, the higher breeder dietary energy-to-protein ratio was realized by decreasing the CP level in the diet. It remains unclear whether or not a higher breeder dietary energy-to-protein ratio, by increasing dietary energy, during both the rearing and production phase affects chick quality and offspring performance.

Besides the maternal dietary energy-to-protein ratio, also severity of feed restriction might affect day-old chick quality and offspring performance. It has been observed that maternal feed restriction resulted in an increased risk of chronic metabolic diseases in offspring in mammals (Roseboom et al., 2006) and broilers (Van der Waaij et al., 2011). Broiler breeders are commonly fed restricted quantities of feed to control the growth trajectory and BW in order to ensure reproductive performance (Robinson et al., 1991; Bruggeman et al., 1999; Hocking et al., 2002; Sun et al., 2006). Recently, it is suggested that a higher growth curve, by means of an increased feed intake, is possible in modern broiler breeders without negative effects on egg production (Van der Klein et al., 2018; Heijmans et al., 2021; Zukiwsky et al., 2021b).

The aim of this study was to evaluate effects of growth curve and dietary energy-to-protein ratio of broiler breeder hens during rearing and production on day-old chick quality and offspring performance.

## MATERIALS AND METHODS

### Experimental Design

Female Ross 308 broiler breeder pullets ( $n = 1,536$ ) were assigned to 1 of 8 treatments from hatch to 60 wk of age, according to a  $2 \times 4$  factorial arrangement with 2 growth curves (**GC**) (standard growth curve = **SGC** or elevated growth curve = **EGC**) and 4 diets, differing in energy-to-protein ratio by step-wise increase in energy content from 96 to 108% AME<sub>n</sub> at a similar CP content (further defined as 96%, 100%, 104% and 108% AME<sub>n</sub> diet), where the 100% AME<sub>n</sub> treatment was the AME<sub>n</sub> recommended by the breeding company (Aviagen, 2016a). The weekly growth target of the SGC was according to the breeder recommendation (Aviagen, 2016b), whereas the EGC targeted a 15% higher weekly growth relative to the SGC throughout rearing and production. Pair-gain of pullets within each GC was achieved by weekly adaptation of feed allocation per diet based on weekly BW measurement. Treatments were randomly assigned at the start of the experiment to 24 pens (64 pullets per pen) within 3 blocks ( $n = 3$  pens per treatment). A detailed description of this experiment, including diet composition, was reported by Heijmans et al. (2021). At 28 and 36 wk of age, hatching eggs produced by these broiler breeders were incubated and broiler performance was recorded until slaughter. All experimental protocols were approved by the Central Commission on Animal Experimentation (The Hague, the Netherlands), approval number 2018.W-0023.001 and 2018.W-0023.002.

### Incubation

At 28 and 36 wk of age of the breeders, 60 clean settable hatching eggs per maternal pen ( $n = 1,440$ ) were selected for incubation. Of each maternal pen, 20 hatching eggs of 3 consecutive days were selected based on the average egg weight per treatment  $\pm 2.5$  g. The eggs were stored at the breeder farm at 17 °C for 10 to 12 d before incubation. Eggs were transported for approximately 1 h to the hatchery (Lagerwey, Lunteren, The Netherlands). Hatching eggs were incubated in a single-stage incubator with a maximum capacity of 4,800 hatching eggs (HatchTech, Veenendaal, the Netherlands). The incubator contained 1 trolley with 2 rows of 16 setter trays. Per maternal pen, the 60 selected hatching eggs were distributed evenly over 1 setter tray, resulting in 24 setter trays in total. The setter trays were randomly divided over 3 blocks in the incubator (top, middle, bottom of the trolley). Per row, the bottom 2 setter trays and the top 2 setter trays were kept empty. Eggs were warmed linearly

in 10 h from storage temperature to an eggshell temperature (**EST**) of 37.8 °C. The moment the eggs reached an EST of 37.8 °C was considered as embryonic day (**E**)0 and the start of incubation. The EST was monitored throughout incubation, from start of the warming profile, using 4 sensors (NTC Thermistors: type DC 95; Thermometrics, Somerset, UK) that were attached to 4 individual eggs from different treatments. The EST sensors were attached to the eggshell at the equator of the egg, using a small piece of tape (Tesa BV, Almere, The Netherlands) in silicone heat sink compound paste (Type 340; Dow Corning, Midland, MI). The air temperature of the incubator was continuously adapted to maintain an EST of 37.8 °C, based on the median temperature of the 4 EST sensors. At E8, all eggs were candled and clear eggs and eggs containing a dead embryo were removed. Eggs were turned over 90° every hour until E18.

At E18, EST sensors were removed and all eggs were candled again and clear eggs or eggs containing a dead embryo were removed. Eggs containing viable embryos were transferred per setter tray to 1 hatching basket, resulting in 24 hatching baskets in total. The hatching baskets were placed on a trolley containing 3 rows of 11 hatching baskets. Per row, the bottom 2 hatching baskets and the top basket were kept empty. The hatching baskets were randomly divided over the top, bottom, middle, front, and back of the trolley. The trolley was placed in another incubator (HatchTech, Veenendaal, the Netherlands), where 6 EST sensors were attached to 6 individual eggs from different treatments as described above. Again, the air temperature of the incubator was continuously adapted to maintain an EST of 37.8 °C based on the median temperature of the 6 EST sensors. From 467 h after the start of incubation (E19h11), the EST sensors were removed and the air temperature of the incubator was fixed at the current settings until pull of the hatched chicks (E21h13). Relative humidity was maintained between 50 and 65% until E4, between 50 and 60% from E4 to E7, between 50 and 55% from E7 to E10, and between 40 and 45% thereafter. Carbon dioxide was maintained below 0.35% throughout incubation.

## Hatching

From E19h11 until pull (E21h13), every 6 h the incubator was opened to check whether or not chicks had hatched. All chicks that hatched were marked with a permanent marker on the head. Six hours later, marked chicks were collected and chick quality was scored as described below. After assessing chick quality, first grade chicks were transferred to another similar incubator (HatchTech, Veenendaal, the Netherlands), where they were placed in 24 hatching baskets until pull. After pulling, all chicks were feather sexed and pooled per maternal treatment and sex.

## Broilers, Housing and Management

At each maternal age (28 and 36 wk of age), 384 female and 384 male first-grade chicks were transported for 1 h to the broiler facility (Eerde, the Netherlands) in a climate controlled truck. At the start of the experiment (d 0), the maternal treatments were randomly assigned to 64 floor pens within 4 blocks ( $n = 8$  pens per treatment) in a climate controlled room. In each pen, 6 female and 6 male broilers were placed, originating from the same maternal treatment. Broilers were marked with a unique neck tag number. At 7 d of age, 1 female and 1 male broiler were removed per pen, euthanized by cervical dislocation and stored until further analysis. At 14 d of age, 2 adjacent pens of the same maternal treatment were merged ( $n = 4$  pens per treatment). Each pen (1 m<sup>2</sup> from 0 to 14 d of age and 2 m<sup>2</sup> from 14 d of age onwards) contained wood shavings as bedding. Water and feed were supplied *ad libitum* via drinking nipples and a feeding trough, respectively. At d 0, photoperiod was 23L:1D (40 lux), which gradually changed to 18L:6D at d 3, which was maintained until slaughter. Temperature was set at 33 °C at d 0 and decreased gradually to 21 °C at d 32. Broilers were fed a standard commercial available broiler diet according to a 4-phase feeding program. A starter diet (2,925 kcal of AME<sub>n</sub>/kg, 198 g/kg CP and 11.6 g digestible lysine/kg) from d 0 to 7, a grower I diet (3,000 kcal of AME<sub>n</sub>/kg, 187 g/kg CP and 10.7 g digestible lysine/kg) from d 7 to 21, a grower II diet (3,050 kcal of AME<sub>n</sub>/kg, 180 g/kg CP and 10.0 g digestible lysine/kg) from d 21 to 28 and a finisher diet (3,100 kcal of AME<sub>n</sub>/kg, 180 g/kg CP and 9.8 g digestible lysine/kg) from d 28 to 32.

## Measurements

**Egg Weight.** Selected hatching eggs were weighed individually before storage, at start of incubation (E0) and at E18. Egg weight (EW) loss during storage was calculated as the difference between EW before storage and E0. Egg weight loss during incubation was calculated as the difference between EW at E0 and EW at E18.

**Fertility, Hatchability, Embryonic Mortality.** Clear eggs and eggs containing a dead embryo at E8 and E18 and unhatched eggs at pull were opened to determine infertility or stage of embryonic mortality. The following classifications were used: 1) unfertilized eggs showing no signs of development, 2) very early mortality (E0-E3): area vasculosa until start development black eye (< 0.5 mm), 3) early embryonic mortality (E4-E10); black eye (> 0.5 mm) until feather development, 4) mid embryonic mortality (E11-E18): small embryo with feathers, 5) late embryonic mortality (E19-E21.5): full grown embryo. Embryos showing clear deformities were noted as abnormal embryos. Fertility was calculated as a percentage of set eggs. Hatchability was calculated as a percentage of set eggs and of fertile eggs. Embryonic mortality was calculated as a percentage of fertile eggs.



**Chick Quality and Hatch Window.** From E19h11, every 6 h chick quality of just hatched chicks was determined. Chicks were classified as first or second grade. A chick was classified as first grade when it was dry, clean, free of deformities and with bright eyes. The other chicks were classified as second grade, including the chickens that died in the hatching basket after emergence from the egg shell. Second grade chicks were euthanized by cervical dislocation. Percentage first and second grade chicks were calculated relative to the total number of hatched chicks. Body weight of all chicks was determined. Hereafter, first grade chicks were scored on activity and navel, beak, and leg quality. Activity was scored as good or weak, after placing the chick on its back. If the chick returned to the standing position within 2 seconds, it was noted as good; longer than 2 seconds was noted as weak. Navel quality was scored as 0 (closed and clean navel), 1 (black button up to 2 mm or black string), or 2 (black button exceeding 2 mm) (Molenaar et al., 2010a). Beak quality was scored as 0 (normal beak), or 1 (red dot or nostrils contaminated with albumen). Leg quality was scored as 0 (normal legs, toes and hocks), 1 (red or swollen hock of 1 leg) or 2 (red or swollen hocks from both legs). Every fifth first grade chick was euthanized by cervical dislocation, followed by decapitation and the residual yolk (**RY**) was removed and weighed. In total, 10 chicks per setter tray were euthanized for determination of RY weight. Yolk-free body mass (**YFBM**) was calculated as chick weight minus RY weight. Start of hatch was determined per setter tray as the time of hatch of the first chick. The hatch window was calculated per setter tray as the time of hatch of the last chick minus the time of hatch of the first chick.

**Broiler Performance.** Broilers were weighed individually at d 0, 4, 7, 14, 21, 28 and 32 and feed intake was determined per pen on those weighing days. Average daily gain (**ADG**), average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**) were calculated between those days. Feed conversion ratio over the whole period (d 0-32) was corrected for differences in BW at day 32 (**FCRc**). Heavier birds are assumed to have a higher maintenance requirement and a higher feed intake. Therefore, the equation is based on the assumption that 0.03 FCR is equivalent to a 100 g difference in BW (Van Krimpen et al., 2019). A standard BW of 2100 g was used for calculation of FCRc:

$$FCRc = FCR + \frac{2100 - \text{actual BW d 32}}{100} * 0.03$$

**Processing yields and myopathies.** At d 32, 2 male and 2 female broilers per pen were randomly selected and weighed. Hereafter, these broilers were euthanized by a percussive blow to the head, followed by cervical dislocation. The head, skin, legs, tips of the wing (*manus*), tail, and visceral organs were removed, leaving the wet carcass to be weighed. The pectoralis major, pectoralis minor, thighs plus drums and wings were

removed and weighed separately. Slaughter yield of each of these components was calculated as a percentage of the wet carcass. The pectoralis major was scored on appearance of wooden breast and white striping (adopted from Kuttappan et al., 2016). White striping was scored as 0 (no white striations), 1 (small thin white striations <1mm) or 2 (thick white striations 1-2 mm). Wooden breast was scored as 0 (soft breast muscle), 1 (part of the breast muscle is hardened), or 2 (whole breast muscle is hardened). Prevalence of white striping or wooden breast was calculated as percentage of broilers with a score 1 or 2 of the total broilers slaughtered.

### Statistical Analysis

All continuous and binomial data were analyzed, using the Restricted Maximum Likelihood variance component analysis procedure within a generalized linear mixed model (Genstat 19<sup>th</sup> Edition, 2019). Means and model residuals were checked on homogeneity of variance prior to analyses. Not-normal distributed data (early embryonic mortality and abnormal embryos) were log transformed before analyses. None of the models included the interaction of GC or diet with breeder age, as this was confounded with season and incubator. For statistical analysis of incubation parameters, the experimental unit was setter tray. The model used for incubation parameters was:

$$Y_{ijk} = \mu + GC_i + Diet_j + GC_i \times Diet_j + Age_k + e_{ijk} [1]$$

Where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the overall mean,  $GC_i$  is the growth curve of the breeders ( $i = \text{SGC or EGC}$ ),  $Diet_j$  is the energy-to-protein ratio in the diet of the breeders ( $j = 96\%, 100\%, 104\% \text{ or } 108\% \text{ AME}_n$ ),  $GC_i \times Diet_j$  is the interaction between growth curve and diet,  $Age_k$  is age of the breeder flock ( $k = 28 \text{ or } 36 \text{ wk of age}$ ), and  $e_{ijk}$  is the residual error. Block in the incubator was added to the model as a random factor. For ADG, ADFI, FCR and FCRc model 1 was also used, without Block. Pen was considered as the experimental unit.

For analysis of chick quality at hatch, model 1 was used, added with sex and its interactions with Diet and GC:

$$Y_{ijkl} = \mu + GC_i + Diet_j + GC_i \times Diet_j + Age_k + Sex_l + GC_i \times Sex_l + Diet_j \times Sex_l + GC_i \times Diet_j \times Sex_l + e_{ijkl} [2]$$

where  $Y_{ijkl}$  is the dependent variable,  $\mu$  is the overall mean,  $GC_i$  is the growth curve of the breeders ( $i = \text{SGC or EGC}$ ),  $Diet_j$  is the energy-to-protein ratio in the diet of the breeders ( $j = 96\%, 100\%, 104\% \text{ or } 108\% \text{ AME}_n$ ),  $GC_i \times Diet_j$  is the interaction between growth curve and diet,  $Age_k$  is age of the breeder flock ( $k = 28 \text{ or } 36 \text{ wk of age}$ ),  $Sex_l$  is the sex of the chick ( $l = \text{male or female}$ ),  $GC_i \times Sex_l$  is the interaction

between growth curve and sex,  $\text{Diet}_j \times \text{Sex}_i$  is the interaction between diet and sex,  $\text{GC}_i \times \text{Diet}_j \times \text{Sex}_i$  is the interaction between growth curve, diet and sex, and  $e_{ijkl}$  is the residual error. Hatching tray was considered as the experimental unit and was added to the model as a random factor.

For broiler BW data and slaughter characteristics model 2 was used, with pen ( $n = 16$  per treatment up to d 14;  $n = 8$  per treatment after d 14) added to the model as a random factor instead of hatching tray. Pen was considered as the experimental unit. Preliminary analysis showed that interactions between GC and Sex, Diet and Sex, and between GC, Diet and Sex were not significant for any of the variables. Furthermore, pre-liminary analysis showed that inclusion of Sex in the model did not affect results of the other factors. Consequently, the factor Sex, the interaction with Sex and the random factor were excluded from the model.

Fisher adjustments were used for multiple comparisons of factorial analysis. Additionally, linear and quadratic contrasts of Diet and Diet  $\times$  GC interaction were analyzed. If linear effects of dietary energy-to-protein ratio were observed, also within GC, the slope ( $\beta$ ) is presented. If quadratic effects of dietary energy-to-protein ratio, also within GC, were observed, the estimated  $\text{AME}_n$  percentage at which the dependent variable was at the maximum (concave quadratic relation) or minimum (convex quadratic relation) was calculated and presented. Data are presented as  $\text{LSmeans} \pm \text{SEM}$ . For transformed data,  $\text{LSmeans}$  of original data are presented, combined with  $P$ -values of the transformed data. Differences were reported where  $P \leq 0.05$ .

## RESULTS

### Incubation

No interaction between breeder GC and dietary energy-to-protein ratio was observed on hatching EW and EW loss during storage and incubation (Table 1). Hatching eggs obtained from EGC breeders were 2.4 g heavier before storage, 2.4 g heavier at E0, and 2.3 g heavier at E18 than hatching eggs from SGC breeders ( $P < 0.001$ ; Table 1). A negative linear effect of an increasing breeder dietary energy-to-protein ratio was observed on hatching EW before storage ( $\beta = -0.06$  g per %  $\text{AME}_n$ ), at E0 ( $\beta = -0.06$  g per %  $\text{AME}_n$ ), and at E18 ( $\beta = -0.04$  g per %  $\text{AME}_n$ ;  $P \leq 0.03$ ; Table 1). No differences were observed between breeder GC or dietary energy-to-protein ratio on egg weight loss during storage and incubation (Table 1).

No interaction between breeder GC and dietary energy-to-protein ratio was observed on fertility, hatchability, very early (E0-E3), early (E4-E10) or mid (E11-E18)

Table 1. Average egg weight (EW) and EW loss during storage and incubation of hatching eggs obtained from broiler breeders at 2 different ages (28 and 36 wk of age), which were fed to reach one of two targeted growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>) from hatch onwards

Item		EW (g) <sup>1</sup>			EW loss % <sup>1</sup>	
		Before storage <sup>2</sup>	E0 <sup>3</sup>	E18 <sup>3</sup>	During storage	During incubation
Growth curve (n=24)						
SGC		58.1 <sup>b</sup>	57.6 <sup>b</sup>	52.3 <sup>b</sup>	0.89	9.09
EGC		60.5 <sup>a</sup>	60.0 <sup>a</sup>	54.6 <sup>a</sup>	0.87	8.98
SEM		0.1	0.1	0.1	0.03	0.05
Diet (n=12)						
96% AME <sub>n</sub>		59.6 <sup>a</sup>	59.1 <sup>a</sup>	53.6	0.92	9.15
100% AME <sub>n</sub>		59.6 <sup>a</sup>	59.1 <sup>a</sup>	53.7	0.85	9.08
104% AME <sub>n</sub>		59.0 <sup>b</sup>	58.5 <sup>b</sup>	53.2	0.89	8.94
108% AME <sub>n</sub>		59.0 <sup>b</sup>	58.5 <sup>b</sup>	53.2	0.86	8.97
SEM		0.2	0.2	0.1	0.03	0.07
Treatment (n=6)						
SGC	96% AME <sub>n</sub>	58.4	57.8	52.4	0.95	9.23
	100% AME <sub>n</sub>	58.1	57.7	52.4	0.80	9.18
	104% AME <sub>n</sub>	58.0	57.4	52.2	0.90	9.00
	108% AME <sub>n</sub>	58.0	57.4	52.3	0.89	8.96
EGC	96% AME <sub>n</sub>	60.9	60.4	54.9	0.90	9.07
	100% AME <sub>n</sub>	61.0	60.5	55.0	0.90	9.00
	104% AME <sub>n</sub>	60.1	59.6	54.3	0.87	8.87
	108% AME <sub>n</sub>	60.1	59.6	54.1	0.83	8.97
SEM		0.2	0.2	0.2	0.05	0.10
Hen age (n=24)						
28 weeks		55.6 <sup>b</sup>	55.1 <sup>b</sup>	50.2 <sup>b</sup>	0.94 <sup>a</sup>	8.74 <sup>b</sup>
36 weeks		63.1 <sup>a</sup>	62.5 <sup>a</sup>	56.7 <sup>a</sup>	0.82 <sup>b</sup>	9.33 <sup>a</sup>
SEM		0.12	0.11	0.04	0.03	0.04
P-value						
Growth curve (GC)		<0.001	<0.001	<0.001	0.68	0.10
Diet (factorial)		0.003	0.004	0.08	0.31	0.12
Diet (linear)		<0.001	0.001	0.03	0.28	0.23
Diet (quadratic)		0.89	0.98	0.84	0.42	0.37
GC x Diet (factorial)		0.16	0.24	0.25	0.22	0.73
GC x Diet (linear)		0.12	0.130	0.09	0.59	0.55
GC x Diet (quadratic)		0.55	0.66	0.53	0.16	0.12
Hen age		<0.001	<0.001	<0.001	<0.001	0.001

<sup>ab</sup> LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>EW = egg weight; EW loss during storage (10 to 12 days) = (EW before storage – EW E0)/(EW before storage)\*100%; EW loss during incubation = (EW E0 – EW E18)/(EW E0)\*100%.

<sup>2</sup>Per replicate 60 hatching eggs of 3 consecutive days (20 hatching eggs per day) were selected and stored at 17 °C for 10 to 12 days before incubation.

<sup>3</sup>Embryonic day (E).

embryonic mortality, start of hatch, hatch window or percentage of second grade chicks (Table 2). Increasing dietary energy-to-protein ratio linearly reduced embryonic mortality within embryos from SGC breeders ( $\beta = -0.3$  % per % AME<sub>n</sub>), but not in embryos from EGC breeders ( $\beta = 0.1$  % per % AME<sub>n</sub>;  $P = 0.03$ ; Table 2). This occurred mainly during the last 3 days of incubation ( $P = 0.05$ ; Table 2). Very early (E0-E3) embryonic mortality was not affected by breeder GC, but there was a linear effect of breeder dietary energy-to-protein ratio. An increase in breeder dietary energy-to-protein ratio resulted in a linear decrease in very early embryonic mortality ( $\beta = -0.2$  % per % AME<sub>n</sub>;  $P = 0.05$ ; Table 2). No effect of breeder GC or dietary energy-to-protein ratio was observed on fertility, hatchability, early or mid-embryonic mortality, start of hatch, hatch window or percentage of second grade chicks (Table 2).

### Chick Quality

In total 2,598 first grade chicks hatched and were scored on chick quality, of which 480 chicks were dissected for RY and YFBM weight. No interaction between breeder GC and dietary energy-to-protein ratio was observed on hatchling weight, RY weight, YFBM, activity or beak score (Table 3). Increasing dietary energy-to-protein ratio linearly increased percentage of chicks with navel score 1 within chicks from SGC breeders ( $\beta = 0.4$  % per % AME<sub>n</sub>), but not in chicks from EGC breeders ( $\beta = -0.5$  % per % AME<sub>n</sub>;  $P = 0.03$ ; Table 3). A quadratic interaction between breeder GC and dietary energy-to-protein ratio on percentage of chicks with leg score 1 was observed ( $P = 0.04$ ; Table 3). The lowest percentage of chicks with leg score 1 was estimated at 103% AME<sub>n</sub> ( $\Delta_{\max} = -8.8\%$ ) for chicks obtained from SGC breeders, whereas this was estimated at 103% AME<sub>n</sub> ( $\Delta_{\max} = 8.0\%$ ) for chicks from EGC breeders. Percentage of chicks with leg score 2 did not differ between treatments (Table 3).

Hatchlings obtained from EGC breeders were 1.9 g heavier, with a 0.6 g heavier RY and 1.2 g heavier YFBM, compared to hatchlings from SGC breeders ( $P < 0.001$ ; Table 3). An increase in breeder dietary energy-to-protein ratio resulted in a linear decrease in hatchling weight ( $\beta = -0.1$  g per % AME<sub>n</sub>;  $P = 0.001$ ), but did not affect RY weight or YFBM (Table 3). Percentage of chicks with navel score 2 was 3.8 % higher in chicks from EGC breeders, compared to SGC breeders ( $P = 0.04$ ; Table 3). Breeder dietary energy-to-protein ratio did not affect chick navel quality.

### Broiler Performance

A linear interaction between breeder GC and dietary energy-to-protein ratio was observed on broiler BW at 0, 4, 7, 14 and 21 d of age ( $P < 0.05$ ; Table 4). At 0 and 4 d of age, broiler BW decreased with an increasing breeder dietary energy-to-protein ratio, but this was more profound in broilers from EGC breeders ( $\beta = -0.19$  g per %

Table 2. Fertility, hatchability, embryonic mortality, start of hatch, hatch window and percentage second grade chicks obtained from broiler breeders at 2 different ages (28 and 36 wk of age), which were fed to reach one of two targeted growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>) from hatch onwards

Item	Fertility of set eggs (%)	Hatch. of set eggs (%)	Hatch. of fertile eggs (%)	Embryonic mortality (% of fertile eggs)					Start hatch (h)	Hatch window (h)	Second grade chicks (% of total chicks)	
				E0-E3 <sup>1</sup>	E4-E10	E11-E17	E18-E21.5	E0-E21.5				Abnormal
Growth curve (n=24)												
SGC	98.7	91.9	93.1	3.5	0.8	0.3	1.4	6.1	485.5	24.8	0.8	
EGC	97.9	91.6	93.6	3.1	0.8	0.5	1.7	6.0	485.5	26.3	0.6	
SEM	0.4	0.8	0.6	0.5	0.3	0.1	0.4	0.6	0.7	0.8	0.2	
Diet (n=12)												
96% AME <sub>n</sub>	98.2	91.5	93.2	4.1	0.7	0.3	1.3	6.4	486.5	24.0	0.3	
100% AME <sub>n</sub>	98.7	90.9	92.3	3.7	0.9	0.3	1.8	6.8	485.0	24.5	0.5	
104% AME <sub>n</sub>	98.2	91.8	93.5	3.1	0.8	0.3	2.0	6.2	485.0	27.0	1.1	
108% AME <sub>n</sub>	98.2	92.8	94.5	2.3	0.6	0.7	1.1	4.7	485.5	26.5	0.9	
SEM	0.6	1.1	0.9	0.7	0.4	0.2	0.5	0.8	1.0	1.2	0.3	
Treatment (n=6)												
SGC	98.1	90.3	92.1	4.5	0.9	0.3	1.7	7.4	486.0	23.0	0.3	
96% AME <sub>n</sub>												
100% AME <sub>n</sub>	99.4	90.8	91.3	4.5	0.8	0.3	2.0	7.6	484.0	25.0	0.6	
104% AME <sub>n</sub>	98.1	91.9	93.8	3.1	1.1	0.3	1.4	5.9	486.0	27.0	1.5	
108% AME <sub>n</sub>	99.2	94.4	95.2	2.0	0.3	0.6	0.6	3.3	486.0	24.0	0.9	
EGC	98.3	92.7	94.3	3.7	0.6	0.3	0.9	5.4	487.0	25.0	0.3	
96% AME <sub>n</sub>												
100% AME <sub>n</sub>	97.9	91.1	93.3	2.9	1.1	0.4	1.6	6.0	486.0	24.0	0.5	
104% AME <sub>n</sub>	98.3	91.6	93.1	3.1	0.6	0.3	2.6	6.5	484.0	27.0	0.6	
108% AME <sub>n</sub>	97.2	91.1	93.7	2.6	0.9	0.9	1.7	6.0	485.0	29.0	0.9	
SEM	0.8	1.6	1.3	1.0	0.6	0.3	0.7	1.2	1.3	1.7	0.5	

Table 2. Continued

Item	Fertility of set eggs (%)	Hatch. of set eggs (%)	Hatch. of fertile eggs (%)	Embryonic mortality (% of fertile eggs)					Start hatch (h)	Hatch window (h)	Second grade chicks (% of total chicks)	
				E0-E3 <sup>1</sup>	E4-E10	E11-E17	E18-E21.5	E0-E21.5				Abnormal
Hen age (n=24)												
28 weeks	98.7	91.4	92.6	3.9	0.7	0.2	2.0	6.9	485.8	25.3	0.8	
36 weeks	97.9	92.1	94.1	2.6	0.8	0.6	1.0	5.1	485.2	25.8	0.6	
SEM	0.4	0.7	0.6	0.5	0.3	0.1	0.5	0.6	0.8	0.7	0.2	
P-value												
Growth curve (GC)	0.23	0.81	0.60	0.59	0.87	0.68	0.50	1.00	1.00	0.22	0.37	
Diet (factorial)	0.86	0.65	0.30	0.26	0.87	0.45	0.41	0.23	0.66	0.24	0.27	
Diet (linear)	0.83	0.32	0.17	0.05	0.54	0.21	0.90	0.10	0.49	0.07	0.09	
Diet (quadratic)	0.63	0.44	0.23	0.68	0.60	0.34	0.10	0.19	0.30	0.68	0.50	
GC x Diet (factorial)	0.38	0.29	0.31	0.74	0.77	0.96	0.23	0.16	0.45	0.32	0.70	
GC x Diet (linear)	0.34	0.06	0.07	0.34	0.54	0.66	0.05	0.03	0.25	0.36	0.82	
GC x Diet (quadratic)	0.82	0.85	0.88	0.67	0.94	0.76	0.74	0.63	1.00	0.10	0.35	
Hen age	0.23	0.52	0.15	0.13	0.51	0.11	0.23	0.09	0.67	0.66	0.67	

<sup>1</sup>Embryonic day (E).

Table 3. Hatchling weight (HW), residual yolk (RY) weight, yolk-free body mass (YFBM), activity, navel, beak and leg score of chicks obtained from broiler breeders at 2 different ages (28 and 36 wk of age), which were fed to reach one of two targeted growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from hatch onwards

		HW (g)	RY (g)	YFBM (g)	Activity <sup>1</sup> (%)	Navel score <sup>2</sup> (%)		Beak <sup>3</sup> (%)	Leg score <sup>4</sup> (%)		
Item						Score 1	Score 2		Score 1	Score 2	
Growth curve (n=24)											
	SGC	41.9 <sup>b</sup>	5.1 <sup>b</sup>	36.9 <sup>b</sup>	11.1	43.8	12.5 <sup>b</sup>	11.0	23.7	8.2	
	EGC	43.8 <sup>a</sup>	5.7 <sup>a</sup>	38.1 <sup>a</sup>	12.5	44.0	16.2 <sup>a</sup>	9.6	21.9	8.4	
	SEM	0.1	0.1	0.1	0.9	0.9	1.4	1.1	1.5	1.1	
Diet (n=12)											
	96% AME <sub>n</sub>	43.1 <sup>a</sup>	5.3	37.5	13.0	46.0	14.2	11.1	23.3	8.6	
	100% AME <sub>n</sub>	43.1 <sup>a</sup>	5.5	37.8	11.8	41.2	15.1	10.2	20.7	7.4	
	104% AME <sub>n</sub>	42.7 <sup>b</sup>	5.4	37.3	12.3	44.1	13.1	9.6	25.1	8.2	
	108% AME <sub>n</sub>	42.6 <sup>b</sup>	5.3	37.3	10.1	44.1	15.2	10.3	22.1	8.9	
	SEM	0.1	0.1	0.2	1.5	1.5	1.8	1.4	2.1	1.5	
Treatment (n=6)											
	SGC	96% AME <sub>n</sub>	42.1	5.1	37.0	13.1	41.6 <sup>b</sup>	13.6	10.6	26.3	7.9
		100% AME <sub>n</sub>	42.0	5.2	36.9	8.9	42.9 <sup>b</sup>	11.8	11.1	17.5	8.6
		104% AME <sub>n</sub>	41.8	5.2	36.9	13.9	44.6 <sup>ab</sup>	10.1	8.9	22.0	5.9
		108% AME <sub>n</sub>	41.8	5.0	36.6	8.4	45.9 <sup>ab</sup>	14.6	13.2	21.8	10.2
	EGC	96% AME <sub>n</sub>	44.2	5.6	38.1	12.8	50.5 <sup>a</sup>	14.7	11.6	20.3	9.3
		100% AME <sub>n</sub>	44.2	5.7	38.7	14.7	39.5 <sup>b</sup>	18.4	9.3	23.8	6.3
		104% AME <sub>n</sub>	43.5	5.7	37.6	10.8	43.5 <sup>b</sup>	16.1	10.2	28.3	10.6
		108% AME <sub>n</sub>	43.4	5.6	38.1	11.7	42.3 <sup>b</sup>	15.8	7.3	22.5	7.7
	SEM	0.2	0.2	0.2	2.2	2.2	2.5	2.0	2.9	2.1	
Hen age (n=24)											
	28 weeks	40.0 <sup>b</sup>	4.8 <sup>b</sup>	35.1 <sup>b</sup>	10.6	48.9 <sup>a</sup>	9.5 <sup>b</sup>	7.1 <sup>b</sup>	15.4 <sup>b</sup>	7.8	
	36 weeks	45.7 <sup>a</sup>	6.0 <sup>a</sup>	39.9 <sup>a</sup>	12.9	38.8 <sup>b</sup>	19.3 <sup>a</sup>	13.4 <sup>a</sup>	30.2 <sup>a</sup>	8.8	
	SEM	0.1	0.1	0.1	0.6	0.5	1.5	1.1	1.4	1.1	
P-value											
	Growth curve (GC)	<0.001	<0.001	<0.001	0.38	0.91	0.04	0.33	0.39	0.85	
	Diet (factorial)	0.007	0.75	0.11	0.61	0.24	0.82	0.89	0.50	0.90	
	Diet (linear)	0.001	0.83	0.17	0.28	0.71	0.89	0.62	0.92	0.79	
	Diet (quadratic)	0.76	0.27	0.62	0.75	0.16	0.74	0.56	0.93	0.54	
	GC x Diet (factorial)	0.29	0.99	0.13	0.23	0.03	0.53	0.25	0.14	0.26	
	GC x Diet (linear)	0.12	0.83	0.77	0.92	0.03	0.98	0.16	0.30	0.73	
	GC x Diet (quadratic)	0.53	0.79	0.86	0.97	0.15	0.14	0.45	0.04	0.57	
	Hen age	<0.001	0.004	<0.001	0.07	<0.001	0.009	0.02	0.002	0.56	

<sup>ab</sup> LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Percentage of chicks scored as weak.

<sup>2</sup>Percentage of chicks with a score 1 (black button up to 2 mm or black string) or a score 2 (black button exceeding 2 mm or open navel).

<sup>3</sup>Percentage of chicks with a red dot on beak or nostrils contaminated with albumen.

<sup>4</sup>Percentage of chicks with a score 1 (red or swollen hock of 1 leg) or a score 2 (red or swollen hocks from both legs).



AME<sub>n</sub> on average) than from SGC breeders ( $\beta = -0.03$  g per % AME<sub>n</sub> on average). At 7, 14 and 21 d of age, broiler BW increased linearly with an increasing dietary energy-to-protein ratio within broilers from SGC breeders ( $\beta = 1.2$  g per % AME<sub>n</sub> on average), whereas this was not observed within broilers from EGC breeders ( $\beta = -0.3$  g per % AME<sub>n</sub> on average). At 28 and 32 d of age, this interaction was not observed anymore, but broilers obtained from EGC breeders were 33 g ( $P < 0.001$ ) and 36 g ( $P = 0.001$ ) heavier, respectively, than broilers from SGC breeders (Table 4). Furthermore, at 28 and 32 d of age, an increase in breeder dietary energy-to-protein ratio resulted in a linear increase in broiler BW ( $\beta = 2.3$  g per % AME<sub>n</sub> and  $\beta = 3.2$  g per % AME<sub>n</sub>;  $P = 0.02$  and  $P = 0.007$ , respectively).

Weekly broiler ADG, ADFI and FCR can be found in supplementary Table S1. No interaction was observed on ADG, ADFI or FCR over the whole period (0 – 32 d of age; Table 5). Broilers originating from EGC breeders had an 1.0 g/d higher ADG and 1.5 g/d higher ADFI over the whole period, compared to broilers originating from SGC breeders ( $P \leq 0.006$ ; Table 5). This was mainly due to a higher ADG and ADFI observed in the first 21 d of age (Supplementary Table S1). FCR did not differ between broilers from EGC and SGC breeders.

An increase in breeder dietary energy-to-protein ratio resulted in a linear increase in ADG over the whole period ( $\beta = 0.1$  g/d per % AME<sub>n</sub>;  $P = 0.004$ ; Table 5). This was mainly due to a linear increase in ADG from 7 to 14 d of age ( $\beta = 4.4$  g/d per % AME<sub>n</sub>;  $P = 0.03$ ) and a quadratic relation with the highest ADG estimated at 103% AME<sub>n</sub> ( $\Delta_{\max} = 2.2$  g/d;  $P = 0.04$ ) from 14 to 21 d of age (Supplementary Table S1). An increase in breeder dietary energy-to-protein ratio resulted in a linear increase in ADFI over the whole period ( $\beta = 0.1$  g/d per % AME<sub>n</sub>;  $P = 0.02$ ; Table 5). This was mainly due to a linear increase in ADFI from 14 to 21 d of age ( $\beta = 0.2$  g/d per % AME<sub>n</sub>;  $P = 0.04$ ) and from 21 to 28 d of age ( $\beta = 0.3$  g/d per % AME<sub>n</sub>;  $P = 0.02$ ; Supplementary Table S1). FCR did not differ between dietary energy-to-protein ratio. FCR<sub>c</sub>, however, decreased linearly with an increasing dietary energy-to-protein ratio ( $\beta = -0.002$  per % AME<sub>n</sub>;  $P = 0.002$ ; Table 5).

## Slaughter Characteristics

A linear interaction between breeder GC and dietary energy-to-protein ratio was observed on carcass yield percentage ( $P = 0.02$ ) and thighs plus drums as percentage of the carcass ( $P < 0.001$ ; Table 6). Carcass yield percentage increased linearly with an increasing breeder dietary energy-to-protein ratio in broilers from SGC breeders ( $\beta = 0.08$  % per % AME<sub>n</sub>), whereas it decreased linearly in broilers from EGC breeders ( $\beta = -0.09$  % per % AME<sub>n</sub>). Thighs plus drums as percentage of the carcass decreased linearly with an increasing breeder dietary energy-to-protein ratio in broilers from

Table 4. BW at different ages of broilers obtained from broiler breeders at 2 different ages (28 and 36 wk of age), which were fed to reach one of two targeted growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from hatch onwards

Item		BW <sup>1</sup> (g)						
		0 d	4 d	7 d	14 d	21 d	28 d	32 d
Growth curve								
SGC		39.3	96.8 <sup>b</sup>	158.2	453.9 <sup>b</sup>	935.9 <sup>b</sup>	1,632 <sup>b</sup>	2,090 <sup>b</sup>
EGC		41.7	101.7 <sup>a</sup>	164.9	468.1 <sup>a</sup>	957.9 <sup>a</sup>	1,665 <sup>a</sup>	2,126 <sup>a</sup>
SEM		0.1	0.3	0.5	1.7	3.4	6	7
Diet								
96% AME <sub>n</sub>		40.9	100.3 <sup>a</sup>	163.2	458.7 <sup>b</sup>	934.0 <sup>b</sup>	1,627 <sup>b</sup>	2,077 <sup>b</sup>
100% AME <sub>n</sub>		40.7	99.1 <sup>ab</sup>	160.8	458.1 <sup>b</sup>	947.8 <sup>a</sup>	1,650 <sup>a</sup>	2,115 <sup>a</sup>
104% AME <sub>n</sub>		40.2	99.3 <sup>ab</sup>	161.4	467.6 <sup>a</sup>	959.3 <sup>a</sup>	1,666 <sup>a</sup>	2,124 <sup>a</sup>
108% AME <sub>n</sub>		40.2	98.3 <sup>b</sup>	160.7	459.8 <sup>b</sup>	946.4 <sup>ab</sup>	1,652 <sup>a</sup>	2,117 <sup>a</sup>
SEM		0.1	0.4	0.7	2.3	4.8	8	11
Treatment								
SGC	96% AME <sub>n</sub>	39.6 <sup>c</sup>	96.9	158.7 <sup>c</sup>	449.3	916.9	1,603	2,052
	100% AME <sub>n</sub>	39.3 <sup>cd</sup>	96.7	156.9 <sup>c</sup>	447.4	930.2	1,628	2,089
	104% AME <sub>n</sub>	39.1 <sup>d</sup>	97.1	158.1 <sup>c</sup>	462.1	952.8	1,656	2,112
	108% AME <sub>n</sub>	39.3 <sup>cd</sup>	96.5	159.1 <sup>c</sup>	456.8	943.5	1,642	2,108
EGC	96% AME <sub>n</sub>	42.2 <sup>a</sup>	103.7	167.8 <sup>a</sup>	468.1	951.1	1,650	2,101
	100% AME <sub>n</sub>	42.0 <sup>a</sup>	101.5	164.7 <sup>b</sup>	468.6	965.4	1,672	2,141
	104% AME <sub>n</sub>	41.3 <sup>b</sup>	101.5	164.6 <sup>b</sup>	473.2	965.8	1,677	2,135
	108% AME <sub>n</sub>	41.1 <sup>b</sup>	100.1	162.4 <sup>b</sup>	462.7	949.3	1,662	2,126
SEM		0.2	0.6	1.0	3.3	6.8	12	15
Hen age								
28 weeks		37.8 <sup>b</sup>	97.4 <sup>b</sup>	156.9 <sup>b</sup>	450.1 <sup>b</sup>	923.6 <sup>b</sup>	1,618 <sup>b</sup>	2,074 <sup>b</sup>
36 weeks		43.2 <sup>a</sup>	101.1 <sup>a</sup>	166.2 <sup>a</sup>	472.0 <sup>a</sup>	970.1 <sup>a</sup>	1,679 <sup>a</sup>	2,142 <sup>a</sup>
SEM		0.1	0.3	0.5	1.7	3.4	6	7
P-value								
Growth curve (GC)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
Diet (factorial)		<0.001	0.02	0.06	0.02	0.006	0.02	0.02
Diet (linear)		<0.001	0.003	0.03	0.24	0.03	0.02	0.007
Diet (quadratic)		0.36	0.83	0.23	0.14	0.008	0.03	0.04
GC x Diet (factorial)		0.02	0.08	0.04	0.09	0.08	0.51	0.58
GC x Diet (linear)		0.004	0.02	0.005	0.03	0.02	0.16	0.20
GC x Diet (quadratic)		0.23	0.57	0.50	0.44	0.67	0.98	0.83
Hen age		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

<sup>a-d</sup> LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>At 14 d of age, 2 adjacent pens from the same treatment were merged. n = 16 per treatment for d 0, 4, 7, and 14, and n = 8 per treatment for d 21, 28, and 32.

Table 5. Average daily gain (ADG; g/d), average daily feed intake (ADFI; g/d) and feed conversion ratios (FCR; kg of feed/kg of BW gain) of broilers from 0 to 32 d of age, obtained from broiler breeders at 2 different ages (28 and 36 wk of age), which were fed to reach one of two targeted growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from hatch onwards

Item		d 0-32			
		ADG	ADFI	FCR	FCR <sub>c</sub> <sup>1</sup>
Growth curve (n = 32)					
SGC		64.1 <sup>b</sup>	89.5 <sup>b</sup>	1.40	1.40
EGC		65.1 <sup>a</sup>	91.0 <sup>a</sup>	1.40	1.39
SEM		0.2	0.4	0.01	0.01
Diet (n = 16)					
96% AME <sub>n</sub>		63.6 <sup>b</sup>	89.0 <sup>b</sup>	1.40	1.42 <sup>a</sup>
100% AME <sub>n</sub>		64.9 <sup>a</sup>	90.3 <sup>ab</sup>	1.39	1.39 <sup>b</sup>
104% AME <sub>n</sub>		65.1 <sup>a</sup>	91.0 <sup>a</sup>	1.40	1.39 <sup>b</sup>
108% AME <sub>n</sub>		64.9 <sup>a</sup>	90.7 <sup>a</sup>	1.39	1.39 <sup>b</sup>
SEM		0.3	0.5	0.01	0.01
Treatment (n = 8)					
SGC	96% AME <sub>n</sub>	62.9	87.9	1.40	1.43
	100% AME <sub>n</sub>	64.1	89.2	1.40	1.40
	104% AME <sub>n</sub>	64.8	90.6	1.40	1.39
	108% AME <sub>n</sub>	64.7	90.4	1.39	1.39
EGC	96% AME <sub>n</sub>	64.3	90.2	1.40	1.41
	100% AME <sub>n</sub>	65.6	91.5	1.39	1.38
	104% AME <sub>n</sub>	65.4	91.3	1.41	1.40
	108% AME <sub>n</sub>	65.2	90.9	1.40	1.38
SEM		0.5	0.7	0.01	0.01
Hen age (n = 32)					
28 weeks		63.7 <sup>b</sup>	87.6 <sup>b</sup>	1.36 <sup>b</sup>	1.37 <sup>b</sup>
36 weeks		65.6 <sup>a</sup>	92.9 <sup>a</sup>	1.43 <sup>a</sup>	1.42 <sup>a</sup>
SEM		0.2	0.4	0.01	0.01
P-value					
Growth curve (GC)		0.003	0.006	0.33	0.18
Diet (factorial)		0.006	0.05	0.14	0.008
Diet (linear)		0.004	0.02	0.21	0.002
Diet (quadratic)		0.03	0.12	0.78	0.28
GC x Diet (factorial)		0.55	0.41	0.62	0.48
GC x Diet (linear)		0.19	0.12	0.62	0.25
GC x Diet (quadratic)		0.84	0.98	0.94	0.53
Hen age		<0.001	<0.001	<0.001	<0.001

<sup>ab</sup> LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Corrected FCR to a standard BW of 2100 g, calculated as  $FCR - (2100 - \text{actual BW d 32})/100 \times 0.03$ .

Table 6. Carcass yields and prevalence of breast myopathies of broilers at slaughter age (32 d of age) obtained from broiler breeders at 2 different ages (28 and 36 wk of age), which were fed to reach one of two targeted growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from hatch onwards

Item	BW <sup>1</sup> (g)	Carcass yield (% of BW)	Pectoralis major (% carcass)	Pectoralis minor (% carcass)	Thighs+drums (% carcass)	Wings (% carcass)	Wooden breast (%) <sup>2</sup>	White striping (%) <sup>3</sup>
Growth curve (n=64)								
SGC	2,090	64.0	26.1	4.4	28.5	8.8	24.5	22.9
EGC	2,097	64.5	26.2	4.4	28.4	8.9	21.1	28.9
SEM	13	0.2	0.2	0.1	0.1	0.1	3.9	4.3
Diet (n=32)								
96% AME <sub>n</sub>	2,082	64.2	26.1	4.4	28.4	8.9	18.8	25.0
100% AME <sub>n</sub>	2,095	64.3	26.2	4.3	28.4	8.8	22.4	27.1
104% AME <sub>n</sub>	2,092	64.2	26.1	4.5	28.3	8.8	25.0	23.4
108% AME <sub>n</sub>	2,105	64.2	26.2	4.3	28.6	8.7	25.0	28.1
SEM	18	0.3	0.2	0.1	0.2	0.1	5.5	6.2
Treatment (n=16)								
SGC 96% AME <sub>n</sub>	2,059	63.3	25.6	4.4	29.0 <sup>a</sup>	8.9	15.6	9.4
100% AME <sub>n</sub>	2,096	64.1	26.2	4.3	28.4 <sup>abc</sup>	8.8	22.9	22.9
104% AME <sub>n</sub>	2,098	64.1	26.2	4.6	28.2 <sup>c</sup>	8.7	31.3	28.1
108% AME <sub>n</sub>	2,107	64.4	26.4	4.3	28.3 <sup>bc</sup>	8.6	28.1	31.3
EGC 96% AME <sub>n</sub>	2,106	65.1	26.6	4.4	27.9 <sup>c</sup>	8.8	21.9	40.6
100% AME <sub>n</sub>	2,094	64.6	26.1	4.4	28.3 <sup>abc</sup>	8.9	21.9	31.3
104% AME <sub>n</sub>	2,086	64.2	26.0	4.4	28.5 <sup>abc</sup>	8.9	18.8	18.8
108% AME <sub>n</sub>	2,102	64.0	26.1	4.3	28.9 <sup>ab</sup>	8.8	21.9	25.0
SEM	26	0.4	0.3	0.1	0.2	0.1	7.9	8.7
Hen age (n=64)								
28 weeks	2,083	64.1	26.4	4.4	28.2 <sup>b</sup>	9.1 <sup>a</sup>	13.5 <sup>b</sup>	20.6
36 weeks	2,104	64.3	25.9	4.4	28.7 <sup>a</sup>	8.5 <sup>b</sup>	32.0 <sup>a</sup>	31.3
SEM	13	0.2	0.2	0.1	0.1	0.1	3.9	4.3
P-value								
Growth curve (GC)	0.70	0.11	0.67	0.47	0.76	0.36	0.55	0.34
Diet (factorial)	0.86	0.99	0.97	0.16	0.66	0.55	0.84	0.95
Diet (linear)	0.44	0.97	0.77	0.96	0.56	0.18	0.39	0.84
Diet (quadratic)	1.00	0.92	0.86	0.47	0.29	0.60	0.74	0.84
GC x Diet (factorial)	0.66	0.09	0.23	0.43	0.005	0.71	0.67	0.10
GC x Diet (linear)	0.31	0.02	0.07	0.63	<0.001	0.30	0.32	0.02
GC x Diet (quadratic)	0.44	0.55	0.31	0.88	0.30	0.67	0.54	0.29
Hen age	0.27	0.55	0.09	1.00	0.008	<0.001	0.01	0.09

<sup>a-c</sup> LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup> Average BW of randomly selected broilers for slaughter (per pen 2 male and 2 female broilers).

<sup>2</sup> Percentage of broilers with score 1 (part of breast muscle is hardened) or score 2 (whole breast muscle is hardened) wooden breast.

<sup>3</sup> Percentage of broilers with score 1 (small white lines <1 mm) or score 2 (large white lines 1-2 mm) white striping.

SGC breeders ( $\beta = -0.06$  % per % AME<sub>n</sub>), whereas it increased linearly in broilers from EGC breeders ( $\beta = 0.08$  % per % AME<sub>n</sub>). No effect of treatments was observed on pectoralis major, pectoralis minor or leg percentage, nor on prevalence of wooden breast (Table 6). A linear interaction was observed on prevalence of white striping ( $P = 0.02$ ; Table 6). Prevalence of white striping increased linearly with an increasing breeder dietary energy-to-protein ratio in broilers from SGC breeders ( $\beta = 1.8$  % per % AME<sub>n</sub>), whereas it decreased linearly in broilers from EGC breeders ( $\beta = -1.5$  % per % AME<sub>n</sub>).

## DISCUSSION

The objective of this study was to evaluate effects of growth curve and dietary energy-to-protein ratio of broiler breeder hens on offspring quality and performance.

### Breeder Growth Curve

In the current study, hatching eggs were selected based on average EW per treatment. Selected hatching eggs from EGC breeders were heavier than from SGC breeders, due to a higher average EW for EGC breeders (Heijmans et al., 2021). A higher EW is probably due to a higher feed allowance of EGC breeders, compared to SGC breeders, which has been discussed previously by Heijmans et al. (2021). Although eggs were heavier from EGC breeders, no difference was observed in relative EW loss during incubation between GC. Egg weight loss is mainly determined by water loss through the eggshell during incubation and is optimal between 6.5 and 14.0 % (Molenaar et al., 2010b). Egg weight loss during incubation is determined by water vapor pressure differences between the egg and its surrounding, which was similar for all eggs in the current study, and eggshell characteristics, such as eggshell thickness, number of pores or membrane characteristics (Molenaar et al., 2010b). It can be speculated that these characteristics were similar for eggs from different GC, as relative EW loss did not differ between eggs from different GC. To our knowledge, no studies are available on the impact of breeder GC on eggshell characteristics.

Broiler breeders are commonly fed restricted quantities of feed to control BW development and ensure reproductive performance (Robinson et al., 1991; Bruggeman et al., 1999; Hocking et al., 2002; Sun et al., 2006). In the current study, no effect of GC was observed on fertility, hatchability or embryonic mortality for breeders at 28 and 36 wk of age. Other studies also did not observe an effect of a higher breeder GC, compared to breeder recommendations, during rearing (28 to 200% higher; Hocking et al., 2002; Zuidhof et al., 2007) or during production (14% higher; Hocking et al., 2002) on fertility or hatchability. Van Emous et al. (2015a) did observe a 3.5% higher

fertility and a 2.3% lower embryonic mortality at 29 wk of age of a 7.5% higher GC during rearing, but these carry-over effects disappeared at a later age (33 and 37 wk of age). In contrast to our study, other studies observed a 22% lower fertility (Hocking et al., 2002) or a 2.2 to 7.9% lower hatchability (Renema et al., 2001; Hocking et al., 2002) when breeders were on a higher GC (8% higher; Renema et al., 2001) or fed *ad libitum* (Hocking et al., 2002) during rearing and production. The latter studies, however, were performed over 20 years ago and might not be applicable to modern broiler breeders. The current results suggest that feed restriction level might be reduced in modern broiler breeders without negative effects on reproductive performance.

Breeder GC did not impact chick quality scores, except for navel score. Chicks from EGC breeders had a higher prevalence of navels with black buttons (> 2 mm; score 2), than chicks from SGC breeders. Elevated growth curve breeders produced heavier eggs with larger yolk, which resulted in relative and absolute larger RY at hatch. Embryos might have had more difficulties to insert the larger remaining yolk properly into the body, leading to a poorer navel closure, as observed by Molenaar et al. (2010a). A poorer navel closure is an indicator for a lower chick quality and might result in a lower post hatch performance and a higher mortality (Fasenko and O'Dea, 2008). However, this was not observed in the current study. Chicks from EGC breeders even had a better post hatch performance than chicks from SGC breeders in the current study, suggesting that other factors outbalanced the potential negative effect of navel closure on post hatch performance.

An 1.9 g heavier hatchling from EGC breeders compared to SGC breeders seems consequential to a 2.4 g larger hatching egg (Ulmer-Franco et al., 2010; Nangsuay et al., 2011; Iqbal et al., 2017). A heavier day-old chick, more specifically a heavier YFBM, is an indicator for better chick quality (reviewed by Narinç and Aydemir, 2021) and a predictor for slaughter weight (Willemsen et al., 2008). In the current study, chicks from EGC breeders maintained a higher BW up to slaughter age compared to chicks from SGC breeders, due to an 1.0 g/d higher ADG and 1.5 g/d higher ADFI. Bowling et al. (2018) also observed a higher growth, leading to a higher slaughter weight, of offspring from heavier breeders (+15% BW compared to standard), although exact growth and slaughter weight numbers are not reported in this study. In other studies, a 2.5 to 22.5% higher BW of breeders during the rearing and laying phase had no effect on hatchling weight or BW gain of the offspring (Afrouziyeh et al., 2021; Zukiwsky et al., 2021a). In these studies, however, the authors also did not observe an effect of breeder BW on EW (Afrouziyeh et al., 2021; Zukiwsky et al., 2021b). Discrepancy between these studies and the current study might be due to feeding frequency. In the studies of Afrouziyeh et al. (2021) and Zukiwsky et al. (2021a;b) breeders were fed continuously during the day with a precision feeding system, whereas in the cur-

rent study breeders were fed once a day. It has been shown that continuous feeding, compared to once a day feeding, can induce metabolic changes (Van der Klein et al., 2018; Zuidhof, 2018). In turn, it has been proposed that metabolic status plays an important role in reproduction (Bédécarrats et al., 2016; Van der Klein et al., 2020), although mechanisms are not fully elucidated yet (Bédécarrats et al., 2016; Van der Klein et al., 2020).

### Breeder Dietary Energy-to-Protein Ratio

In order to achieve a similar BW, feed allocation decreased with an increasing dietary energy-to-protein ratio (Heijmans et al., 2021). With an increasing dietary energy-to-protein ratio CP intake decreased up to 14.5% and energy intake increased up to 2.3% during rearing and production (Heijmans et al., 2021). Increased breeder dietary energy-to-protein ratio decreased size of selected hatching eggs. This is most probably due to a decreasing total CP intake when dietary energy-to-protein ratio increases (Heijmans et al., 2021). No effect of dietary energy-to-protein ratio was observed on EW loss. As discussed before, this suggests eggshell characteristics, such as eggshell thickness, number of pores or membrane characteristics (Molenaar et al., 2010b) are not affected by dietary energy-to-protein ratio.

No effect of dietary energy-to-protein ratio was observed on fertility. This is in line with other studies, where authors also did not find an effect of 1.0 to 5.4% reduction in dietary CP intake during rearing (Hocking et al., 2002; Van Emous et al., 2015a; b), or 9.6 to 17.5% reduction in dietary CP intake during production (Mohiti-Asli et al., 2012; Van Emous et al., 2018), or 1.0% higher or 2.0% lower dietary energy intake during production (Van Emous et al., 2015b) on fertility. Ekmay et al. (2013) observed an effect of specifically lysine and isoleucine intake on fertility. An oversupply of either of these amino acids resulted in a decreased fertility, probably due to an increase in pH around the sperm storage tubules of the breeder hen (Ekmay et al., 2013). Lesuisse et al. (2017) observed a 14.5% lower fertility when dietary CP intake was severely reduced with 22.8% during rearing and production. These results suggest that dietary energy-to-protein ratio does not affect fertility, as long as diets have a balanced amino acid profile and CP intake is not that severely reduced (maximal 17.5%) compared to breeder recommendations.

Different breeder dietary energy-to-protein ratios resulted in a similar hatchability of fertile eggs. Several other studies also did not observe an effect of breeder dietary CP intake during rearing (Hocking et al., 2002; Van Emous et al., 2015a; b), during production (Mohiti-Asli et al., 2012; Van Emous et al., 2018), during rearing and production (Lesuisse et al., 2017) or breeder dietary energy level during production (Van Emous et al., 2015b) on hatchability of fertile eggs. Van Emous et al. (2015b)

observed a 1.1% higher hatchability in the first laying phase (wk 22 to 45) when breeders had a 3.7% lower dietary CP intake during rearing compared to high dietary CP intake. In that same study, they also observed a 1.5% higher hatchability for breeders with a 8 to 10% lower CP intake during the second laying phase (wk 45 to 60). Although hatchability did not differ in the current study, very early embryonic mortality (E0-E3) decreased with an increasing breeder dietary energy-to-protein ratio. These results support the observations from Van Emous et al. (2015b), indicating a reduction in breeder CP intake might be beneficial for hatchability, due to a lower early embryonic mortality. A lower CP intake may have resulted in a lower albumen pH (Silversides and Budgell, 2004). In turn, a lower albumen pH has been related to an improved hatchability (Walsh et al., 1995; Reijrink et al., 2008). To protect the embryo from a suboptimal albumen pH, an effective barrier is formed between the ectodermal and endodermal epithelia of the embryo (Gillespie and McHanwell, 1987). Maintenance of this barrier might cause a depletion of energy reserves, particularly glucose, of the embryo (Walsh et al., 1995). During the first days of incubation an embryo mainly uses glucose as energy source (Moran, 2007). It can therefore be speculated that embryos, originating from breeders fed with a higher dietary energy-to-protein ratio, have a higher availability of glucose as they need less energy for maintenance of the barrier, due to a lower albumen pH. The higher availability of glucose for these embryos might have led to a higher survivability.

An increase in breeder dietary energy-to-protein ratio, and thus a decrease in dietary CP intake, resulted in a lower hatchling weight as a result of a lower hatching egg weight. This was also observed by Lesuisse et al. (2017). Breeders with a 22.8% lower CP intake during rearing and production produced eggs and hatchlings with a lower weight (Lesuisse et al., 2017). Van Emous et al. (2015a; b, 2018) did not find an effect of breeder dietary energy-to-protein ratio on EW nor on hatchling weight. In these studies, however, dietary energy-to-protein ratio was altered during either the rearing phase (Van Emous et al., 2015a; b) or the production phase (Van Emous et al., 2015b, 2018) alone and not in both phases, like the current study. Possibly, a lower breeder dietary energy-to-protein ratio can be beneficial for hatchling weight, but only when a lower breeder dietary energy-to-protein ratio is fed during both rearing and production.

Breeder dietary energy-to-protein ratio affected prevalence of chicks with a poorer navel closure. Prevalence of chicks with poorer navel closure (navel score 1) increased with increasing dietary energy-to-protein ratio in chicks from SGC breeders, whereas it decreased in chicks from EGC breeders. As discussed before, a larger RY might lead to a poorer navel closure (Molenaar et al., 2010a). However, RY size did not differ between treatments. It remains unclear why this interaction occurred.



Willemsen et al. (2008) observed a weak correlation ( $r = 0.3$ ) between hatchling weight and market weight. In the current study, however, hatchlings were heaviest from the 96% AME<sub>n</sub> breeders, compared to the other AME<sub>n</sub> levels, whereas they had the lowest BW at market age. At hatch, BW decreased linearly with an increasing breeder dietary energy-to-protein ratio, whereas at market age, BW increased linearly with increasing breeder dietary energy-to-protein ratio. Broilers originating from breeders fed a higher dietary energy-to-protein ratio had a higher growth, a higher feed intake and were more efficient, than broilers originating from breeders fed a lower dietary energy-to-protein ratio. Several other studies also observed an effect of breeder dietary energy-to-protein ratio on progeny performance (Spratt and Leeson, 1987; Peebles et al., 2002; Moraes et al., 2014, 2019; Lesuisse et al., 2017, 2018). These results indicate that the maternal diet influences offspring performance, which is often referred to as transgenerational epigenetic programming (Berghof et al., 2013), where the phenotype of the offspring is matched to the maternal environment. Phenotypic changes in offspring can be induced by a modification in gene expression in specific tissues (Rao et al., 2009). Breeders that were fed a higher energy-to-protein ratio had a higher feed restriction and lower CP intake (Heijmans et al., 2021). We speculate that broilers originating from breeders with a high energy-to-protein ratio were programmed for an environment poor in CP and use dietary CP more efficiently, as this nutrient was poorly available in the maternal environment. This has led to a lower FCR and a higher growth for these broilers. In line with this hypothesis, Lesuisse et al. (2018) observed an enhanced nitrogen retention in broilers originating from breeders fed a low CP diet, compared to a high CP diet. Nitrogen is mainly retained as breast muscle tissue in broilers. Long term breast muscle growth is regulated by myogenic precursor cells, satellite cells (Halevy et al., 2000; Sklan et al., 2003; Halevy, 2020). It has been observed that satellite cell activity depends on expression of specific genes (Halevy et al., 2004) and can be altered by a change in prenatal or early post hatch environment (Halevy, 2020). It is speculated that epigenetic effects have been triggered in the current study causing an enhanced nitrogen retention by upregulation of satellite cell activity.

An increase in breeder dietary energy-to-protein ratio resulted in a higher carcass yield in offspring from SGC breeders. Moraes et al. (2019) also observed a higher carcass yield for offspring from breeders fed a higher dietary energy-to-protein ratio during rearing. As speculated before, a low CP availability in breeders, might have resulted in epigenetic changes in satellite cell activity and leading to a higher muscle growth. This might have happened in offspring from SGC breeders. However, within offspring from EGC breeders, an increase in breeder dietary energy-to-protein ratio resulted in a lower carcass yield. It remains unclear why this interaction occurred. A higher breast muscle growth has been associated with a higher occurrence of myopathies (Velle-

man, 2015). In line with a higher carcass yield, prevalence of white striping increased with an increasing dietary energy-to-protein ratio in broilers from SGC breeders and decreased in broilers from EGC breeders.

## CONCLUSIONS

It can be concluded that an elevated growth curve of broiler breeders during both rearing and production had no effect on fertility or hatchability, but was beneficial for hatchling weight and offspring growth up to market age. Increasing breeder dietary energy-to-protein ratio led to a significantly lower very early embryonic mortality, but had minor effects on chick quality parameters. Increasing breeder dietary energy-to-protein ratio enhanced feed intake and growth and lowered FCRc. This might be due to transgenerational epigenetic effects and an altered CP efficiency.

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SUPPLEMENTARY INFORMATION

Table S1. Weekly average daily gain (ADG; g/d), average daily feed intake (ADFI; g/d) and feed conversion ratios (FCR; kg of feed/kg of BW gain) of broilers obtained from broiler breeders at 2 different ages (28 and 36 wk of age), which were fed to reach one of two targeted growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%)) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>), from hatch onwards.

Item	Period <sup>1</sup>											
	d 0-7			d 7-14			d 14-21			d 21-28		
	ADG	ADFI	FCR	ADG	ADFI	FCR	ADG	ADFI	FCR	ADG	ADFI	FCR
Growth curve												
SGC	17.0 <sup>b</sup>	19.1 <sup>b</sup>	1.13	41.5 <sup>b</sup>	55.3 <sup>b</sup>	1.34	68.2	97.1	1.42	97.8	140.0	1.43
EGC	17.6 <sup>a</sup>	19.7 <sup>a</sup>	1.12	42.6 <sup>a</sup>	56.7 <sup>a</sup>	1.33	69.3	98.5	1.42	99.0	141.8	1.43
SEM	0.1	0.1	0.01	0.2	0.2	0.01	0.4	0.4	0.01	0.6	0.7	0.01
Diet												
96% AME <sub>n</sub>	17.5	19.6 <sup>a</sup>	1.12	41.4 <sup>b</sup>	55.4 <sup>b</sup>	1.34	67.4	96.5	1.43	97.0	138.6	1.43
100% AME <sub>n</sub>	17.2	19.2 <sup>b</sup>	1.12	41.7 <sup>b</sup>	55.8 <sup>b</sup>	1.34	69.2	97.8	1.41	98.4	140.8	1.43
104% AME <sub>n</sub>	17.3	19.5 <sup>ab</sup>	1.12	43.0 <sup>a</sup>	57.0 <sup>a</sup>	1.33	69.6	98.8	1.42	98.9	142.2	1.44
108% AME <sub>n</sub>	17.2	19.3 <sup>b</sup>	1.12	41.9 <sup>b</sup>	55.9 <sup>b</sup>	1.33	68.8	98.2	1.42	99.3	142.0	1.43
SEM	0.1	0.1	0.01	0.3	0.3	0.01	0.6	0.6	0.01	0.9	1.0	0.01
Treatment												
SGC	17.0	19.2	1.13	40.5	54.4	1.34	66.4	95.0 <sup>c</sup>	1.43	95.4	136.4	1.43
100% AME <sub>n</sub>	16.8	18.9	1.13	40.9	54.8	1.34	67.6	96.0 <sup>bc</sup>	1.42	97.5	139.4	1.43
104% AME <sub>n</sub>	17.0	19.0	1.12	42.8	56.6	1.33	70.1	99.3 <sup>a</sup>	1.41	99.3	142.4	1.44
108% AME <sub>n</sub>	17.1	19.2	1.12	41.7	55.5	1.33	68.7	98.1 <sup>ab</sup>	1.43	98.9	141.7	1.43
EGC	17.9	20.1	1.12	42.3	56.5	1.34	68.4	97.9 <sup>ab</sup>	1.43	98.5	140.8	1.43
100% AME <sub>n</sub>	17.5	19.5	1.12	42.4	56.8	1.34	70.7	99.7 <sup>a</sup>	1.41	99.3	142.1	1.43
104% AME <sub>n</sub>	17.6	19.8	1.12	43.3	57.3	1.32	69.1	98.3 <sup>ab</sup>	1.42	98.5	142.1	1.44
108% AME <sub>n</sub>	17.3	19.4	1.12	42.2	56.3	1.33	69.0	98.2 <sup>ab</sup>	1.42	99.7	142.2	1.43
SEM	0.1	0.2	0.01	0.4	0.5	0.01	0.8	0.9	0.01	1.2	1.5	0.01

Table S1. Continued

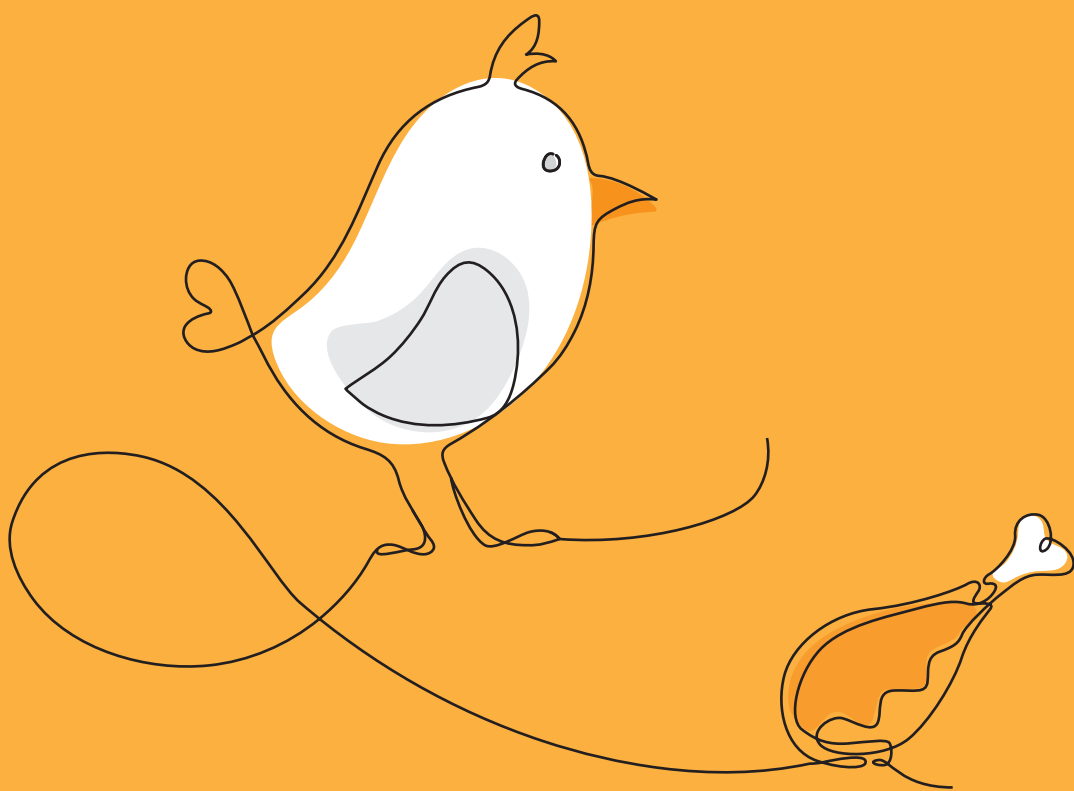
Item	Period <sup>1</sup>											
	d 0-7			d 7-14			d 14-21			d 21-28		
	ADG	ADFI	FCR	ADG	ADFI	FCR	ADG	ADFI	FCR	ADG	ADFI	FCR
Hen age												
28 weeks	17.0 <sup>b</sup>	18.4 <sup>b</sup>	1.08 <sup>b</sup>	41.2 <sup>b</sup>	55.9	1.36 <sup>a</sup>	66.8 <sup>b</sup>	87.4 <sup>b</sup>	1.31 <sup>b</sup>	97.4 <sup>b</sup>	140.5	1.44 <sup>a</sup>
36 weeks	17.6 <sup>a</sup>	20.4 <sup>a</sup>	1.16 <sup>a</sup>	42.8 <sup>a</sup>	56.2	1.31 <sup>b</sup>	70.7 <sup>a</sup>	108.2 <sup>a</sup>	1.53 <sup>a</sup>	99.3 <sup>a</sup>	141.3	1.42 <sup>b</sup>
SEM	0.1	0.1	0.01	0.2	0.2	0.01	0.4	0.4	0.01	0.6	0.7	0.01
P-value												
Growth curve (GC)	<0.001	<0.001	0.23	<0.001	<0.001	0.77	0.07	0.03	0.69	0.15	0.09	0.95
Diet (factorial)	0.14	0.03	0.99	0.001	0.02	0.17	0.07	0.07	0.44	0.25	0.08	0.68
Diet (linear)	0.17	0.11	0.87	0.03	0.11	0.15	0.09	0.04	0.79	0.06	0.02	0.76
Diet (quadratic)	0.28	0.18	0.83	0.03	0.05	0.41	0.04	0.11	0.12	0.54	0.25	0.47
GC x Diet (factorial)	0.09	0.13	0.70	0.35	0.26	0.99	0.08	0.03	0.87	0.46	0.39	0.84
GC x Diet (linear)	0.02	0.06	0.38	0.10	0.09	0.72	0.09	0.03	0.98	0.23	0.12	0.79
GC x Diet (quadratic)	0.62	0.53	0.85	0.81	0.87	0.96	0.91	0.92	0.87	0.42	0.57	0.51
Hen age	<0.001	<0.001	<0.001	<0.001	0.34	<0.001	<0.001	<0.001	<0.001	0.03	0.45	<0.001

<sup>a-c</sup> LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>At 14 d of age, 2 adjacent pens from the same treatment were merged. n = 16 per treatment for d 0-7 and d 7-14, and n = 8 per treatment for d 14-21, d 21-28, and d 28-32.







# Chapter 6

## Interaction between broiler breeder and broiler dietary energy-to-protein ratio

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## ABSTRACT

The interaction between maternal rearing and production dietary energy-to-protein ratio and offspring dietary protein content on chick quality and broiler performance was investigated. Pullets ( $n = 1,440$ ) were randomly assigned over 24 pens in a  $2 \times 2$  factorial design with 2 rearing diets fed from hatch to 21 wk of age (96R and 104R) and 2 production diets fed from 21 to 40 wk of age (96P and 104P), differing in energy-to-protein ratio by a different energy content (96% and 104% AME<sub>n</sub>) fed on a pair-gain basis. At 29 and 38 wk of age, hatching eggs were selected and incubated per maternal pen and 864 broilers were divided over 72 floor pens according to maternal treatment. For each maternal treatment, broiler pens were divided over 3 dietary treatments, which consisted of 90%, 100% and 110% digestible lysine (dLys) diets. Within the 90% dLys treatment, broilers from the 104R-96P breeders had on average a 3.5 g/d lower growth and 115 g lower BW at d 32 compared to the other breeder dietary treatments ( $P \leq 0.04$ ). Broilers within the 90% dLys treatment from the 104R breeders had on average a 3.9 g/d lower feed intake, compared to broilers from the 96R breeders ( $P = 0.002$ ). Within the 100% dLys treatment, no effect of breeder dietary treatment on growth, feed intake, feed conversion ratio or BW was observed. Within the 110% dLys treatment, broilers from the 104R-96P breeders had a 2.4 g/d higher growth, compared to broilers from the 104R-104P breeder diet, with the other 2 breeder dietary treatments in between ( $P = 0.03$ ). Within the 110% dLys, no effect of breeder dietary treatment on feed intake, feed conversion ratio or BW was observed. Overall, it can be concluded that maternal diets mainly affect broiler performance when broilers are fed a low protein diet.

**Key words:** broiler breeder, broiler, offspring, feed strategy

## INTRODUCTION

The maternal diet has been shown to affect the phenotype of the offspring in both mammals and birds (Roseboom et al., 2006; Van der Waaij et al., 2011; Lesuisse et al., 2017, 2018; Heijmans et al., 2022a). This so-called maternal effect is also referred to as transgenerational epigenetic programming (Berghof et al., 2013). The purpose of this maternal effect is to prepare the offspring for the environment in which it will develop, in order to increase the chance of survival.

Several studies have investigated the impact of maternal breeder dietary energy-to-protein ratio on chick quality and broiler performance (Moraes et al., 2014, 2019; Van Emous et al., 2015a; Lesuisse et al., 2017; Heijmans et al., 2022a). Adjustments of breeder dietary CP level during the rearing phase alone did not affect chick quality and broiler performance (Van Emous et al., 2015a; Moraes et al., 2019). Broilers hatched from 30 wk old breeders that were had a standard dietary CP intake during both rearing and production had a 82 g lower male broiler BW and a 0.03 higher female feed conversion ratio, compared a 23% lower maternal CP intake (Lesuisse et al., 2017). In line with this, Heijmans et al. (2022a) observed a 1.3 g/d lower growth, a 1.7 g/d lower feed intake and a 0.03 higher feed conversion ratio in offspring that originated from a relative high breeder dietary CP intake during both rearing and production, compared to a 15% lower breeder dietary CP intake. It can be speculated that a lower breeder dietary CP intake 'programmed' the offspring to be efficient with protein (Lesuisse et al., 2018). It was also observed, however, that a lower breeder dietary CP intake negatively impacted breeder performance, as these breeders had a lower egg production and a lower egg weights (Lesuisse et al., 2017; Heijmans et al., 2021).

In all these studies, a standard CP diet was fed to the offspring. It therefore remains unclear whether or not a higher dietary CP content in the broiler diet could potentially further improve performance in broilers from low dietary CP intake breeders. Only few studies have investigated the interaction between maternal and offspring diet in broilers (Van der Waaij et al., 2011; Lesuisse et al., 2018). It has been observed that either a match (Van der Waaij et al., 2011) or a mismatch (Lesuisse et al., 2018) between maternal and offspring diet might further optimize offspring development. In a matched maternal and offspring dietary environment, the offspring is fed a comparable diet or feeding program as the breeder hen. In a mismatched maternal and offspring dietary environment, the offspring is fed a contradictory diet or feeding program than the breeder hen. Van der Waaij et al. (2011) observed a lower growth and potentially a higher risk for metabolic disorders in *ad libitum* fed offspring that originated from restricted fed mothers, compared to *ad libitum* fed offspring that originated from *ad*

*libitum* fed mothers. Lesuisse et al. (2018) observed a 158 g higher BW and 2.8% higher nitrogen retention in offspring of breeders that were fed low CP diets during both rearing and production, followed by a standard CP broiler diet, whereas this was not observed when broilers were fed a low CP broiler diet. These results suggest a mismatch between maternal low dietary CP and offspring high dietary CP protein might be beneficial for offspring performance. In the study of Lesuisse et al. (2018) a lower maternal CP intake was achieved by feeding a diet with a lower dietary CP content. It remains unclear whether or not a lower maternal CP intake, as a result of a higher maternal dietary energy content (Heijmans et al., 2021), also interacts with broiler dietary CP content. Furthermore, Lesuisse et al. (2018) hypothesized the maternal effect of a lower CP intake is only transferable to the offspring when a lower CP intake is realized during the production phase, whereas these effects are not transferable to the offspring when a lower CP intake is realized in the rearing phase alone. In the study of Lesuisse et al. (2018), however, dietary treatments were applied during both rearing and production phase and therefore it remains unclear whether or not this hypothesis is true.

The aim of this study was to evaluate the interaction effect between the maternal and offspring dietary energy-to-protein ratio on offspring performance.

## MATERIALS AND METHODS

### Experimental Design

Female Ross 308 broiler breeder pullets were randomly assigned to 4 treatments over 24 pens in a 2 x 2 factorial arrangement from hatch to 40 wk of age ( $n = 6$  per treatment). Treatments were 2 rearing diets fed from hatch to 21 wk of age (further defined as **96R** and **104R**) and 2 production diets fed from 21 to 40 wk of age (further defined as **96P** and **104P**), differing in energy-to-protein ratio by a different energy content (96% and 104% AME<sub>n</sub> relative to breeder recommendations of Aviagen (2016a)). Pair-gain of pullets was achieved by weekly adaptation of feed allocation per diet based on weekly BW measurement. At 29 and 38 wk of age, hatching eggs produced by these breeders were incubated and per maternal age 864 broilers were divided over 72 floor pens according to maternal treatment ( $n = 18$  pens per maternal treatment). For each maternal treatment, pens were divided over 3 dietary treatments, which consisted of low, standard and high digestible lysine (**dLys**) diets (90%, 100% and 110% relative to breeder recommendations of Aviagen (2019)); ( $n = 6$  pens per treatment) and broiler performance was recorded until slaughter. All experimental protocols were approved by the Central Commission on Animal Experimentation (The Hague, the Netherlands), approval number 2018.W-0023.003 and 2018.W-0023.004.

## Experimental diets

Breeders were fed according to 5 phases, a starter 1 diet was fed from 0 to 21 d of age, a starter 2 diet from 22 to 42 d of age, a grower diet from 43 to 112 d of age, a pre-breeder diet from 113 to 160 d of age and a breeder 1 diet from 161 to 280 d of age. Breeder experimental diets were formulated per phase with a dietary AME<sub>n</sub> level at 96% and 104% AME<sub>n</sub>, relative to breeder recommendations (Aviagen, 2016a). Diets were formulated isonitrogenous and with a similar starch-to-fat ratio. An increase in dietary AME<sub>n</sub> level was reached by exchanging fibrous ingredients (cellulose and finely ground oat hulls) for energy rich ingredients (soy oil, lard and maize starch). Diets were analyzed on CP (NEN-EN-ISO 16634-1), crude fat (NEN-EN-ISO 6492-1999) and starch (NEN-ISO 6493) content. Ingredient composition with calculated and analyzed nutrient contents of the breeder diets are presented in Table 1. All breeder diets were fed as mash and provided *ad libitum* for the first 14 d of age. Hereafter, breeders were fed once a day. Daily feed allocation for each treatment was calculated and adjusted weekly to achieve pair-gaining among treatments and growth according to the elevated growth curve (+15% relative to standard breeder growth curve recommendations of Aviagen (2016b)) as presented by Heijmans et al. (2021). Growth and egg production in the week prior were directives for calculations of daily feed allocation.

Broilers were fed according to 4 phases, a starter diet was fed from 0 to 7 d of age, a grower 1 diet from 7 to 21 d of age, a grower 2 diet from 21 to 28 d of age, and a finisher diet from 28 to 32 d of age. Broiler diets were formulated per phase with step-wise increment in dietary dLys level at 90%, 100% and 110% relative to breeder recommendations of Aviagen (2019). Diets were formulated isoenergetic with a balanced amino acid profile according to breeder recommendations (Aviagen, 2019). An increase in dietary dLys level was reached by exchanging peas for soybean meal, supplemented with synthetic amino acids. First, the 90% and 110% dLys diets were produced. The intermediate diet 100% dLys was produced by homogenous mixing of 90% and 110% dLys diets in a 1:1 ratio. The starter diet was fed as crumble and all other diets were fed as pellets. Diets were analyzed on CP (NEN-EN-ISO 16634-1) and total lysine (NEN-EN-ISO 13902). Ingredient composition and analyzed nutrient contents of the broiler diets are presented in Table 2.

## Breeders, Housing and Management

A total of 1,440 female Ross 308 day-old breeder pullets were obtained from a 39 wk old grandparent flock (Aviagen-EPI, Roermond, The Netherlands). Day-old pullets were randomly divided over 24 pens (n = 60 pullets per pen) in a climate controlled room in 6 blocks of 4 pens (n = 6 per treatment). Each pen consisted of an elevated slatted floor area (1.75 x 2.90 m) with a 9 m track feeding system, drinking nipples,

Table 1. Dietary ingredients, and calculated and analyzed nutrients of breeder diets (g/kg, as-fed basis)

Item	Starter 1 (0-21 days)			Starter 2 (22-42 days)			Grower (43-112 days)			Pre-breeder (113-160 days)			Breeder 1 (161-280 days)		
	96% AME <sub>n</sub>	104% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>	104% AME <sub>n</sub>
Ingredient															
Maize	400.0	400.0	400.0	450.0	450.0	450.0	400.0	400.0	400.0	460.0	460.0	460.0	430.0	430.0	430.0
Wheat	150.0	150.0	150.0	150.0	150.0	150.0	135.1	135.1	135.1	150.0	150.0	150.0	100.0	100.0	100.0
Soybean meal	223.3	225.5	225.5	122.7	124.6	124.6	30.0	33.3	33.3	38.8	38.8	39.6	161.3	161.3	162.6
Sunflower meal	75.0	75.0	75.0	115.0	115.0	115.0	133.4	133.4	133.4	175.0	175.0	175.0	72.1	72.1	72.1
Wheat middlings	-	-	-	-	-	-	150.0	150.0	150.0	25.0	25.0	25.0	-	-	-
Oat hulls (fine)	46.5	16.6	16.6	54.8	23.5	23.5	64.7	34.2	34.2	50.0	50.0	18.0	55.0	55.0	27.4
Cellulose	45.0	14.0	14.0	50.0	20.0	20.0	45.0	15.0	15.0	45.3	45.3	16.0	50.0	50.0	19.0
Soya oil	6.4	8.5	8.5	4.6	6.6	6.6	4.2	6.1	6.1	4.5	4.5	7.0	4.8	4.8	8.0
Lard	3.7	6.3	6.3	5.9	8.6	8.6	3.0	6.0	6.0	5.0	5.0	7.3	27.9	27.9	32.1
Maize starch	9.0	63.3	63.3	6.1	61.0	61.0	1.0	53.6	53.6	1.0	1.0	57.0	7.9	7.9	58.0
Limestone (fine)	14.8	14.9	14.9	14.7	14.7	14.7	14.6	14.7	14.7						
Limestone (coarse)	-	-	-	-	-	-	-	-	-	24.4	24.4	24.4	70.9	70.9	71.0
Monocalcium phosphate	8.4	8.1	8.1	9.2	8.8	8.8	4.3	3.9	3.9	4.5	4.5	4.1	4.7	4.7	4.4
Sodium bicarbonate	1.8	1.8	1.8	1.9	1.9	1.9	1.5	1.5	1.5	1.8	1.8	1.8	1.0	1.0	1.0
Salt	1.7	1.7	1.7	1.6	1.6	1.6	1.8	1.8	1.8	1.5	1.5	1.5	2.3	2.3	2.3
L-Lysine	1.93	1.91	1.91	2.05	2.03	2.03	1.30	1.24	1.24	1.58	1.58	1.60	-	-	-
L-Threonine	0.80	0.80	0.80	0.64	0.65	0.65	-	-	-	0.56	0.56	0.58	0.53	0.53	0.54
DL-Methionine	2.23	2.25	2.25	1.57	1.59	1.59	0.81	0.81	0.81	1.01	1.01	1.02	1.66	1.66	1.68
L-Tryptophane	-	-	-	-	-	-	-	-	-	0.07	0.07	0.07	-	-	-
Choline Chloride-50%	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.5	1.5	1.4	1.4	1.4	1.3
Formic acid	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Xylanase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02



Table 1. Continued

Item	Starter 1 (0-21 days)		Starter 2 (22-42 days)		Grower (43-112 days)		Pre-breeder (113-160 days)		Breeder 1 (161-280 days)	
	96% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>	96% AME <sub>n</sub>	104% AME <sub>n</sub>
Premix rearing <sup>1</sup>	2.5	2.5	2.5	2.5	2.5	2.5	-	-	-	-
Premix laying <sup>2</sup>	-	-	-	-	-	-	2.5	2.5	2.5	2.5
Calculated content <sup>3</sup>										
AME <sub>n</sub> (kcal/kg)	2,570	2,780	2,570	2,780	2,545	2,760	2,640	2,860	2,735	2,965
Crude protein	177.5	176.9	146.2	145.6	130.0	130.0	128.8	127.7	142.1	141.3
Crude fat	38.5	42.9	39.7	44.0	38.0	42.5	38.0	42.4	58.4	65.5
Crude fibre	84.7	53.9	98.3	67.8	110.6	80.5	108.0	77.9	87.8	57.8
Starch	376.4	422.2	403.1	449.3	382.9	427.1	401.3	448.3	356.6	398.7
Starch:fat	9.8	9.8	10.2	10.2	10.1	10.1	10.6	10.6	6.1	6.1
Linoleic acid	16.0	17.1	16.0	17.0	16.0	17.0	15.7	16.9	16.5	18.3
Digestible lysine	9.2	9.2	7.2	7.2	5.0	5.0	5.3	5.3	6.1	6.1
Calcium	9.8	9.8	9.8	9.8	8.9	8.9	13.1	13.1	31.0	31.0
Retainable phosphorus	4.1	4.1	4.1	4.1	3.3	3.3	3.2	3.2	3.2	3.2
Analyzed content										
Crude protein	186.9	184.0	147.3	151.8	126.4	129.0	126.0	123.7	142.2	141.6
Crude fat	31.8	37.0	32.4	34.5	33.0	37.4	30.6	38.1	53.7	61.4
Starch	373.9	418.4	410.1	450.0	387.6	427.5	416.7	462.4	367.7	411.7

<sup>1</sup>Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 3.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 6.0 mg; Vitamin B<sub>6</sub> 4.0 mg; Vitamin B<sub>12</sub> 20 µg; Niacinamide 35 mg; D-pantothenic acid 15 mg; Folic acid 1.5 mg; Biotin 0.20 mg; Iron 40 mg; Copper 16 mg; Manganese 120 mg; Zinc 90 mg; Iodine 1.25 mg; Selenium 0.3 mg.

<sup>2</sup>Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 3000 IU; Vitamin E 100 IU; Vitamin K 5.0 mg; Vitamin B<sub>1</sub> 3.0 mg; Vitamin B<sub>2</sub> 12.0 mg; Vitamin B<sub>6</sub> 5.0 mg; Vitamin B<sub>12</sub> 40 µg; Niacinamide 55 mg; D-pantothenic acid 15 mg; Folic acid 2.0 mg; Biotin 0.40 mg; Iron 50 mg; Copper 10 mg; Manganese 120 mg; Zinc 90 mg; Iodine 2.0 mg; Selenium 0.3 mg.

<sup>3</sup>Calculated according to CVB (2012).

Table 2. Dietary ingredients, and calculated and analyzed nutrients of the 90% and 110% dLys broiler diets (g/kg, as-fed basis). The intermediate 100% dLys diet was produced by mixing the 90% and 110% dLys diet in a 1:1 ratio.

Item	Starter (0-7 days)		Grower 1 (7-21 days)		Grower 2 (21-28 days)		Finisher (28-32 days)	
	90% dLys	110% dLys	90% dLys	110% dLys	90% dLys	110% dLys	90% dLys	110% dLys
Ingredient								
Maize	350.0	350.0	380.0	380.0	400.0	400.0	400.0	400.0
Wheat	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Sunflower meal	3.8	3.8	-	-	-	-	-	-
Soybean meal	233.2	263.6	205.4	238.5	185.5	220.0	175.1	211.6
Peas	64.3	21.4	64.7	20.0	60.0	15.0	67.2	20.0
Soya oil	5.1	5.1	9.8	10.2	14.6	15.3	20.6	21.5
Lard	3.7	3.8	3.9	4.0	4.0	4.2	4.3	4.4
Chalk	14.3	14.1	12.9	12.8	13.0	12.9	12.2	12.1
Monocalcium phosphate	8.8	8.8	7.0	7.0	7.2	7.1	5.4	5.4
Sodium bicarbonate	1.3	1.3	1.3	1.3	1.4	1.3	1.3	1.3
Salt	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
L-Lysine	3.32	7.30	3.12	6.65	3.01	6.12	2.96	6.02
DL-Methionine	2.47	4.09	2.33	3.82	2.05	3.38	2.17	3.52
L-Threonine	1.11	2.55	0.97	2.24	0.86	1.97	0.84	1.94
L-Valine	-	1.60	-	1.42	-	1.26	-	1.23
L-Isoleucine	-	1.32	0.04	1.23	0.01	1.03	0.11	1.13
L-Arginine	-	2.33	-	2.06	-	1.80	-	1.77
L-Tryptophane	-	0.36	-	0.31	-	0.25	-	0.25
Salinomycin	0.6	0.6	0.6	0.6	0.6	0.6	-	-
Xylanase	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Premix <sup>1</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Table 2. Continued

Item	Starter (0-7 days)		Grower 1 (7-21 days)		Grower 2 (21-28 days)		Finisher (28-32 days)	
	90% dLys	110% dLys	90% dLys	110% dLys	90% dLys	110% dLys	90% dLys	110% dLys
Calculated content <sup>2</sup>								
AME <sub>n</sub> (kcal/kg)	2,925	2,925	3,000	3,000	3,050	3,050	3,100	3,100
Crude protein	195.7	213.4	183.2	200.6	173.8	190.4	170.4	187.5
Crude fat	36.4	36.6	41.7	42.4	46.9	47.9	53.0	54.2
Crude fibre	27.4	26.2	26.1	24.9	25.5	24.4	25.5	24.4
Calcium	9.6	9.6	8.7	8.7	8.7	8.7	7.9	7.9
Available phosphorus	4.8	4.8	4.4	4.4	4.4	4.4	4.0	4.0
Total lysine	11.6	14.0	10.7	12.9	10.0	12.1	9.8	11.8
Digestible lysine	10.6	13.0	9.8	12.0	9.2	11.2	9.0	11.0
Digestible M+C	7.8	9.6	7.5	9.1	7.0	8.5	7.0	8.6
Digestible threonine	7.1	8.7	6.6	8.0	6.2	7.5	6.0	7.4
Digestible arginine	11.3	13.9	10.5	12.8	9.8	12.0	9.6	11.8
Digestible tryptophane	2.0	2.5	1.9	2.3	1.8	2.1	1.7	2.1
Analyzed content								
Crude protein	197.0	212.4	187.6	200.5	177.1	187.4	171.6	188
Lysine total	11.5	13.7	10.2	12.5	10.2	11.9	10.1	11.9

<sup>1</sup>Provided per kg diet: Vitamin A 10,000 IU; Vitamin D<sub>3</sub> 2500 IU; Vitamin E 50 mg; Vitamin K 1.5 mg; Vitamin B<sub>1</sub> 2.0 mg; Vitamin B<sub>2</sub> 7.5 mg; Vitamin B<sub>6</sub> 3.5 mg; Vitamin B<sub>12</sub> 20 µg; Nicotinamide 35 mg; D-pantothenic acid 20 mg; Choline chloride 460 mg; Folic acid 1.0 mg; Biotin 0.20 mg; Iron 80 mg; Copper 12 mg; Manganese 85 mg; Zinc 60 mg; Iodine 0.8 mg; Selenium 0.15 mg.

<sup>2</sup>Calculated according to CVB (2012).

7.2 m perches and laying nests (1.75 x 0.60 m) and a floor area with wood shavings as bedding (1.75 x 2.80 m). The elevated slatted floor area was covered with rearing paper (MS Schippers, Bladel, The Netherlands) and wood shavings until 6 wk of age. The laying nests were covered with black plastic to prevent access or sight to the laying nest until 20 wk of age. A grill was placed on the track feeding system to prevent rooster access to the feed after 20 wk of age. Two additional feeding pans were placed per pen during the first 2 wk of age to stimulate feed intake. Water was *ad libitum* available. Breeders were vaccinated according to a standard vaccination schedule (Poultry Vets, Diessen, The Netherlands). Room temperature was 36 °C for the first 3 d of age. Hereafter, temperature was gradually reduced to 20 °C at 4 wk of age and was maintained thereafter. Photoperiod was 23L:1D (20 lux) at arrival of the pullets, which gradually changed to 8L:16D (10 lux) at 3 wk of age and was maintained until 21 wk of age. Pullets were photo-stimulated instantly by increasing the photoperiod to 11L:13D (20 lux) at 21 wk of age, which was then gradually increased to 13L:11D (40 lux) at 23 wk of age. All pens were standardized to 45 breeders per pen closest to the average pen weight at 20 wk of age and 4 20 wk old Ross 308 roosters were introduced per pen. Roosters were fed once a day a commercial available rooster diet (2,725 kcal of AME<sub>n</sub>/kg, 134 g of CP/kg, 5 g digestible lysine/kg) in a rooster feeding pan. Rooster body weight, body condition, and mating activity were assessed every other week according to breeder recommendations (Aviagen, 2018). Inactive roosters were instantly replaced by sexually mature spike roosters.

## Incubation

At 29 and 38 wk of age of the breeders, settable hatching eggs were incubated to obtain chicks. At 29 wk of age of the breeders, 20 clean settable hatching eggs were selected per day per maternal pen for 3 consecutive days ( $n = 60$  per maternal pen). At 38 wk of age of the breeders, 16 clean settable hatching eggs were selected per day per maternal pen for 4 consecutive days per maternal pen ( $n = 64$  per maternal pen). At each breeder age, eggs were selected based on the average egg weight per treatment  $\pm 2.5$  g at that specific age. The hatching eggs were stored at the breeder farm at 17 °C for 10 to 13 d before incubation. Hatching eggs were transported for approximately 1 h to the hatchery (Lagerwey, Lunteren, The Netherlands), where they were incubated in a single-stage incubator with a maximum capacity of 4,800 hatching eggs (PicoClimer setter HT-150, HatchTech, Veenendaal, the Netherlands). The incubator contained 1 trolley with 16 levels of setter trays, with 2 rows per level (total 32 setter trays). The selected hatching eggs were distributed evenly over 1 setter tray per maternal pen, resulting in 24 setter trays in total. The bottom and top 2 levels were filled with empty setter trays. The setter trays with eggs were randomly divided over 6 blocks (top, middle, and bottom and front and back row) on the trolley. A temperature sensor (NTC Thermistors: type DC 95; Thermometrics, Somerset, UK)

was attached to 4 individual eggs from the 4 different maternal treatments to monitor eggshell temperature (**EST**). The EST sensors were attached at the equator of the egg using a small piece of tape (Tesa BV, Almere, The Netherlands) in silicone heat sink compound paste (Type 340; Dow Corning, Midland, MI). Eggs were warmed linearly in 10 h from storage temperature to an EST of 37.8 °C. The moment the eggs reached an EST of 37.8 °C was considered as start of incubation and embryonic day (**E**)0. Hereafter, the air temperature in the incubator was continuously and automatically adapted to maintain an EST of 37.8 °C, based on the median of the 4 EST sensors. Relative humidity was maintained between 50 and 65% until E4, between 50 and 60% from E4 to E7, between 50 and 55% from E7 to E10, and between 40 and 45% thereafter. Carbon dioxide was maintained below 0.35% throughout incubation. Eggs were turned hourly over 70° until E18. At E8 and E18, all eggs were candled and clear eggs or eggs containing a dead embryo were removed. At E18, EST sensors were removed and eggs containing a viable embryo were transferred to hatching baskets per setter tray, resulting in 24 hatching baskets in total. These 24 hatching baskets were placed on a trolley with 3 rows and 11 levels of hatching baskets, where the bottom 3 levels of hatching baskets were kept empty. The hatching baskets were randomly divided over 6 blocks (top and middle and front, middle, and back row) on the trolley and placed in a hatcher (PicoClimer Hatcher HT-150, HatchTech, Veenendaal, the Netherlands). In this hatcher, 6 EST were attached to 6 individual eggs from different treatments. The air temperature of the hatcher was manually adapted to maintain an EST of 37.8 °C based on the median temperature of the 6 EST sensors. From 473 h after the start of incubation (E19h17), the EST sensors were removed and the air temperature of the incubator was fixed at the current settings until pull of the hatched chicks (E21h13).

## Hatching

From E19h17 until pull, the incubator was opened every 6 h to check whether or not chicks had hatched. All chicks that hatched were marked with a permanent marker on the head. Marked chicks were collected 6 h later to assess chick quality. Hereafter, all first grade chicks were transferred to another similar hatcher (PicoClimer Hatcher HT-150, HatchTech, Veenendaal, the Netherlands), where they were placed in 24 hatching baskets until pull. After pulling, all chicks were feather sexed and pooled per maternal treatment and sex.

## Broilers, Housing and Management

At each maternal age, 29 and 38 wk of age, 360 female and 504 male first-grade broiler chicks were transported for 1 h in a climate controlled van to the broiler facility (Eerde, the Netherlands). At start of each experiment (d 0), the 4 maternal dietary treatments and the 3 broiler dietary treatments were randomly assigned to 72 floor pens within 3

blocks of 24 pens ( $n = 6$  pens per treatment). In each pen, 5 female and 7 male broilers were placed, originating from the same maternal treatment. Broilers were marked with a unique neck tag number. At 4 and 7 d of age, 1 male broiler was removed per pen, euthanized by percussive blow to the head followed by cervical dislocation and stored until further analysis. At 14 d of age, 2 adjacent pens of the same maternal and broiler treatment were merged ( $n = 3$  pens per treatment), in order to fulfil the housing requirements of the Central Commission on Animal Experimentation. Each pen ( $1 \text{ m}^2$  from 0 to 14 d of age and  $2 \text{ m}^2$  from 14 d of age onwards) contained wood shavings as bedding. Feed was supplied *ad libitum* in a feeding trough. Water was supplied *ad libitum* via drinking nipples. At d 0, photoperiod was 23L:1D (40 lux), which gradually changed to 18L:6D at d 3, which was maintained until slaughter. Temperature was set at  $33^\circ\text{C}$  at d 0 and decreased gradually to  $21^\circ\text{C}$  at d 32. Broilers were vaccinated according to a standard vaccination schedule (AdVee Dierenartsen, Heeswijk Dinther, The Netherlands).

## Measurements

**Egg Weight.** Selected hatching eggs were weighed individually before storage, at E0 and at E18. Egg weight (**EW**) loss during storage was calculated as the difference between EW before storage and E0. Egg weight loss during incubation was calculated as the difference between EW at E0 and EW at E18.

**Fertility, Hatchability, Embryonic Mortality.** Clear eggs and eggs containing a dead embryo at E8 and E18 and unhatched eggs at pull were opened to determine infertility or stage of embryonic mortality. The following classifications were used: 1) unfertilized eggs showing no signs of development, 2) early embryonic mortality (E0-E10): area vasculosa until feather development, 4) mid embryonic mortality (E11-E18): small embryo with feathers, 5) late embryonic mortality (E19-E21.5): full grown embryo. Embryos showing clear deformities were noted as abnormal embryos. Fertility was calculated as a percentage of set eggs. Hatchability was calculated as a percentage of set eggs and of fertile eggs. Embryonic mortality per classification was calculated as a percentage of fertile eggs.

**Chick Quality.** From E19h17 until pull, every 6 h chick quality of just hatched chicks was determined. Chicks were classified as first or second grade. A chick was classified as first grade when it was dry, clean, free of deformities and with bright eyes. The other chicks were classified as second grade, including the chickens that died in the hatching basket after emergence from the egg shell. Second grade chicks were euthanized by cervical dislocation. Percentage second grade chicks was calculated relative to the total number of hatched chicks. Body weight of all chicks was determined. Hereafter, first grade chicks were scored on activity and navel, beak, and leg quality. Activity was

scored as good or weak, after placing the chick on its back. If the chick returned to the standing position within 2 seconds, it was noted as good; longer than 2 seconds was noted as weak. Navel quality was scored as 0 (closed and clean navel), 1 (black button up to 2 mm or black string) or 2 (black button exceeding 2 mm) (Molenaar et al., 2010a). Beak quality was scored as 0 (normal beak) or 1 (red dot or nostrils contaminated with albumen). Leg quality was scored as 0 (normal legs, toes and hocks), 1 (red or swollen hock of 1 leg) or 2 (red or swollen hocks from both legs).

Every sixth first grade chick was euthanized by cervical dislocation, followed by decapitation. In total, 8 chicks per setter tray were euthanized. From all euthanized chicks the residual yolk (**RY**) was removed and weighed. Yolk-free body mass (**YFBM**) was calculated as chick weight minus RY weight.

**Broiler Performance.** Broilers were weighed individually at d 0, 7, 14, 21, 28 and 32 and feed intake was determined per pen on those weighing days. Average daily gain (**ADG**), average daily feed intake (**ADFI**) and feed conversion ratio (**FCR**), corrected for mortality, were calculated between those days. Feed conversion ratio over the whole period (d 0-32) was corrected for differences in BW at day 32 (**FCRc**). Heavier birds are assumed to have a higher maintenance requirement and a higher feed intake. Therefore, the equation is based on the assumption that 0.03 FCR is equivalent to a 100 g difference in BW (Van Krimpen et al., 2019). A standard BW of 2000 g was used for calculation of FCRc:

$$FCRc = FCR + \left( \frac{2000 - \text{actual BW d 32}}{100} * 0.03 \right)$$

Mortality was recorded daily per pen. First week (d 0 to 7) and overall (d 0 to 32) mortality was calculated as a percentage of chicks at placement.

### Breast Meat Yield, Myopathies and Body Composition.

At d 32, 1 male and 1 female broiler per pen were selected closest to average pen weight. Selected broilers were weighed and euthanized by a percussive blow to the head, followed by cervical dislocation. These broilers were then scalded in water of 65 °C for approximately 30 seconds and defeathered in a plucking machine (BRM1800, Beeketal Levensmitteltechnik GmbH & Co., Rastdorf, Germany). Broilers were then weighed, dissected and potential feed residues from the gastrointestinal tract were removed. The pectoralis major was visually scored on appearance of wooden breast and white striping (adopted from Kuttappan et al., 2016). White striping was scored as 0 (no white striations), 1 (small thin white striations <1mm) or 2 (thick white striations 1-2 mm). Wooden breast was scored as 0 (soft breast muscle), 1 (part of the breast muscle is hardened), or 2 (whole breast muscle is hardened). Prevalence of

white striping or wooden breast was calculated as percentage of broilers with a score 1 or 2 of the total broilers slaughtered. The pectoralis major and minor were removed, weighed, and placed back on the carcass. Hereafter, the defeathered empty carcass was weighed and grinded to a homogenous mixture of which a sample was analyzed on moisture (103 °C for 16 h), CP (NEN-ISO-8968-1) and crude fat (NEN-ISO-1735) content.

### Statistical Analysis

All continuous and binomial data were analyzed using the Restricted Maximum Likelihood variance component analysis procedure within a generalized linear mixed model (Genstat 19<sup>th</sup> Edition, 2019). Means and model residuals were checked on homogeneity of variance prior to analyses. Not-normal distributed data (first week mortality) were arcsine transformed before analyses. None of the models included the interaction with breeder age, as this was confounded with season and incubator. For statistical analysis of egg weight, fertility, hatchability, embryonic mortality, chick quality and chick composition the experimental unit was setter tray. The model used for incubation parameters was:

$$Y_{ijk} = \mu + R_i + P_j + R_i \times P_j + Age_k + e_{ijk} \quad [1],$$

where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the overall mean,  $R_i$  is the energy-to-protein ratio in the rearing diet of the breeders ( $i = 96\%$  or  $104\%$  AME<sub>n</sub>),  $P_j$  is the energy-to-protein ratio in the production diet of the breeders ( $j = 96\%$ , or  $104\%$  AME<sub>n</sub>),  $R_i \times P_j$  is the interaction between rearing and production diet,  $Age_k$  is age of the breeder flock ( $k = 29$  or  $38$  wk of age), and  $e_{ijk}$  is the residual error. Block in the incubator was added to the model as a random factor, except for analysis of egg weight parameters.

For analysis of broiler BW, ADG, ADFI, FCR, mortality, slaughter characteristics and body composition, model 1 was used, added with dLys and its interactions with the rearing and production diet:

$$Y_{ijkl} = \mu + R_i + P_j + dLys_k + R_i \times P_j + R_i \times dLys_k + P_j \times dLys_k + R_i \times P_j \times dLys_k + Age_l + e_{ijkl} \quad [2],$$

where  $Y_{ijkl}$  is the dependent variable,  $\mu$  is the overall mean,  $R_i$  is the energy-to-protein ratio in the rearing diet of the breeders ( $i = 96\%$  or  $104\%$  AME<sub>n</sub>),  $P_j$  is the energy-to-protein ratio in the production diet of the breeders ( $j = 96\%$  or  $104\%$  AME<sub>n</sub>), dLys<sub>k</sub> is the digestible lysine content in the diet of the broilers ( $k = 90\%$ ,  $100\%$  or  $110\%$  dLys),  $R_i \times P_j$  is the interaction between rearing and production diet,  $R_i \times dLys_k$  is the interaction between rearing breeder diet and broiler diet,  $P_j \times dLys_k$  is the interaction between production breeder diet and broiler diet,  $R_i \times P_j \times dLys_k$  is the interaction between rearing breeder diet, production breeder diet and broiler diet,  $Age_l$  is age of



the breeder flock ( $l = 29$  or  $38$  wk of age), and  $e_{ijk}$  is the residual error. Block in the farm was added to the model as a random factor.

Fisher adjustments were used for multiple comparisons of factorial analysis. Data are presented as LSmeans  $\pm$  SEM. For transformed data, LSmeans of original data are presented, combined with  $P$ -values of the transformed data. Differences were reported where  $P \leq 0.05$ .

## RESULTS

### Egg Weight, Incubation and Chick Quality

No interaction was observed between breeder rearing and production diet on selected hatching egg weight before storage, at E0, and at E18 or on egg weight loss during storage and incubation (data not shown). Selected hatching eggs obtained from breeders fed the 96R diet were  $0.2$  g larger before storage ( $60.7$  vs.  $60.5$  g;  $P = 0.004$ ), and at E0 ( $60.3$  vs.  $60.1$  g;  $P = 0.004$ ), but did not differ significantly at E18 ( $55.3$  vs.  $55.1$  g;  $P = 0.09$ ), compared to eggs obtained from breeders fed the 104R diet. Selected hatching eggs obtained from breeders fed the 96P diet were  $0.3$  g larger before storage ( $60.8$  vs.  $60.5$  g;  $P < 0.001$ ), and at E0 ( $60.4$  vs.  $60.1$  g;  $P < 0.001$ ), and  $0.2$  g larger at E18 ( $55.3$  vs.  $55.1$  g;  $P = 0.02$ ), compared to breeders fed the 104P diet. Egg weight loss during storage did not differ between treatments (data not shown) and was on average  $0.65 \pm 0.02\%$ . Egg weight loss between E0 and E18 was  $0.19\%$  higher for eggs obtained from breeders fed the 96P diet compared to breeders fed the 104P diet ( $8.47$  vs.  $8.28\%$ , respectively,  $P = 0.04$ ). No differences were observed between treatments on fertility, hatchability, embryonic mortality or percentage of second grade chicks (Table 3).

In total,  $2,681$  first grade chicks hatched and were scored on chick quality, of which  $384$  chicks were dissected for RY and YFBM weight and composition. No interaction was observed between breeder rearing and production diet on hatchling weight, RY weight, YFBM, activity, beak or leg score (Table 4). An interaction was observed between breeder rearing and production diet on percentage of chicks with navel score 2 ( $P = 0.05$ ; Table 4). However, after Fisher adjustments for multiple comparisons, no significant differences were observed. No effect was observed of breeder rearing diet on hatchling weight, RY weight or YFBM. Hatchlings obtained from breeders fed the 96P diet were  $0.2$  g heavier and had a  $0.2$  g smaller RY, compared to hatchlings obtained from breeders fed the 104P diet ( $P \leq 0.04$ ; Table 4). No differences were observed between rearing or production diets on activity, navel, beak or leg score (Table 4).

Table 3. Fertility, hatchability, embryonic mortality, and percentage second grade chicks obtained from broiler breeders at 2 different ages (29 and 38 wk of age), which were fed 2 rearing diets from hatch to 21 wk of age (96R and 104R) and 2 production diets from 21 wk of age onward (96P and 104P), differing in energy-to-protein ratio (96% AME<sub>n</sub> or 104% AME<sub>n</sub>).

Item	Fertility of set eggs (%)		Hatch. of set eggs (%)		Hatch. of fertile eggs (%)		Embryonic mortality (% of fertile eggs)				Second grade chicks (% of total chicks)	
							E0-E10 <sup>1</sup>	E11-E17	E18-E21.5	E0-E21.5	Abnormal	
Rearing diet (n=24)												
96R	98.7	91.2	92.5	92.5	5.0	1.2	1.0	7.5	0.3	1.0		
104R	98.3	91.1	92.7	92.7	5.0	0.8	1.2	7.3	0.4	0.6		
SEM	0.4	0.8	0.7	0.7	0.6	0.3	0.3	0.7	0.2	0.2		
Production diet (n=24)												
96P	98.3	91.3	92.8	92.8	4.8	0.8	1.2	7.2	0.3	0.7		
104P	98.6	91.1	92.3	92.3	5.2	1.1	1.0	7.7	0.4	0.9		
SEM	0.4	0.8	0.7	0.7	0.6	0.3	0.3	0.7	0.2	0.2		
Rearing diet x Production diet (n=12)												
96R	98.7	91.1	92.3	92.3	5.1	1.3	1.1	7.7	0.3	1.2		
104P	98.6	91.3	92.6	92.6	4.9	1.1	1.0	7.4	0.4	0.8		
96P	98.0	91.4	93.3	93.3	4.5	0.4	1.4	6.7	0.4	0.2		
104P	98.6	90.8	92.1	92.1	5.4	1.1	1.0	7.9	0.4	1.0		
SEM	0.6	1.1	1.0	1.0	0.9	0.4	0.3	1.0	0.2	0.3		
Breeder age (n=24)												
29 weeks	98.0 <sup>b</sup>	90.3	92.2	92.2	5.3	1.1	1.2	7.8	0.2	0.8		
38 weeks	99.0 <sup>a</sup>	92.0	92.9	92.9	4.7	0.9	1.0	7.1	0.5	0.8		
SEM	0.3	0.8	0.7	0.7	0.7	0.3	0.3	0.7	0.2	0.2		
P-value												
Rearing diet	0.24	0.91	0.84	0.84	1.00	0.25	0.69	0.84	0.73	0.28		
Production diet	0.30	0.86	0.64	0.64	0.65	0.49	0.44	0.64	0.76	0.51		
Rearing diet x Production diet	0.39	0.72	0.47	0.47	0.54	0.27	0.67	0.47	0.71	0.06		
Breeder age	0.03	0.19	0.49	0.49	0.52	0.67	0.60	0.49	0.27	1.00		

<sup>ab</sup> LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>Embryonic day (E).

Table 4. Hatchling weight (HW), residual yolk (RY) weight, yolk-free body mass (YFBM), activity, navel, beak and leg score of chicks obtained from broiler breeders at 2 different ages (29 and 38 wk of age), which were fed 2 rearing diets from hatch to 21 wk of age (96R and 104R) and 2 production diets from 21 wk of age onward (96P and 104P), differing in energy-to-protein ratio (96% AME<sub>n</sub> or 104% AME<sub>n</sub>).

Item		HW (g)	RY (g)	YFBM (g)	Activity <sup>1</sup> (%)	Navel score <sup>2</sup> (%)		Beak <sup>3</sup> (%)	Leg score <sup>4</sup> (%)	
						Score 1	Score 2		Score 1	Score 2
Rearing diet (n=24)										
96R		44.5	5.9	38.5	16.7	43.4	10.9	16.5	23.6	24.3
104R		44.3	5.9	38.3	18.5	44.9	10.5	15.4	24.5	25.2
SEM		0.1	0.1	0.1	1.1	1.6	0.9	1.0	1.4	1.7
Production diet (n=24)										
96P		44.5 <sup>a</sup>	5.8 <sup>b</sup>	38.6	16.9	45.9	10.5	15.5	24.3	25.5
104P		44.3 <sup>b</sup>	6.0 <sup>a</sup>	38.3	18.3	42.4	10.9	16.4	23.9	24.1
SEM		0.1	0.1	0.1	1.1	1.6	0.9	1.0	1.4	1.7
Rearing diet x Production diet (n=12)										
96R	96P	44.6	5.8	38.7	16.7	46.9	9.6 <sup>a</sup>	15.5	24.1	24.9
	104P	44.4	6.0	38.3	16.6	40.0	12.1 <sup>a</sup>	17.5	23.1	23.8
104R	96P	44.5	5.8	38.4	17.1	44.9	11.4 <sup>a</sup>	15.5	24.4	26.1
	104P	44.2	6.1	38.2	20.0	44.9	9.6 <sup>a</sup>	15.3	24.6	24.4
SEM		0.1	0.1	0.2	1.7	2.2	1.2	1.7	1.9	2.4
Breeder age (n=24)										
29 weeks		41.1 <sup>b</sup>	5.3 <sup>b</sup>	36.0 <sup>b</sup>	18.6	50.9 <sup>a</sup>	13.2 <sup>a</sup>	16.1	27.3 <sup>a</sup>	30.9 <sup>a</sup>
38 weeks		47.4 <sup>a</sup>	6.6 <sup>a</sup>	40.8 <sup>a</sup>	16.6	37.5 <sup>b</sup>	8.2 <sup>b</sup>	15.8	20.1 <sup>b</sup>	18.7 <sup>b</sup>
SEM		0.1	0.1	0.1	0.9	1.6	1.1	0.7	1.3	1.8
P-value										
Rearing diet		0.10	0.77	0.17	0.30	0.52	0.74	0.55	0.65	0.70
Production diet		0.003	0.04	0.07	0.44	0.13	0.72	0.62	0.83	0.55
Rearing diet x Production diet		0.84	0.63	0.53	0.40	0.14	0.05	0.56	0.76	0.88
Breeder age		<0.001	<0.001	<0.001	0.16	<0.001	0.01	0.78	0.006	<0.001

<sup>ab</sup> LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Percentage of chicks scored as weak (after placing the chick on its back, the chick does not return to the standing position within 2 seconds).

<sup>2</sup>Percentage of chicks with a score 1 (black button up to 2 mm or black string) or a score 2 (black button exceeding 2 mm or open navel).

<sup>3</sup>Percentage of chicks with a red dot on beak or nostrils contaminated with albumen.

<sup>4</sup>Percentage of chicks with a score 1 (red or swollen hock of 1 leg) or a score 2 (red or swollen hocks from both legs).

Table 5. Protein and fat percentage of the residual yolk (RY) and yolk-free body mass (YFBM) of chicks 6 h after hatching, obtained from broiler breeders at 2 different ages (29 and 38 wk of age), which were fed 2 rearing diets from hatch to 21 wk of age (96R and 104R) and 2 production diets from 21 wk of age onward (96P and 104P), differing in energy-to-protein ratio (96% AME<sub>n</sub> or 104% AME<sub>n</sub>).

Item	Protein RY (%)	Fat RY (%)	Protein YFBM (%)	Fat YFBM (%)	
Rearing diet (n=24)					
96R	26.2	18.9	14.0	5.0	
104R	26.1	19.6	14.0	5.0	
SEM	0.2	0.3	0.1	0.1	
Production diet (n=24)					
96P	26.4	18.7	14.0	4.9	
104P	25.9	19.9	14.0	5.0	
SEM	0.2	0.1	0.1	0.1	
Rearing diet x Production diet (n=12)					
96R	96P	26.3	18.8b	14.0	5.0
	104P	26.0	19.0b	14.0	5.0
104R	96P	26.4	18.6b	14.0	5.1
	104P	25.8	20.7a	14.0	4.9
SEM	0.2	0.5	0.1	0.1	
Breeder age (n=24)					
29 weeks	27.5 <sup>a</sup>	16.3 <sup>b</sup>	13.9 <sup>a</sup>	4.6 <sup>b</sup>	
38 weeks	24.8 <sup>b</sup>	22.3 <sup>a</sup>	14.1 <sup>b</sup>	5.4 <sup>a</sup>	
SEM	0.2	0.3	0.1	0.1	
P-value					
Rearing diet	0.73	0.12	0.96	0.97	
Production diet	0.07	0.02	0.96	0.31	
Rearing diet x Production diet	0.62	0.04	0.88	0.57	
Breeder age	<0.001	<0.001	0.005	<0.001	

<sup>ab</sup> LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

No treatment effects were observed on protein or fat content of the YFBM or protein content in the RY (Table 5). An interaction between breeder rearing and production diets was observed on fat content of the RY. Fat content was maximum 1.9% higher in the RY of chicks obtained from breeders fed the 104R-104P diet, compared to chicks obtained from breeders fed any of the other dietary treatments ( $P = 0.04$ ; Table 5).

## Broiler Performance

Weekly broiler BW can be found in supplementary Table S1. At slaughter age (d 32), a three-way interaction was observed on broiler BW and ADG between breeder rearing diet, breeder production diet and broiler diet ( $P \leq 0.04$ ; Figure 1 and Table 6). Within the 90% dLys treatment, broilers from the 104R-96P breeders had on

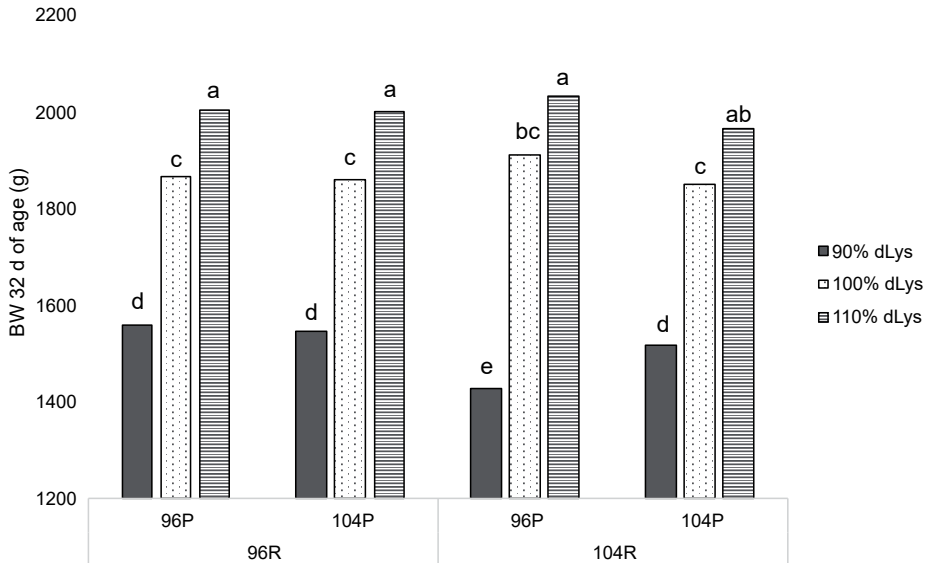


Figure 1. BW at 32 d of age of broilers obtained from broiler breeders at 2 different ages (29 and 38 wk of age), which were fed 2 rearing diets from hatch to 21 wk of age (96R and 104R) and 2 production diets from 21 wk of age onward (96P and 104P), differing in energy-to-protein ratio (96% AME<sub>n</sub> or 104% AME<sub>n</sub>). Broilers were fed 3 diets from hatch to 32 d of age differing in digestible lysine (dLys) content (90%, 100% or 110% dLys).

average a 115 g lower BW and a 3.5 g/d lower ADG, compared to the other breeder dietary treatments. Within the 100% dLys, no effect of breeder dietary treatment was observed on broiler BW or ADG. Within the 110% dLys, no effect of breeder dietary treatment was observed on broiler BW, whereas broiler originating from the 104R-96P breeders, had a 2.4 g/d higher ADG, compared to broilers from the 104R-104P breeder diet, with the other 2 breeder dietary treatments in between (Table 6).

An two-way interaction was observed on ADFI between breeder rearing diet and broiler diet ( $P = 0.002$ ; Table 6). Broilers from the 104R breeders, that were fed a 90% dLys diet, had on average a 3.9 g lower ADFI, compared to broilers from 96R breeders, whereas this difference was not observed when broilers were fed a 100% or 110% dLys diet. No interaction was observed between breeder rearing diet, breeder production diet and broiler diet on FCR or FCR<sub>c</sub> (Table 6). Broilers on 100% dLys had a 0.105 lower FCR than broilers on 90% dLys and broilers on 110% dLys had a 0.056 lower FCR than broilers on 100% dLys (Table 6).

No interactions were observed breeder rearing diet, breeder production diet and broiler diet on first week or overall mortality (data not shown). First week mortality

Table 6. Average daily gain (ADG; g/d), average daily feed intake (ADFI; g/d) and feed conversion ratios (FCR; kg of feed/kg of BW gain) of broilers from 0 to 32 d of age, obtained from broiler breeders at 2 different ages (29 and 38 wk of age), which were fed 2 rearing diets from hatch to 21 wk of age (96R and 104R) and 2 production diets from 21 wk of age onward (96P and 104P), differing in energy-to-protein ratio (96% AME<sub>n</sub> or 104% AME<sub>n</sub>). Broilers were fed 3 diets from hatch to 32 d of age differing in digestible lysine (dLys) content (90%, 100% or 110% dLys).

		d 0-32			
Item		ADG	ADFI	FCR	FCRc
Rearing diet (n=36)					
96R		55.1	76.9	1.403	1.461
104R		54.4	76.1	1.407	1.473
SEM		0.3	0.4	0.005	0.007
Production diet (n=36)					
96P		54.9	76.6	1.403	1.463
104P		54.6	76.4	1.407	1.471
SEM		0.3	0.4	0.005	0.007
dLys (n=24)					
90%		46.0	68.6	1.494 <sup>a</sup>	1.640 <sup>a</sup>
100%		57.2	79.4	1.389 <sup>b</sup>	1.427 <sup>b</sup>
110%		61.1	81.5	1.333 <sup>c</sup>	1.333 <sup>c</sup>
SEM		0.4	0.5	0.006	0.008
Rearing diet x Production diet (n=18)					
96R	96P	55.2	77.0	1.402	1.459
	104P	55.0	76.8	1.404	1.463
104R	96P	54.6	76.2	1.404	1.467
	104P	54.1	76.0	1.411	1.478
SEM		0.5	0.6	0.006	0.009
Rearing diet x dLys (n=12)					
96R	90%	47.2	70.6 <sup>c</sup>	1.496	1.630
	100%	56.9	78.6 <sup>b</sup>	1.382	1.423
	110%	61.2	81.5 <sup>a</sup>	1.331	1.331
104R	90%	44.7	66.7 <sup>d</sup>	1.493	1.651
	100%	57.4	80.2 <sup>ab</sup>	1.395	1.431
	110%	61.0	81.5 <sup>a</sup>	1.334	1.336
SEM		0.6	0.7	0.008	0.011
Production diet * dLys (n=12)					
96P	90%	45.3	67.8	1.494	1.646
	100%	57.7	80.0	1.387	1.420
	110%	61.8	82.0	1.328	1.322
104P	90%	46.6	69.5	1.494	1.635
	100%	56.7	78.8	1.390	1.434
	110%	60.5	80.9	1.338	1.344
SEM		0.6	0.7	0.008	0.011

Table 6. Continued

				d 0-32			
Item				ADG	ADFI	FCR	FCR <sub>c</sub>
Rearing diet * Production diet * dLys (n=6)							
96R	96P	90%	47.4 <sup>c</sup>	70.8	1.494	1.625	
		100%	57.0 <sup>d</sup>	78.8	1.383	1.423	
		110%	61.3 <sup>ab</sup>	81.4	1.329	1.328	
96R	104P	90%	47.0 <sup>c</sup>	70.4	1.498	1.634	
		100%	56.8 <sup>d</sup>	78.5	1.380	1.422	
		110%	61.2 <sup>ab</sup>	81.6	1.334	1.334	
104R	96P	90%	43.3 <sup>f</sup>	64.7	1.495	1.667	
		100%	58.4 <sup>cd</sup>	81.2	1.390	1.417	
		110%	62.2 <sup>a</sup>	82.7	1.327	1.317	
104R	104P	90%	46.1 <sup>c</sup>	68.7	1.490	1.635	
		100%	56.5 <sup>d</sup>	79.1	1.401	1.446	
		110%	59.8 <sup>bc</sup>	80.3	1.342	1.354	
SEM				0.8	1.1	0.010	0.015
Breeder age (n=24)							
29 weeks				53.6	73.9	1.389 <sup>b</sup>	1.463
38 weeks				56.0	79.1	1.421 <sup>a</sup>	1.470
SEM				0.3	0.4	0.005	0.007
P-value							
Rearing diet				0.12	0.19	0.44	0.17
Production diet				0.45	0.78	0.44	0.35
dLys				<0.001	<0.001	<0.001	<0.001
Rearing diet x Production diet				0.77	0.96	0.69	0.65
Rearing diet x dLys				0.02	0.002	0.47	0.70
Production diet x dLys				0.06	0.10	0.78	0.24
Rearing diet x Production diet x dLys				0.03	0.06	0.65	0.13
Breeder age				<0.001	0.003	<0.001	0.40

<sup>a-f</sup>LSmeans within a column and factor lacking a common superscript differ ( $P \leq 0.05$ ).

was significantly higher for broilers on the 100% dLys diet (7.5%), compared to the 90% dLys diet (2.8%), with the 110% dLys diet in between (5.6%;  $P = 0.02$ ). Overall mortality (d 0-32) was lower for broilers on the 90% dLys diet (4.0%), compared to the 100% and 110% dLys diet (8.7% and 8.4%, respectively;  $P = 0.008$ ). Breeder dietary treatments did not affect first week or overall mortality. First week mortality was 9.8% for broilers from the 29 wk old breeders, compared to 0.8% first week mortality for broilers from the 38 wk old breeders ( $P < 0.001$ ). First week mortality was mainly due to yolk-sac inflammation.

## Breast Meat Yield, Myopathies and Body Composition

An interaction was observed between breeder rearing diet, breeder production diet and broiler diet on prevalence of wooden breast. However, after Fisher adjustments for multiple comparisons, no significant differences were observed (data not shown). Furthermore, no interactions were observed between breeder rearing diet, breeder production diet and broiler diet on breast meat yield, prevalence of white striping, or broiler body composition (Table 7). Therefore, only main effects are presented in Table 7. Breeder dietary treatments did not affect breast meat yield, prevalence of white striping, wooden breast or broiler body composition at slaughter age (Table 7). Breast meat yield was affected by broiler diet ( $P < 0.001$ ), where broilers on 100% dLys had a 110 g heavier breast filet than broilers on 90% dLys and broilers on 110% dLys had a 69 g heavier breast filet than broilers on 100% dLys (Table 7). Prevalence of white striping or wooden breast was not affected by broiler diet. Broilers on 100% dLys had a 64 g larger protein mass than broilers on 90% dLys and broilers on 110% dLys had a 27 g larger protein mass than broilers on 100% dLys ( $P < 0.001$ ; Table 7). Broilers on 90% dLys had on average a 32 g smaller fat mass than broilers on 100% or 110% dLys ( $P < 0.001$ ; Table 7). After corrections for body weight differences, broilers on 100% dLys had a 0.5% higher protein content (16.7%) than broilers on 90% dLys (16.2%) and broilers on 110% dLys had a 0.3% higher protein content (17.0%) than broilers on 100% dLys ( $P < 0.001$ ). Broilers on 100% dLys had a 0.7% lower fat content (13.5%) than broilers on 90% dLys (14.2%) and broilers on 110% dLys had a 1.2% lower fat content (12.3%) than broilers on 100% dLys ( $P < 0.001$ ).



Table 7. BW, empty BW, breast weight, prevalence of breast myopathies and body composition of broilers at slaughter age (d 32), obtained from broiler breeders at 2 different ages (29 and 38 wk of age), which were fed 2 rearing diets from hatch to 21 wk of age (96R and 104R) and 2 production diets from 21 wk of age onward (96P and 104P), differing in energy-to-protein ratio (96% AME<sub>n</sub> or 104% AME<sub>n</sub>). Broilers were fed 3 diets from hatch to 32 d of age differing in digestible lysine (dLys) content (90%, 100% or 110% dLys).

Item	BW <sup>1</sup> (g)	Empty BW <sup>1</sup> (g)	Breast filet (g)	White striping <sup>2</sup> (%)	Wooden breast <sup>3</sup> (%)	Body composition	
						Protein (g)	Fat (g)
Rearing diet (n=36)							
96R	1820	1669	352	40.3	12.5	275	220
104R	1810	1642	339	30.6	15.3	276	217
SEM	15	18	5	5.1	7.3	2	5
Production diet (n=36)							
96P	1817	1652	346	36.1	16.7	276	215
104P	1812	1659	345	34.7	11.1	275	221
SEM	15	18	5	5.1	7.3	2	5
dLys (n=24)							
90%	1532 <sup>c</sup>	1387 <sup>c</sup>	249 <sup>c</sup>	31.3	6.3 <sup>b</sup>	224 <sup>c</sup>	197 <sup>b</sup>
100%	1886 <sup>b</sup>	1714 <sup>b</sup>	359 <sup>b</sup>	37.5	12.5 <sup>ab</sup>	288 <sup>b</sup>	231 <sup>a</sup>
110%	2027 <sup>a</sup>	1866 <sup>a</sup>	428 <sup>a</sup>	37.5	22.9 <sup>a</sup>	315 <sup>a</sup>	227 <sup>a</sup>
SEM	18	24	6	6.2	7.9	3	5
Breeder age (n=24)							
29 weeks	1781 <sup>b</sup>	1610 <sup>b</sup>	327 <sup>b</sup>	25.0 <sup>b</sup>	26.4 <sup>a</sup>	268 <sup>b</sup>	215
38 weeks	1849 <sup>a</sup>	1702 <sup>a</sup>	364 <sup>a</sup>	45.8 <sup>a</sup>	1.4 <sup>b</sup>	283 <sup>a</sup>	221
SEM	20	14	6	5.4	4.8	2	5
P-value							
Rearing diet	0.66	0.28	0.08	0.19	0.66	0.94	0.55
Production diet	0.81	0.54	0.84	0.85	0.37	0.70	0.26
dLys	<0.001	<0.001	<0.001	0.72	0.09	<0.001	<0.001
Rearing diet x Production diet	0.93	0.95	0.78	0.19	0.66	0.69	0.57
Rearing diet x dLys	0.10	0.51	0.14	0.78	0.52	0.10	0.21
Production diet x dLys	0.08	0.29	0.41	0.18	0.70	0.06	0.27
Rearing diet x Production diet x dLys	0.73	0.80	0.56	0.50	0.04	0.84	0.80
Breeder age	0.002	0.003	<0.001	0.005	<0.001	<0.001	0.19

<sup>a-c</sup> LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup> Average (empty) BW of randomly selected broilers for breast filet weight, prevalence of white striping and wooden breast and body composition (per pen 1 male and 1 female broilers).

<sup>2</sup> Percentage of broilers with score 1 (small white lines <1 mm) or score 2 (large white lines 1-2 mm) white striping.

<sup>3</sup> Percentage of broilers with score 1 (part of breast muscle is hardened) or score 2 (whole breast muscle is hardened) wooden breast.

## DISCUSSION

The aim of this study was to evaluate the interaction between maternal dietary energy-to-protein ratio during the rearing and production period and offspring dietary energy-to-protein ratio on offspring performance. First, the effects of maternal dietary energy-to-protein ratio during the rearing and production period will be discussed on incubation parameters and chick quality at hatch. Hereafter, the interaction between maternal and offspring dietary energy-to-protein ratio on offspring performance will be discussed.

### Maternal dietary energy-to-protein ratio

Breeders that were fed a 104% AME<sub>n</sub> diet during either rearing or production had 0.2 to 0.3 g smaller selected hatching eggs compared to a 96% AME<sub>n</sub> breeder diet. Although no interaction between breeder rearing and production diet was observed, breeders that were fed a 104% AME<sub>n</sub> diet during rearing and production had 0.4 g smaller selected hatching eggs compared to breeders that were fed a 96% AME<sub>n</sub> diet during rearing and production. This is in line with observations from Heijmans et al. (2022), who reported a 0.6 g smaller hatching egg when breeders were fed a 104% AME<sub>n</sub> diet compared to a 96% AME<sub>n</sub> diet both during rearing and production. Hatching eggs were most probably smaller due to a 5.7% and 10.4% lower CP intake for breeders fed a 104% AME<sub>n</sub> diet during rearing and production, respectively compared to a 96% AME<sub>n</sub> diet (Heijmans, unpublished data). Van Emous et al. (2013, 2015a) did not observe a carry-over effect of a 3.5 to 5.4% lower CP intake than standard during rearing on egg weight during production. In the current study, only EW at 2 ages was taken into account and hatching eggs were selected based on average EW per treatment, whereas Van Emous et al. (2013, 2015b) measured EW weekly from all eggs produced up to 45 wk of age.

Relative EW loss during incubation was 0.19% lower for eggs originating from breeders fed the 104% AME<sub>n</sub> diet during production, compared to eggs originating from breeders fed the 96% AME<sub>n</sub> diet during production. Heijmans et al. (2022a) did not observe a significant difference in relative EW loss during incubation between the 96% AME<sub>n</sub> diet and 104% AME<sub>n</sub> diet, although numerically a comparable difference of 0.21% was observed. Egg weight loss during incubation is mainly due to water loss. The amount of water lost during incubation is a result of water vapor pressure differences between the egg and its surrounding, which was similar for all eggs in the current study, and eggshell characteristics (Molenaar et al., 2010b). Eggshell characteristics involved in water loss are for example eggshell thickness, porosity, cuticula or membrane characteristics (Narushin and Romanov, 2002; Hincke et al., 2011). Heijmans et al. (2022b) observed a numerical difference of 1 µm thicker eggshell in

eggs of breeders fed a 104% AME<sub>n</sub> diet compared to a 96% AME<sub>n</sub> diet. As eggshell porosity is positively correlated with eggshell thickness (Narushin and Romanov, 2002), it can be speculated that this subtle difference in eggshell thickness might have affected relative EW loss.

Dietary energy-to-protein ratio did not affect breeder fertility or hatchability. Others also did not observe an effect of 1.0 to 17.5% lower CP intake during rearing and/or production (Hocking et al., 2002; Mohiti-Asli et al., 2012; Van Emous et al., 2015a; b, 2018; Heijmans et al., 2022a) on fertility or hatchability. As discussed in Heijmans et al. (2022), breeder fertility and hatchability is most probably not affected by dietary energy-to-protein ratio as long as CP intake is not severely reduced (max. 17.5%) and diets have a balanced amino acid profile.

Hatchling weight was 0.2 g lower when breeders were fed 104% AME<sub>n</sub> production diet (10.4% lower CP intake) compared to breeders fed a 96% AME<sub>n</sub> diet, as a result of a lower hatching egg weight. In line with this, Lesuisse et al. (2017) and Heijmans et al. (2021; 2022a) observed a 4.0 and 0.4 g lower hatchling weight when breeders had a 22.8% and 14.5% lower CP intake during both rearing and production, respectively. Contradictory, Van Emous et al. (2015b, 2018) did not observe an effect on egg weight or hatchling weight of a 9.6% lower CP intake during production (Van Emous et al., 2018) or a 1.0% higher or 2.0% lower dietary energy intake during production (Van Emous et al., 2015b). It can be speculated that in studies of Van Emous et al. (2015b, 2018) relative differences in CP and energy intake were not enough to detect differences on egg or hatchling weight. Rearing diet did not affect hatchling weight in the current study, which is in line with Van Emous et al. (2015a; b) whom did not observe an effect on egg weight or hatchling weight of a 3.5 to 5.4% lower CP intake during rearing (Van Emous et al., 2015a; b). It can be suggested that hatchling weight is affected by dietary energy-to-protein ratio, at least when breeder diets are altered during the production phase.

To our knowledge, no other studies are available that evaluate day-old chick body composition in response to different breeder diets. Yolk-free body composition or residual yolk protein content was not affected by the breeder diet. Fat content in the residual yolk was higher in chicks of breeders that were fed 104% AME<sub>n</sub> diet during both rearing and production, compared to the other breeder dietary treatments. Residual yolk fat content was higher either due to a higher yolk fat content at start of incubation or due to a lower fat uptake from the yolk during incubation. However, as the fat content in the yolk-free body composition did not differ between treatments, it can be assumed that total fat uptake was similar between treatments. This suggests that yolk fat content was higher at start of incubation in hatching eggs obtained from

breeder fed 104% AME<sub>n</sub> diet during both rearing and production. Heijmans et al. (2022b) did not observe a difference in yolk dry matter content in eggs from breeders fed a 96% or 104% AME<sub>n</sub> diet. However, in that study yolk fat and yolk protein content were not determined. It can be speculated that breeders fed a higher dietary energy-to-protein ratio during both rearing and production can partition more fat towards the yolk compared to breeders fed a lower dietary energy-to-protein ratio.

### **Interaction maternal and offspring dietary energy-to-protein ratio**

In the current study, an interaction between maternal and offspring diet was observed on feed intake and growth. Broilers originating from breeders fed the 104R diet, that were fed a low protein broiler diet (90% dLys) had a lower feed intake, compared to broilers from breeders fed the 96R diet, whereas this effect was not observed in broilers fed a standard of high protein diet (100% and 110% dLys). Feed intake in poultry is under control of mechanical (e.g. tension receptors), chemical (e.g. macro nutrients) and hormonal (e.g. ghrelin, leptin, insulin) signals (Richards et al., 2010). Breeders fed a 104% AME<sub>n</sub> rearing diet had a 5.7% lower CP intake compared to a 96% AME<sub>n</sub> rearing diet (Heijmans, unpublished data). The lower maternal CP intake might have triggered epigenetic changes in the offspring (Rao et al., 2009). Potentially, the combination of chemical (low protein) and altered hormonal signals (epigenetics) might have induced a lower feed intake. It can be speculated that these epigenetic changes might only come to expression under a nutritional stressful (low protein) situation in broilers (Price, 1998). The negative effect on feed intake of a 104R maternal diet under low protein broilers diets was enhanced when these breeders were fed a 96P diet. This led to a lower growth and slaughter BW for broilers originating from breeders fed the 104R-96P diet, that were fed a low protein broiler diet (90% dLys) compared to broilers from the other breeder dietary treatments. Breeders fed the 104R diet followed by a 96P diet had a relative low CP intake during rearing (-5.7%) and switched to a higher CP intake during production (+10.7%) compared to the counterparts (Heijmans, unpublished data). Switching from a restricted to a relative abundant environment might have changed hormone status and gene expression of several metabolic hormones in breeders (Richards et al., 2003; Sun et al., 2006). This includes, among others, a lower expression of glucagon (Richards et al., 2003; Sun et al., 2006) and a higher expression of insulin and leptin (Sun et al., 2006). In turn, these hormones can inhibit feed intake (Richards and Proszkowiec-Weglarz, 2007; Richards et al., 2010). As mentioned before, it can be speculated that this information is transferred to the offspring of 104R-96P breeders and only comes to expression under a nutritional stressful situation (Price, 1998).

## CONCLUSIONS

It can be concluded that energy-to-protein ratio in the breeder diet during either rearing or production had no effect on fertility or hatchability and had only minor effects on chick quality parameters. An interaction was observed between broiler breeder and broiler dietary energy-to-protein ratio. Broilers obtained from breeders fed a higher dietary energy-to-protein ratio during rearing and a low dietary energy-to-protein ratio during production had a lower feed intake and growth, compared to other breeder dietary treatments, but only when broilers were fed a low protein diet. This might be due to epigenetic changes in metabolic hormones which regulate feed intake, which only come to expression under a nutritional stressful situation (low protein).

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## SUPPLEMENTARY INFORMATION

Table S1. BW at different ages of broilers obtained from broiler breeders at 2 different ages (29 and 38 wk of age), which were fed 2 rearing diets from hatch to 21 wk of age (96R and 104R) and 2 Production diets from 21 wk of age onward (96P and 104P), differing in energy-to-protein ratio (96% AME<sub>n</sub> or 104% AME<sub>n</sub>). Broilers were fed 3 diets from hatch to 32 d of age differing in digestible lysine (dLys) content (90%, 100% or 110% dLys).

Item		BW <sup>1</sup> (g)					
		0 d	7 d	14 d	21 d	28 d	32 d
Rearing diet							
	96R	42.5 <sup>a</sup>	172.5	444 <sup>a</sup>	877	1,437	1,806
	104R	42.2 <sup>b</sup>	171.0	438 <sup>b</sup>	872	1,431	1,784
	SEM	0.1	0.6	2	5	8	9
Production diet							
	96P	42.6 <sup>a</sup>	172.1	441.0	871	1,432	1,800
	104P	42.2 <sup>b</sup>	171.4	442.0	878	1,435	1,790
	SEM	0.1	0.6	2	5	8	9
dLys							
	90%	42.4	162.8 <sup>c</sup>	391 <sup>c</sup>	754 <sup>c</sup>	1,214 <sup>c</sup>	1,513
	100%	42.3	173.1 <sup>b</sup>	448 <sup>b</sup>	900 <sup>b</sup>	1,485 <sup>b</sup>	1,872
	110%	42.3	179.4 <sup>a</sup>	485 <sup>a</sup>	971 <sup>a</sup>	1,603 <sup>a</sup>	2,000
	SEM	0.1	0.7	2	6	10	12
Rearing diet x Production diet							
	96R 96P	42.7	172.6	445	874	1,436	1,810
	96R 104P	42.3	172.5	444	881	1,438	1,803
	104R 96P	42.5	171.5	438	869	1,428	1,791
	104R 104P	42.0	170.4	439	876	1,433	1,777
	SEM	0.1	0.9	2	7	12	14
Rearing diet x dLys							
	96R 90%	42.7	163.9	398	770 <sup>c</sup>	1,247 <sup>c</sup>	1,554
	96R 100%	42.4	173.0	447	893 <sup>b</sup>	1,470 <sup>b</sup>	1,864
	96R 110%	42.5	180.7	488	968 <sup>a</sup>	1,593 <sup>a</sup>	2,001
	104R 90%	42.2	161.6	385	737 <sup>d</sup>	1,180 <sup>d</sup>	1,472
	104R 100%	42.3	173.3	449	907 <sup>b</sup>	1,499 <sup>b</sup>	1,880
	104R 110%	42.2	178.0	482	973 <sup>a</sup>	1,613 <sup>a</sup>	2,000
	SEM	0.1	1.1	3	8	16	17
Production diet x dLys							
	96P 90%	42.7	161.7	387 <sup>d</sup>	745	1,210	1,494
	96P 100%	42.6	174.7	450 <sup>b</sup>	903	1,491	1,889
	96P 110%	42.5	179.8	487 <sup>a</sup>	965	1,605	2,018
	104P 90%	42.2	163.8	396 <sup>c</sup>	762	1,226	1,532
	104P 100%	42.1	171.5	446 <sup>b</sup>	897	1,479	1,855
	104P 110%	42.2	179.0	482 <sup>a</sup>	976	1,600	1,982
	SEM	0.1	1.1	3	8	16	17

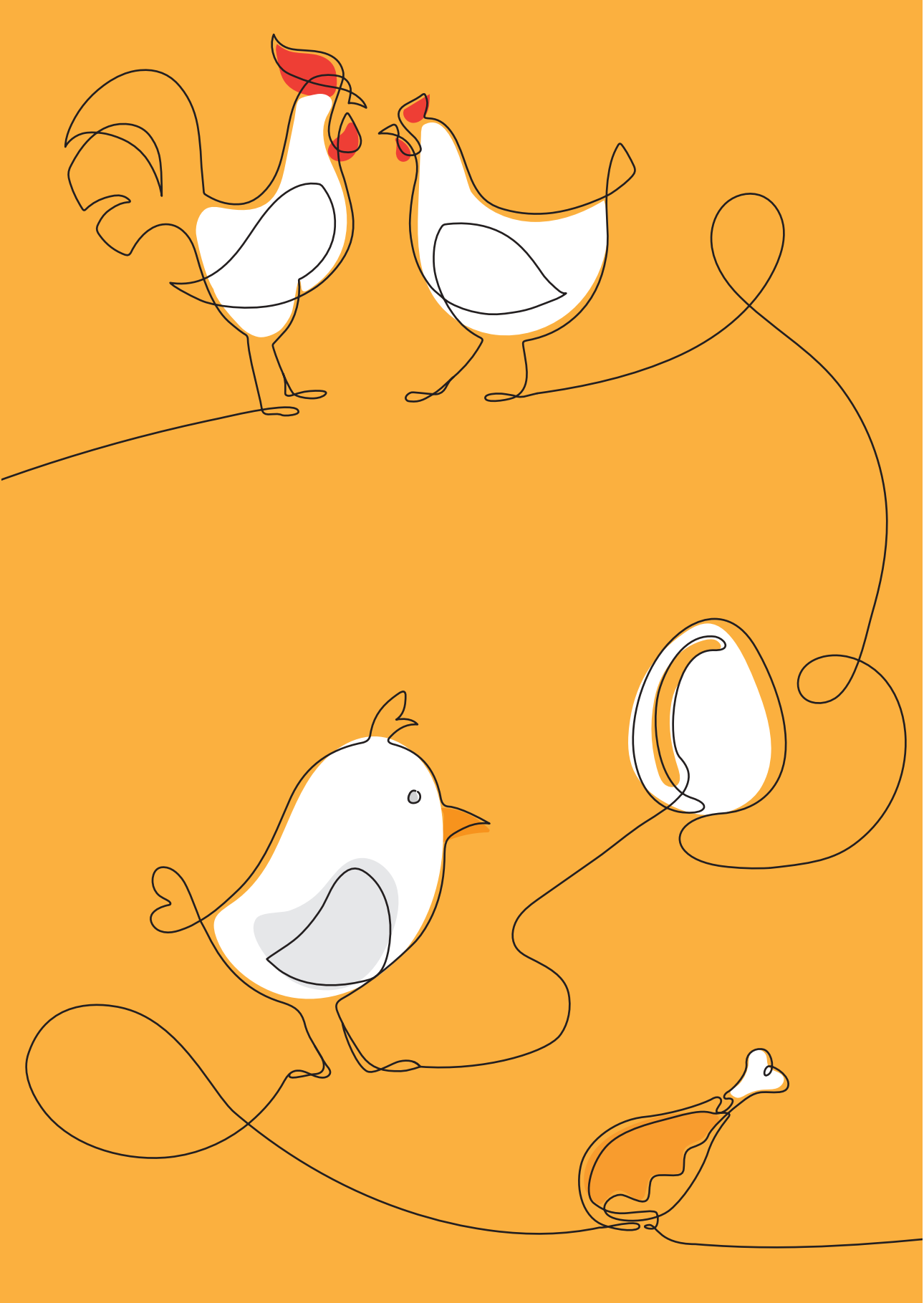


Table S1. Continued

Item			BW <sup>1</sup> (g)					
			0 d	7 d	14 d	21 d	28 d	32 d
Rearing diet x Production diet x dLys								
96R	96P	90%	43.0	162.7	397	772	1,259	1,561 <sup>d</sup>
		100%	42.6	174.2	450	895	1,464	1,866 <sup>c</sup>
		110%	42.6	180.8	489	954	1,585	2,003 <sup>a</sup>
96R	104P	90%	42.5	165.2	400	769	1,236	1,547 <sup>d</sup>
		100%	42.2	171.7	444	892	1,476	1,861 <sup>c</sup>
		110%	42.3	180.6	487	982	1,601	2,000 <sup>a</sup>
104R	96P	90%	42.5	160.7	377	719	1,142	1,427 <sup>c</sup>
		100%	42.5	175.2	450	912	1,517	1,911 <sup>bc</sup>
		110%	42.4	178.7	486	976	1,626	2,034 <sup>a</sup>
104R	104P	90%	41.9	162.5	393	755	1,217	1,517 <sup>d</sup>
		100%	42.0	171.3	448	903	1,482	1,850 <sup>c</sup>
		110%	42.1	177.4	477	970	1,600	1,965 <sup>ab</sup>
SEM			0.1	1.5	5	12	23	25
Breeder age (n=24)								
29 weeks			39.6 <sup>b</sup>	161.9 <sup>b</sup>	428 <sup>b</sup>	870	1,397 <sup>b</sup>	1,755 <sup>b</sup>
38 weeks			45.1 <sup>a</sup>	181.6 <sup>a</sup>	455 <sup>a</sup>	879	1,470 <sup>a</sup>	1,835 <sup>a</sup>
SEM			0.1	0.6	2	5	8	9
P-value								
Rearing diet			<0.001	0.09	0.04	0.59	0.65	0.14
Production diet			<0.001	0.49	0.90	0.29	0.83	0.49
dLys			0.43	<0.001	<0.001	<0.001	<0.001	<0.001
Rearing diet x Production diet			0.41	0.58	0.58	1.00	0.91	0.83
Rearing diet x dLys			0.15	0.35	0.07	0.02	0.008	0.02
Production diet x dLys			0.38	0.06	0.05	0.39	0.49	0.08
Rearing diet x Production diet x dLys			0.75	0.99	0.35	0.10	0.06	0.04
Breeder age			<0.001	<0.001	<0.001	0.19	<0.001	<0.001

<sup>a-d</sup> LSmeans within a column and factor lacking a common superscript differ (P≤0.05).

<sup>1</sup>At 14 d of age, 2 adjacent pens from the same treatment were merged. n = 12 per treatment for d 0, 4, 7, and 14, and n = 6 per treatment for d 21, 28, and 32.



# Chapter 7

## General discussion

*‘Niet meer afgeven nou’*  
*Hans*

An important link in the poultry meat production chain is the quality of day-old chicks (Decuypere et al., 2001). High quality day-old chicks are crucial for performance up to slaughter age, but also for welfare (Tona et al., 2005; Van de Ven et al., 2012). Strategies aiming for an improvement in chick quality therefore help to improve sustainability of poultry meat production. Most of the research on improving chick quality has focused on factors post-oviposition and during incubation. Maternal factors pre-oviposition, however, like maternal nutrition, also showed potential to influence chick quality. A mother passes on both genetic and non-genetic information to her offspring, which might influence her offspring's fitness and developmental plasticity (Mousseau and Fox, 1998). Maternal nutrition plays an important role in the transfer of information from mother to offspring.

The aim of this thesis was to investigate the impact of maternal nutrition on chick quality and broiler performance. More specifically, nutrition was used to influence maternal growth curves (via a change in feeding level and consequently body weight at a given age) and maternal body composition (via a change in dietary energy-to-protein ratio). Effects of these nutritional interventions were investigated on egg characteristics, chick quality and broiler performance. In the *General Introduction*, it was hypothesized that a higher maternal growth curve results in higher egg weights, which will improve chick quality and broiler performance. Furthermore, it was hypothesized that feeding a higher dietary energy-to-protein ratio, by means of a higher dietary energy content, results in a fatter broiler breeder. In turn, a fatter broiler breeder can partition more nutrients towards the egg, which will improve chick quality and broiler performance. Two studies were conducted to challenge these hypotheses. Within each study, one breeder experiment and two consecutive broiler experiments were conducted. In this thesis, results are presented of the broiler breeder experiment as well as the consecutive broiler experiments of the first study (*Chapters 2 to 5*) and the results are presented of the broiler experiments of the second study (*Chapter 6*). In this *General Discussion* the findings from both studies are integrated and discussed. The overall findings of the different maternal treatments will be presented in connecting the dots. Then the impact of maternal nutrition on chick quality will be discussed and how these chick quality parameters potentially influence broiler performance. Furthermore, potential transgenerational epigenetics effects of maternal nutrition on broiler performance will be discussed. Hereafter, the impact of breeder nutrition on egg weight will be discussed. The relationship between breeder body weight, body composition and nutrient intake and egg weight will be further investigated. Then the impact of different breeder feeding strategies on breeder performance and welfare will be discussed. Finally, main conclusions, practical implications and suggestions for future research are presented.

## CONNECTING THE DOTS

Figure 1 gives an overview of the observed effects of a higher breeder growth curve from hatch to 60 wk of age, compared to a standard breeder growth curve (Study I; *Chapters 2 to 5*). Overall, it was observed that a higher breeder growth curve required a higher feed allocation. A higher breeder growth curve does not benefit nor penalize performance of the breeder hens. A higher breeder growth curve resulted in heavier eggs, due to both a larger yolk and a larger albumen. Incubating these heavier eggs resulted in a heavier hatchlings with a larger yolk-free body mass, but hardly affected any of the other chick quality parameters. These heavier hatchlings had a higher feed intake post-hatch, resulting in a higher slaughter weight at 32 d of age.

Figure 2 gives an overview of the observed effects of feeding breeders a low dietary energy-to-protein ratio compared to a high dietary energy-to-protein ratio (Study I; *Chapters 2 to 5*). Overall, it was observed that feeding breeders a low dietary energy-to-protein ratio required a higher feed allocation to achieve the targeted growth compared to a high dietary energy-to-protein ratio. This led to a marginal increase in energy intake, but a substantial increase in protein intake. Feeding breeders a low

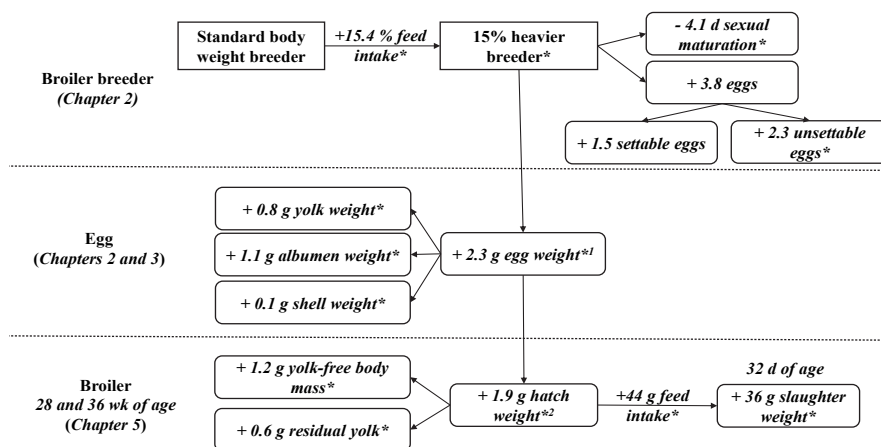


Figure 1. Overview of observed effects of a 15% higher breeder growth curve compared to a standard breeder growth curve from hatch to 60 wk of age. Treatments are represented in squared boxes. Values represent the absolute observed differences between a 15% higher breeder growth curve compared to a standard breeder growth curve. Values are represented in rounded boxes. <sup>1</sup>Egg weight was measured daily from 24 to 60 wk of age. Egg composition (yolk, albumen and shell weight) was measured every other week from a subsample of eggs (n = 4,320). <sup>2</sup>Hatch weight was measured from all first grade chicks at hatch. Yolk-free body mass and residual yolk was measured from a subsample of chicks at hatch (n = 480). \*P ≤ 0.05.

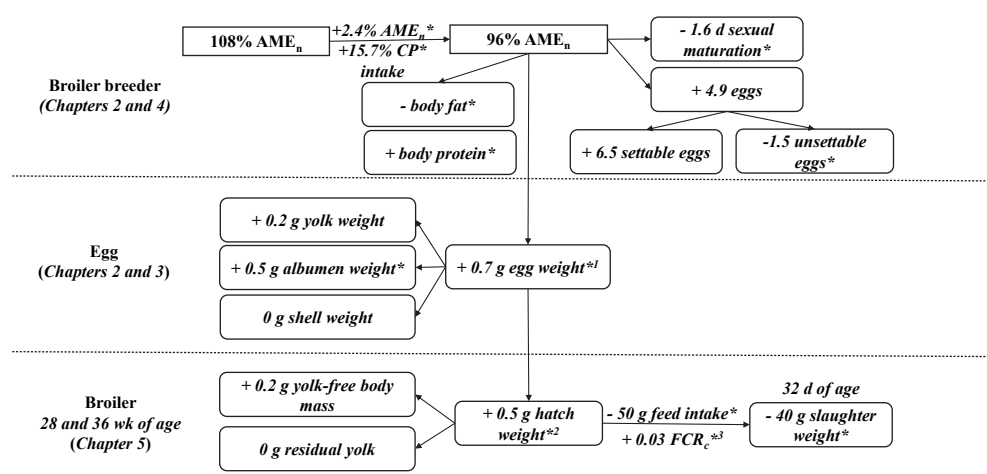


Figure 2. Overview of observed effects in Study I of feeding a breeder diet with 96% AMEn compared to feeding a breeder diet with 108% AMEn from hatch to 60 wk of age. Treatments are represented in squared boxes. Values represent the absolute observed difference between a 96% AMEn breeder diet compared to a 108% AMEn breeder diet, where a plus represents a higher value and a minus a lower value. Values are represented in rounded boxes. <sup>1</sup>Egg weight was measured daily from 24 to 60 wk of age. Egg composition (yolk, albumen and shell weight) was measured every other week from a subsample of eggs (n = 4,320). <sup>2</sup>Hatch weight was measured from all first grade chicks at hatch. Yolk-free body mass and residual yolk was measured from a subsample of chicks at hatch (n = 480). <sup>3</sup>Feed conversion ratio corrected for body weight differences (FCR<sub>c</sub>). \*P ≤ 0.05.

dietary energy-to-protein ratio benefits performance of the breeders hens. Feeding breeders a low dietary energy-to-protein ratio resulted in heavier eggs, mainly due to a larger albumen content. Incubating these heavier eggs resulted in heavier hatchlings, but did not affect any of the other chick quality parameters. These results were confirmed in the second study. In the first study, hatchlings obtained from breeders fed a low dietary energy-to-protein ratio had a lower feed intake and a higher corrected feed conversion ratio compared to hatchlings obtained from breeders fed a high dietary energy-to-protein ratio. This resulted in a lower slaughter weight for broilers obtained from breeders fed a low dietary energy-to-protein ratio compared to broilers obtained from breeders fed a high dietary energy-to-protein ratio. Contradictory to the first study, post-hatch performance in the second study was not affected by maternal diets, when broilers were fed a standard diet. A high maternal dietary energy-to-protein ratio during rearing resulted in lower feed intake, but only when broilers were fed a low protein diet. Furthermore, when broilers were fed a low protein diet, a high maternal dietary energy-to-protein ratio during rearing followed by a low maternal dietary energy-to-protein ratio during production resulted in a lower growth compared to the other maternal treatments (*Chapter 6*).

## IMPACT OF MATERNAL NUTRITION ON CHICK QUALITY AND BROILER PERFORMANCE

Chick quality is a difficult trait to define (Willemsen et al., 2008; Nariñ and Aydemir, 2021), but is mainly focused on quality directly post-hatch. In the current thesis, chick quality at hatch was measured both qualitatively (activity, navel, beak and leg score) and quantitatively (hatch weight, residual yolk weight, percentage second grade chicks). The relationship between chick quality at hatch and post-hatch performance is not consistent (Tona et al., 2003, 2004; Willemsen et al., 2008; Van de Ven et al., 2012). Below, the impact of maternal nutrition on chick quality parameters will be discussed and how these chick quality parameters potentially affect broiler performance. Hereafter, it will be discussed how maternal nutrition might affect broiler performance indirectly.

### Maternal nutrition does not affect qualitative chick quality at hatch

To my knowledge, no other studies are available that evaluated qualitative chick quality (activity, navel, beak and leg score) in response to different maternal nutritional strategies. In both studies, maternal nutrition hardly affected qualitative chick quality parameters. In the following paragraph it will be discussed that these chick quality parameters do not affect broiler performance.

### Qualitative chick quality at hatch does not affect broiler performance

In the current thesis, chicks were pooled after hatch per maternal treatment. Therefore, only average chick quality and body weight at 32 d of age per maternal treatment can be used for further analysis. Pearson correlations were determined per maternal age (= one broiler experiment;  $n = 8$  for study I per maternal age,  $n = 4$  for study II per maternal age) between average chick quality parameters and body weight at 32 d of age. Average chick quality parameters include percentage of chicks scored as weak, percentage of chicks with suboptimal navel closure, percentage of chicks with suboptimal beak quality and percentage of chicks with suboptimal leg quality. No significant correlations were observed between any of the chick quality parameters and body weight at 32 d of age (data not shown). Caution should be taken here, as only average values per maternal treatment were included for analysis, leading to a low number of replicates. Although, observations are in line with observations from Willemsen et al. (2008) and Van de Ven et al. (2012), who also did not observe a correlation between chick quality and post-hatch performance. In these studies and in the current thesis, only first grade chicks were included. Therefore, the lack of correlation between chick quality scoring and post-hatch performance might be due to a lack of chicks with anomalies (and thus suboptimal scores). Van de Ven et al. (2012) concluded that these chick quality parameters at hatch are mainly informative to assess the quality

of incubation. Therefore, for future broiler breeder nutritional research it is advised to particularly include chick weight at hatch as chick quality parameter, rather than qualitative chick quality parameters.

Maternal nutrition affects chick weight at hatch

In both studies of the current thesis, maternal nutrition affected chick weight at hatch, where a higher breeder growth curve or a lower dietary energy-to-protein ratio resulted in a higher chick weight at hatch (*Chapters 5 and 6*). A higher chick weight at hatch is the result of a higher hatching egg weight (*Chapters 2, 3 and 6*; Figure 3;  $R^2 = 0.99$ ). Other studies also observed a higher chick weight at hatch, when larger hatching eggs were incubated from breeders ranging from 29 to 59 wk of age (Wolanski et al., 2007; Ulmer-Franco et al., 2010; Nangsuay et al., 2011). In the current thesis, chick weight was on average 72.7% of the hatching egg weight, whereas this was on average 68.7% in the other studies. Discrepancy between the current thesis and the other studies might be due to moment of measurement of chick weight. In the current thesis, chick weight was measured within 6 hours after hatch, whereas in the other studies chick weight was measured at pulling. The time span between hatch of a chick until pulling could increase up to approximately 33 hours in the current thesis. During that time span, chicks do not have access to feed or water and chicks lose weight due to dehydration (Careghi et al., 2005; Van de Ven et al., 2009). At placement at the broiler farm, which was approximately 4 hours after pulling, chicks lost on average 2.6 g of body

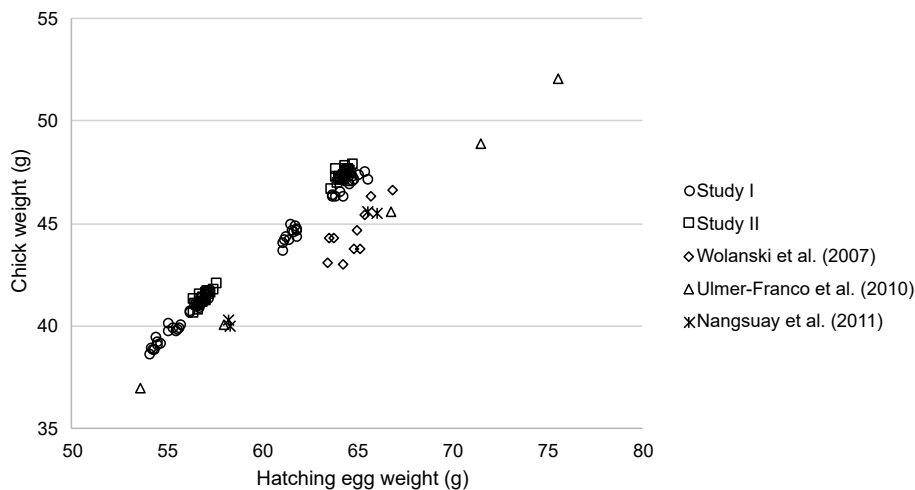


Figure 3. Relationship between hatching egg weight and chick weight at hatch. Each symbol of study I and study II represents the average hatching egg and chick weight per maternal pen. Studies included breeders ranging in age from 28 to 59 wk of age.



weight compared to body weight directly after hatch. In our studies, chick weight at placement at the broiler farm was on average 68.4% of the hatching egg weight, which is comparable to the other studies.

These results indicate that differences in chick weight are mainly due to differences in hatching egg weight. In turn, hatching egg weight was, among others, affected by maternal nutrition. This will be further discussed in the paragraph on egg weight.

### Chick weight at hatch affects broiler performance

Several studies have investigated the correlation between chick weight at hatch and body weight at slaughter age. Willemsen et al. (2008) observed a weak positive correlation ( $r = 0.24 - 0.35$ ) between chick weight at hatch and body weight at 35 d of age of broilers obtained from 3 different maternal ages (39, 42 and 53 wk of age). Van de Ven et al. (2012) also observed a weak positive correlation ( $r = 0.28$ ) between chick weight at hatch and body weight at 42 d of age for broilers obtained from old breeders (53 wk of age), whereas this correlation was not significant ( $r = 0.10$ ) for broilers obtained from younger breeders (35 wk of age). In the current thesis, chicks were pooled after hatch per maternal treatment. Therefore, individual body weight at placement on farm (day 0) was used for further analysis. Pearson correlations were determined between individual body weight at day 0 and individual body weight at 32 d of age at each maternal age (= one broiler experiment; Table 1). Furthermore, Pearson correlations were determined between average body weight at day 0 per pen and average post-hatch performance per pen at each maternal age (Table 2).

Table 1. Correlations between broiler body weight at placement on the research farm (day 0) and broiler body weight at slaughter age (day 32). n represents the number of individual broilers included in each correlation assessment, of which both the body weight at day 0 and body weight at day 32 was determined.

Study	Maternal age (wk)	n	Broiler dietary protein content <sup>1</sup>	Correlation coefficient	P-value
Study I	28	570	standard	0.16	<0.001
	36	562	standard	0.18	<0.001
Study II	29	219	low	0.06	0.37
		206	standard	0.05	0.49
		210	high	0.12	0.08
	38	236	low	-0.01	0.89
		232	standard	0.11	0.10
		225	high	0.25	<0.001

<sup>1</sup>Broiler dietary protein (digestible lysine) content was formulated at 90% (low), 100% (standard), or 110% (high) of breeder recommendations Aviagen (2019).

Table 2. Correlations between broiler body weight at placement on the research farm (day 0) and average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratios (FCR). n represents the number of pens included in each correlation assessment.

Study	Maternal age (wk)	n	Broiler dietary protein content <sup>1</sup>	Correlation coefficient			
				ADG	ADFI	FCR	FCRc <sup>2</sup>
Study I	28	32	standard	0.26	0.31*	0.26	0.12
	36	32	standard	0.42**	0.36**	0.03	-0.32*
Study II	29	12	low	0.29	0.32	0.07	-0.16
		12	standard	0.36	0.47	0.24	-0.05
		12	high	-0.03	-0.27	-0.42	-0.39
	38	12	low	-0.03	0.1	0.36	0.24
		12	standard	0.37	0.31	-0.27	-0.34
		12	high	0.24	0.12	-0.37	-0.35

<sup>1</sup>Broiler dietary protein (digestible lysine) content was formulated at 90% (low), 100% (standard), or 110% (high) of breeder recommendations Aviagen (2019).

<sup>2</sup>Corrected FCR to a standard body weight of 2000 g, calculated as  $FCR - (2000 - \text{actual body weight day 32}) / 100 \times 0.03$ .

\* $0.05 < P \leq 0.10$  and \*\*  $P \leq 0.05$ .

In the first study, a weak correlation ( $r = 0.16$  and  $0.18$ , for 28 and 36 wk of age, respectively; Table 1) was observed between broiler body weights at day 0 and 32. A lower correlation was observed in study I compared to Willemssen et al. (2008) and Van de Ven et al. (2012) as maternal treatments also affected post-hatch performance (*Chapters 5 and 6*), whereas in the other studies day-old chicks were obtained from one breeder flock that were all fed the same diet. In the first study, a higher body weight at day 0 correlated at both maternal ages with a higher feed intake ( $r = 0.31$  and  $0.36$ , for 28 and 36 wk of age, respectively; Table 2). It can be speculated that, in the first study, chicks with a higher body weight at placement on farm have a higher (physical) feed intake capacity, leading to a higher slaughter weight.

In the second study, no significant correlations were observed between body weights at day 0 and 32 (overall,  $r = 0.03$  and  $0.07$ ;  $P = 0.48$  and  $P = 0.07$ ; for 29 and 38 wk of age, respectively). In the second study, broilers were fed diets differing in dietary protein content, which already resulted in approximately 500 g difference in body weight at 32 d of age (*Chapter 6*). In this case, broiler dietary treatments potentially overruled the effect of a higher chick weight at hatch. Therefore, correlation coefficients were also analyzed per broiler dietary treatment (Table 1). At low or standard broiler dietary protein content body weight at placement on farm did not correlate with body weight at 32 d of age, whereas at high broiler dietary protein content a weak correlation ( $r = 0.12$  and  $0.25$ ) was observed (Table 1). In the second study, no



Figure 4. Relationship broiler body weight at placement on the farm (day 0) and broiler body weight at slaughter age (day 32). Each symbol represents an individual broiler.

significant correlations were observed between body weight at day 0 and post-hatch performance (Table 2).

This suggest that day-old chick body weight can partially explain differences in post-hatch performance, where a heavier day-old chick has a potential higher feed intake, leading to a higher slaughter weight. There is still a large variation, however, in post-hatch performance, which cannot be attributed to day-old chick body weight (Figure 4). As mentioned before, maternal nutrition and broiler nutrition also have an effect on post-hatch performance and can interfere with day-old chick body weight. The impact of maternal nutrition on post-hatch performance will be further discussed below.

### Maternal nutrition affects broiler performance potentially via transgenerational epigenesis

In both studies, broiler performance was affected by maternal dietary energy-to-protein ratio, without an effect of maternal nutrition on egg nutrients. Breeders fed a higher dietary energy-to-protein ratio had a higher feed restriction and lower crude protein intake (*Chapter 2*) compared to a lower dietary energy-to-protein ratio, which was comparable in study II (data not shown). It was hypothesized that these changes in maternal feed restriction level and crude protein intake induced transgenerational epigenetic effects, which affected feed intake (*Chapters 5 and 6*) and nutrient efficiency of the offspring (*Chapter 5*). To further investigate this hypothesis, the impact of maternal nutrition on offspring appetite in mammals will be discussed first, as there is a good body of evidence from mammalian models. Hereafter, potential effects in

avian species will be discussed, as there is less knowledge available in avian species. Lastly, the potential reason for discrepancy between obtained results from the first and second study of this thesis will be discussed in this paragraph as well.

### **Maternal undernutrition affects offspring appetite in mammals**

In mammalian models (e.g. rodents and ovine), there is a good body of evidence that maternal feed restriction during gestation affects offspring appetite (Levin, 2008; George et al., 2012; Lagisz et al., 2014, 2015), which are mainly guided by epigenetic changes (Ford and Long, 2012). An undernourished environment for the fetus during gestation can lead to a thrifty phenotype. This means that the fetus attempts to alter function of tissues and organs to maximize its chance of survival in a postnatal malnutritional environment (Ford and Long, 2012). In a meta-analysis of Lagisz et al. (2014, 2015) it was observed that mainly a protein restricted maternal diet induced a higher feed intake in rodents offspring, rather than an energy restricted maternal diet. When dams were fed energy dense, low protein diet, dams were not able to meet their protein requirements and their offspring developed under protein-deficient conditions. The higher feed intake of offspring from malnourished mothers was mainly due to changes in metabolic signals which should inhibit feed intake under normal conditions. This included leptin and insulin resistance and a reduced response to glucose (Levin, 2008; George et al., 2012).

### **Does maternal undernutrition also affect offspring appetite in birds?**

Unlike mammalian fetal development, whereby nutrients are supplied constantly across the placenta, deposition of nutrients into an avian egg are fixed at start of embryonic development. It is therefore unclear if a similar epigenetic mechanism occurs in avian species. A body of evidence is lacking in avian species for this mechanism, although there are some indications that a similar mechanism occurs in avian species as in mammalian species. In wild birds, it was observed that offspring from restricted fed mothers could compensate growth during the post-hatch period, when offered supplemental feed (Giordano et al., 2014), potentially indicating a higher appetite for offspring from restricted fed mothers. Furthermore, others observed a higher (compensatory) growth in offspring from protein restricted mothers (Rao et al., 2009; Lesuisse et al., 2017) and enhanced (nitrogen) efficiency (Lesuisse et al., 2017, 2018). Feed intake data were not provided in these studies (Rao et al., 2009; Lesuisse et al., 2017, 2018). Willems et al. (2015) observed a higher feed intake in offspring which experienced prenatal protein undernutrition. Results from this thesis also indicate a higher feed intake and improved efficiency in offspring from mothers that had higher dietary energy-to-protein ratio and thus a more severe feed (and protein) restriction compared to a lower dietary energy-to-protein ratio (*Chapter 5*), although this effect was not observed in the second study (*Chapter 6*).

Several studies have observed changes in metabolic hormones in response to maternal feed restriction in avian models, which are potentially guided by epigenetic changes (Ford and Long, 2012). This might explain differences in feed intake and consequently growth in offspring from feed or protein restricted hens. Rao et al. (2009) and Lesuisse et al. (2018) observed higher levels of plasma 3,3',5-triiodothyronine (T<sub>3</sub>) in offspring from protein restricted mothers, which may explain part of the higher post-hatch growth (Rao et al., 2009). Li et al. (2019) observed lower circulating levels of glucose and leptin and higher levels of glucagon in offspring from restricted fed mothers. In turn, these hormones can stimulate feed intake (Richards and Proszkowiec-Weglarz, 2007; Richards et al., 2010).

### Discrepancy broiler performance study I and study II

As mentioned before, this potential epigenetic effect of a higher maternal feed and protein restriction on offspring feed intake and feed efficiency was not observed in the second study of this thesis (*Chapter 6*). It remains unclear why these effects were not observed in the second study. It is speculated that these effects were not observed in the second study, as broilers had a lower feed and protein intake compared to the first study. In the first study, incubation and on-farm conditions were optimal for the offspring. This may have led to a high feed intake in all treatment groups (on average 8% higher compared to breeder guidelines; Aviagen, 2022), with an average digestible lysine intake of 947 mg/d per broiler from 0 to 32 d of age. Van der Waaij et al. (2011) only observed metabolic changes when offspring from restricted fed breeders were fed *ad libitum*, whereas this metabolic changes was not observed when offspring from restricted fed breeders were also restricted in feed intake. Also in mammals, metabolic changes of maternal undernutrition come to expression when offspring overeats (Levin, 2008). This might indicate that a high feed intake and high protein intake may have induced the expression of transgenerational epigenetic effects in the offspring from breeders with a higher maternal dietary energy-to-protein ratio.

In the second study, feed intake was on average 9% lower compared to breeder guidelines (Aviagen, 2022). Even in the 110% dLys group, feed intake was on average 3% lower compared to breeder guidelines. Average digestible lysine intake was 652, 836 and 945 mg/d per broiler from 0 to 32 d of age for the 90, 100 and 110% dLys treatments, respectively. As a comparable protein intake was observed in the first study and in the 110% dLys group in the second study, it can be speculated that feed intake capacity is more important than protein intake *per se* for the expression of epigenetic changes.

In *Chapter 6*, it was speculated that the lower maternal crude protein intake might have triggered other transgenerational epigenetic effects in broilers fed a low protein

diet, which induced altered hormonal signals in these broilers related to feed intake regulation. Breeders in the second study that switched at the end of rearing from a restricted to a relative abundant environment might have changed hormone status and gene expression of several metabolic hormones (Richards et al., 2003; Sun et al., 2006). This includes, among others, a lower expression of glucagon (Richards et al., 2003; Sun et al., 2006) and a higher expression of insulin and leptin (Sun et al., 2006). In turn, these hormones can inhibit feed intake (Richards and Proszkowiec-Weglarz, 2007; Richards et al., 2010). It was speculated that these changes were passed on to the offspring. As this low feed intake only occurred in broilers fed a low protein diet, it can be that these transgenerational epigenetic effects might only come to expression under a nutritional stressful (low protein) situation in broilers (Price, 1998).

In both studies, it was thus speculated that maternal dietary energy-to-protein ratio induced transgenerational epigenetic effects, which affected feed intake and nutrient efficiency in the offspring. Potentially these epigenetic effects mainly affected metabolic hormones which regulate feed intake. It is hypothesized that these transgenerational epigenetic effects only come to expression when a stressor (e.g. high feed intake or low protein diet) is applied for the offspring (Price, 1998; Hanson and Gluckman, 2008; Burton et al., 2022).

### **Limitations of the current thesis in relation to chick quality and broiler performance**

Although a linear relationship was observed between maternal dietary energy-to-protein ratio and broiler performance, also a significant quadratic relationship with an optimum at 104% AME<sub>n</sub> on growth and slaughter weight was observed (*Chapter 5*). This indicates that potentially a biological limit might have been reached for an enhanced performance. Further increment in maternal dietary energy-to-protein ratio is therefore not expected to lead to a higher broiler performance. It can be speculated that a decrement in maternal dietary energy-to-protein ratio (below 96% AME<sub>n</sub>) might result in a lower broiler performance. It remains unclear when the biological limit is reached of a lower maternal dietary energy-to-protein ratio on broiler performance.

A factorial approach (standard vs. 15% higher) was used to determine the impact of maternal growth curve on chick quality and broiler performance. It is speculated that a higher difference in maternal growth curve will result in a higher difference in egg weight, chick weight and broiler performance. Furthermore, in both studies, broilers were obtained from breeders which were younger than 40 wk of age. It remains unclear whether or not results are applicable to older breeders as well. It is therefore advised to include offspring from older breeders in future breeder studies. It is speculated that the impact of maternal body weight will be comparable in older breeders

as in younger breeders, as the difference in hatching egg weight is comparable. This is further discussed in the paragraph on egg weight. It is speculated that the impact of maternal dietary energy-to-protein ratio might be similar in older breeders, as a similar feed and protein restriction is applied in older breeders.

In the current thesis, gene expression levels or circulating levels of metabolic hormones were not determined in the offspring as this falls outside the scope of this thesis. It is therefore advised to particularly include these measurements in the offspring in future breeder nutrition research. The focus of these gene expression measurements should be on satellite cell activity (*Chapter 5*) and feed intake regulatory genes (*Chapter 6*). Furthermore, it is advised to include these measurements at least at 2 time points between hatch and slaughter and to apply a stressor, such as high and low dietary protein, in the period in between. At hatch, the difference in gene expression levels might serve as an indicator of the impact of breeder nutrition. If a stressor is applied post-hatch (e.g. low protein diet) and gene expression is measured after application of the stressor (e.g. at slaughter), it can be determined whether or not the stressor interacts with the transgenerational epigenetic changes induced by breeder nutrition. Besides gene expression levels, circulating levels of metabolic hormones, which regulate feed intake, like ghrelin, leptin and cholecystokinin (CCK), should be measured in the offspring as well.

It is questionable whether or not observed results of maternal dietary energy-to-protein ratio are also applicable to other genetic strains. Li et al. (2019) observed an interaction between different metabolic processes and maternal nutrition in fat and lean line breeders. They observed different serum levels of for example  $T_3$ , insulin, glucagon and leptin in the offspring of restricted fed fat and lean line breeders. This indicates that other strains might show a different transgenerational epigenetic response.

Furthermore, in the current thesis altered maternal diets were fed for a longer period (at least 20 wk). In study II, it was observed that altered maternal dietary energy-to-protein ratio during the rearing period alone can potentially induce transgenerational epigenetic changes. It remains unclear whether or not shorter periods (e.g. 0 to 6 wk of age) of altered dietary energy-to-protein ratio can also induce such transgenerational epigenetic changes. Future research should therefore investigate the critical window in which transgenerational epigenetic changes can alter offspring performance.

Overall, it can be concluded that chick weight at hatch is partly indicative for post-hatch performance and was affected by maternal nutrition. The higher chick weight at hatch was due to a higher hatching egg weight, which will be further discussed in the following paragraph. Potential transgenerational epigenetic effects interfere with

chick weight at hatch on post-hatch performance. These transgenerational epigenetic effects are potentially induced by changes in maternal dietary energy-to-protein ratio. Expression levels of these transgenerational effects might depend on stressors encountered during post-hatch life. Future maternal nutrition studies should therefore focus on transgenerational epigenetic pathways.

## IMPACT OF BREEDER NUTRITION ON EGG WEIGHT

A higher chick weight at hatch was due to a higher hatching egg weight (*Chapters 2, 3, 5 and 6*). The higher egg weight was reached by a combination of a higher yolk and albumen weight (*Chapter 3*). Consequently, the embryo had more nutrients available for development, which increased chick weight at hatch and potentially post-hatch performance. It is therefore interesting to further investigate which maternal factors determine egg weight. In the following paragraphs the relationship of maternal body weight, body composition and nutrient intake to egg weight is further investigated and discussed.

### Maternal body weight in relation to egg weight

In the current thesis, the aim for body weight differences between breeder groups was 15% throughout rearing and production. The first study aimed for a 15% difference in body weight between breeder groups, but during the production period the relative difference between breeder groups ranged from 12.7% up to 19.8% (*Chapter 2*). In turn, egg weight differences ranged from 1.7 g up to 3.2 g (*Chapter 3*). Other studies observed a 0.6 g lower to 1.2 g higher egg weight when breeders were 0.7 to 10.8% heavier compared to standard breeder guidelines (Renema et al., 2001; Gous and Cherry, 2004; Sun and Coon, 2005; Renema et al., 2007; Van Emous et al., 2013; Van der Klein et al., 2018; Salas et al., 2019; Afrouzیه et al., 2021). Combining the data of the current thesis with these studies, it appears that a larger relative difference in body weight will result in a larger difference in egg weight (Figure 5). Consequently, it can be hypothesized that if relative differences in growth curves are either smaller or larger than 15%, it will result in smaller or larger differences in egg weight and thus chick weight at hatch and, potentially, also post-hatch performance.

We hypothesized that a higher maternal body weight would result in higher egg weights, which will improve chick quality and offspring performance. *Chapters 2, 3 and 5* show that this hypothesis was correct. As mentioned in *Chapter 2*, the difference in egg weight is only expected when a relative difference in growth curve, and thus feed allowance, is maintained throughout rearing and production (Renema et al., 2001; Afrouzیه et al., 2021). No or smaller differences in egg weight are expected



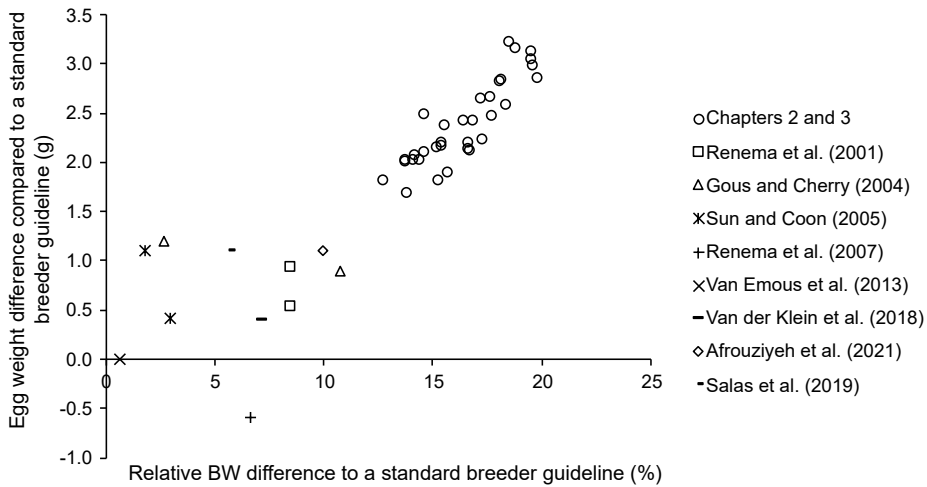


Figure 5. Relationship between relative body weight (BW) difference within an experiment compared to a breeder standard body weight and egg weight difference within an experiment compared to a standard egg weight at a given age. Relative difference in body weight were maintained during rearing and production in Chapters 2 and 3, Renema et al. (2001) and Afrouziyeh et al. (2021). Differences in body weight were converged to a similar body weight during production in Sun and Coon (2005), Renema et al. (2007) and Van Emous et al. (2013). Body weight gain was fixated during production irrespective of body weight differences at the end of rearing in Van der Klein et al. (2018). Feed allowance was fixated during production irrespective of body weight differences at the end of rearing in Gous and Cherry (2004) and Salas et al. (2019).

when different growth curves during rearing are converged to a similar body weight during production (Sun and Coon, 2005; Renema et al., 2007; Van Emous et al., 2013) or when body weight gain (Van der Klein et al., 2018) or feed allowance (Gous and Cherry, 2004; Salas et al., 2019) is fixated during production irrespective of body weight differences at the end of rearing (Figure 5). In these studies, feed allowance was relatively lower for heavier breeders, compared to standard breeders during the production phase. In this way, standard breeders may benefit from this, as they have more nutrients available for egg production and consequently produce egg weights similar to those of as heavier breeders.

In the current thesis, the fast-growing Ross 308 broiler breeder strain was used, whereas other studies also other strains (Shaver Starbro, Ross 708, Hubbard Hi-Yield, Cobb 500). It can be speculated that these effects will also be observed in other broiler breeder strains that have not reached their somatically mature weight yet, due to feed restriction.

From the obtained results, it is clear that breeder body weight has an impact on egg weight at a given age. The relationship between maternal body weight and egg weight in birds had been described already in 1978 by Blueweiss et al. In their study, many different wild avian species were included, which ranged in body weight from approximately 5 g up to 100 kg and egg weight ranged from approximately 0.5 g up to 1 kg. They predicted egg weight (g) using maternal body weight (g) as input with the following formula;  $0.26 * \text{body weight}^{0.77}$  ( $R^2 = 0.83$ ). Using the formula of Blueweiss et al. (1978), predicted egg weights in our study should range from 125 to 169 g, whereas we observed egg weights ranging from 54 to 72 g (28 wk of age onwards). This indicates that modern broiler breeders have a relatively high body weight compared to wild avian species. This is most probably due to genetic selection for high muscle growth, leading to relative heavy breeders. Christians (2002) also investigated the relationship between maternal body weight and egg weight in wild avian species. They observed a similar relationship as Blueweiss et al. (1978), but concluded that only 20% of the variation in egg size was explained by maternal body weight. In the current thesis, when data were analyzed per maternal pen with weekly body weights and egg weights, maternal body weight explained 41.6% of the variation ( $= R^2$  expressed as percentage) in egg weight (Figure 6;  $P < 0.001$ ), where each 100 g of additional body weight resulted in a predicted increase of 0.9 g egg weight. When the analysis was performed per maternal growth curve, 72.3 and 65.0% of the variation in egg weight was explained by body weight, where each 100 g of additional body weight predicted an increase of 2.2 g or 1.8 g egg weight for standard or heavier breeders, respectively ( $P < 0.001$ ). This already indicates that maternal body weight alone does not determine egg weight. Maternal body weight is highly confounded with maternal age. Maternal age already explains 92.0% of the variation observed in egg weight. It is generally accepted that egg weight increases with flock age (Shanawany, 1984; Pinchasov, 1991). As birds become older their sequence length decreases while mean inter-sequence interval increases, and these factors have been reported to influence egg weight (Shanawany, 1984). Therefore, a higher maternal body weight is only expected to lead to a higher egg weight at a given age, rather than that a fixed maternal body weight predicts egg weight.

### Maternal body composition in relation to egg weight

Christians (2002) hypothesized that endogenous protein stores have more potential as determinant for egg size than maternal body weight. In the *General Introduction* it was hypothesized that fatter breeders partition more energy towards the egg, which may benefit egg weight. Therefore, the relationship between body composition and egg weight is further investigated. A simple linear model was fitted between body composition and egg weight (similar to model 4 in *Chapter 4*), with each maternal pen at each age as replicate ( $n = 24$  per age). Overall, body protein mass explained 55.9%

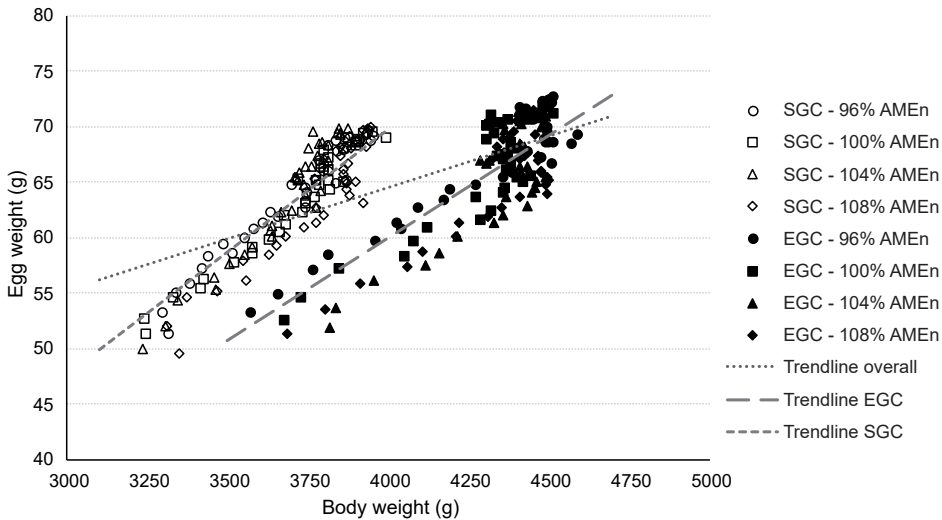
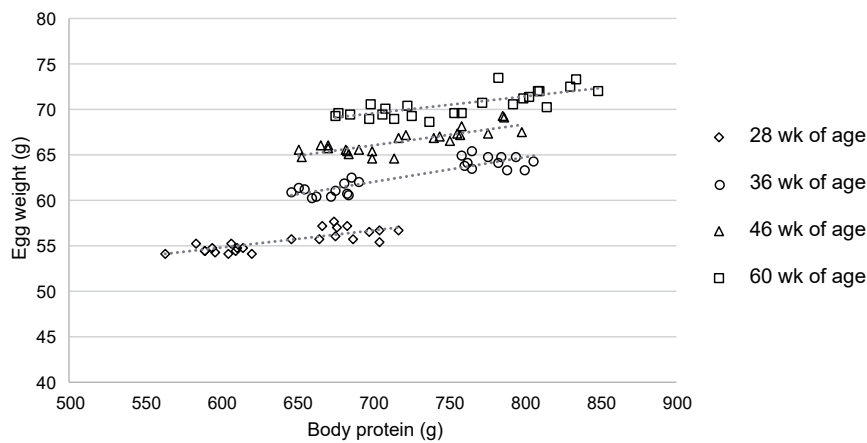


Figure 6. Relationship between body weight of the breeder hen and egg weight of broiler breeders fed at 2 different growth curves (SGC = standard growth curve or EGC = elevated growth curve (+15%) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>) from day 0 onward. Each symbol represents an average weekly value of each treatment. Trendline is based on linear regression analysis with average weekly pen data (n= 24 pens per week).

of the variation in egg weight ( $P < 0.001$ ), whereas body fat mass did not show a linear relationship with egg weight ( $P = 0.92$ ; Figure 7). Each 100 g of additional body protein mass resulted in a predicted increase of 6.5 g in egg weight. As can be seen in Figure 7, maternal age was confounded with body protein mass. Therefore, analyses were performed within each age where body composition was measured (28, 36, 46 and 60 wk of age; *Chapter 4*). Within age, body protein mass even explained 66.7, 80.3, 66.2 and 58.2% (at 28, 36, 46 and 60 wk of age, respectively) of the observed variation in egg weight ( $P \leq 0.02$ ), whereas body fat mass did not significantly affect egg weight ( $P = 0.77$ ,  $P = 0.48$  and  $P = 0.46$ ; at 28, 36 and 60 wk of age, respectively), except at 46 wk of age ( $P = 0.001$ ). Within age, the slope of increment per 100 g of additional body protein was lower (2.0, 2.7, 2.4 and 1.9 g at 28, 36, 46 and 60 wk of age, respectively) compared to the combined data of all ages. Furthermore, at 46 wk of age, each 100 g of additional body fat mass resulted in a predicted increase of 0.7 g in egg weight ( $R^2 = 0.72$ ). These findings thus agree with the hypothesis of Christians (2002), that protein stores have more potential as determinant for egg weight than body weight and that our hypothesis was incorrect. Two potential mechanisms might be involved. Firstly, protein is an important component of the oviduct and ovary (Ricklefs, 1976; Bowmaker and Gous, 1989; Kwakkel et al., 1993). Therefore, it can be speculated that an advanced development of the reproductive tract might have led

A



B

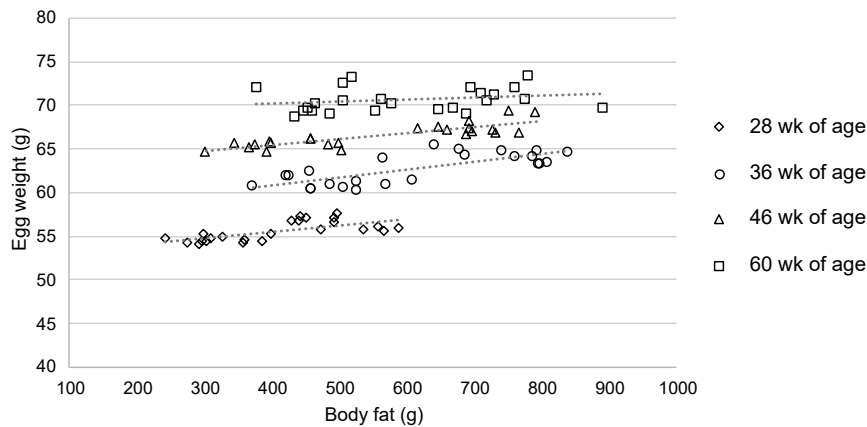


Figure 7. Relationship between body protein (A) and body fat (B) mass of the breeder hen and egg weight of broiler breeders at each given age. Differences in body composition were obtained by feeding breeders at 2 different growth curves (standard growth curve or an elevated growth curve (+15%) and 4 diets, differing in energy-to-protein ratio (96, 100, 104, or 108% AME<sub>n</sub>) from day 0 to 60 wk of age. Each symbol represents 1 maternal pen at each age body composition was measured (Chapter 4; n = 24 per age). Dotted lines represent the linear trendline at each given age.

to a higher total body protein mass and in turn yields higher egg weights. This might play a role in young breeders who just initiate lay, when there is still development of the reproductive tract. Secondly, body protein is an important source for yolk protein (Ekmay et al., 2014) and yolk fat, via gluconeogenesis (Boonsinchai, 2015) and *de novo* lipogenesis (Salas et al., 2017). Vignale et al. (2017, 2018) observed body protein

mobilization during the reproductive phase, indicating that breeders use body protein reserves to support egg production. However, there is still quite a lot of variation in egg weight within each give age that is not explained by body protein mass. This indicates that maternal body protein mass alone does not fully determine egg weight. Consequently, the relationship between feed and nutrient intake and egg weight is further investigated.

### Maternal nutrient intake in relation to egg weight

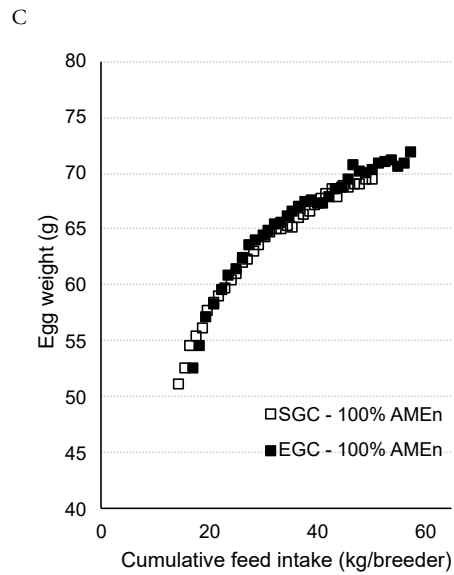
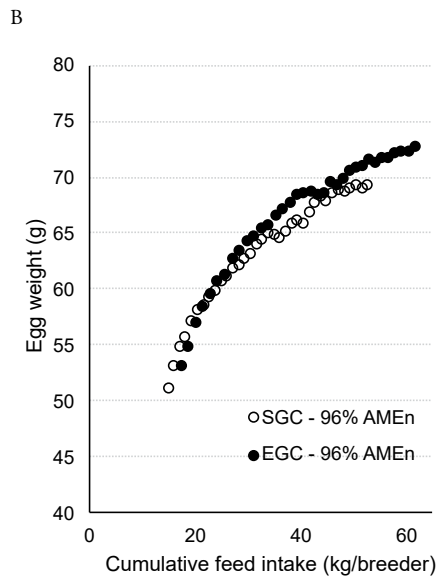
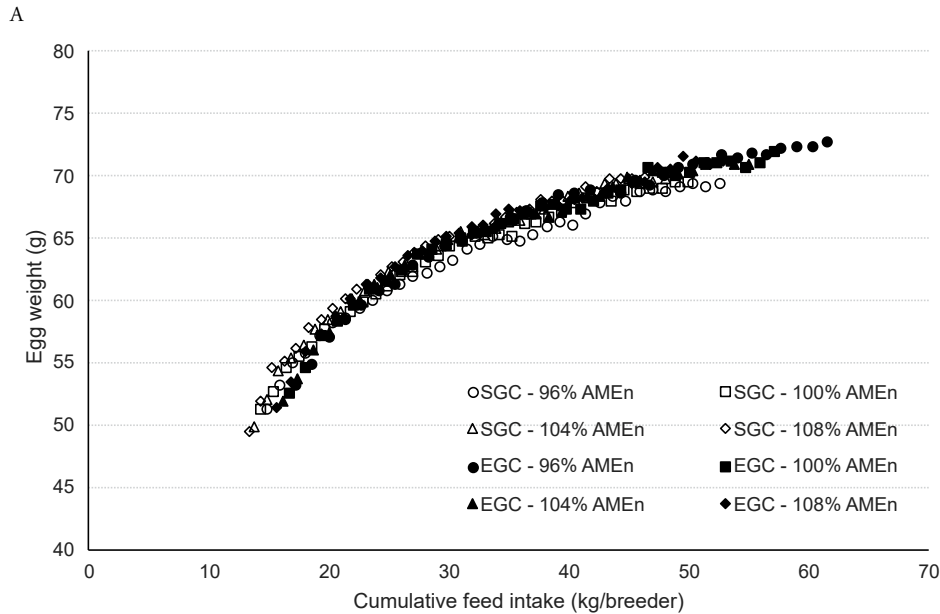
Daily feed intake did not show a relationship with egg weight, neither did daily crude protein or daily energy intake (data not shown). Cumulative feed intake shows a higher potential as predictor for egg weight (Figure 8A). In this way, the egg weight curves for standard and heavier breeders overlap (Figure 8B-E), whereas this is not the case when only maternal ages are taken as an explanatory factor for egg weight (*Chapter 3*). A linear-by-linear curve and an exponential regression curve were fitted to the data of the first study, where each maternal pen was considered as an experimental unit ( $n = 24$  pens per week). Both curves explain a high percentage of the observed variation in egg weight (96.9% and 96.7%, both  $P < 0.001$ , respectively). As the linear-by-linear curve had a higher  $R^2$  and a lower Bayesian Information Criteria (5559 vs. 5618, respectively), this model was used for further evaluation. Replacement of the parameter cumulative feed intake by cumulative crude protein intake did not further improve the model (variance accounted for: 96.9%). Replacement of the parameter cumulative feed intake by cumulative energy intake resulted in a slight improvement of the model (variance accounted for: 97.2%). The predicted model equations of the linear-by-linear curves were as follows:

$$\text{Egg weight (g)} = 80.5 + \frac{-117.0}{1+0.21*\text{cum. feed intake (kg)}} \quad [1]$$

$$\text{Egg weight (g)} = 80.7 + \frac{-107.1}{1+0.136*\text{cum. protein intake (kg)}} \quad [2]$$

$$\text{Egg weight (g)} = 80.8 + \frac{-101.2}{1+6.04*10^{-5}*\text{cum. energy intake (kcal)}} \quad [3]$$

Caution should be taken here, interpreting these models, as cumulative feed or nutrient intake is highly confounded with breeder age, which also explains 92.0% of the variation observed in egg weight. This indicates that incorporation of cumulative feed or nutrient intake in an egg weight model slightly improved the model compared to breeder age alone. The models were compared with to two other studies which varied in dietary energy-to-protein by a reduction in crude protein content (Van Emous et al., 2013; Lesuisse et al., 2017) or in growth curve during rearing (Van Emous et al., 2013) and the average results from study II. Model 2, using cumulative crude protein intake as input parameter, showed the lowest residual variance, compared to model



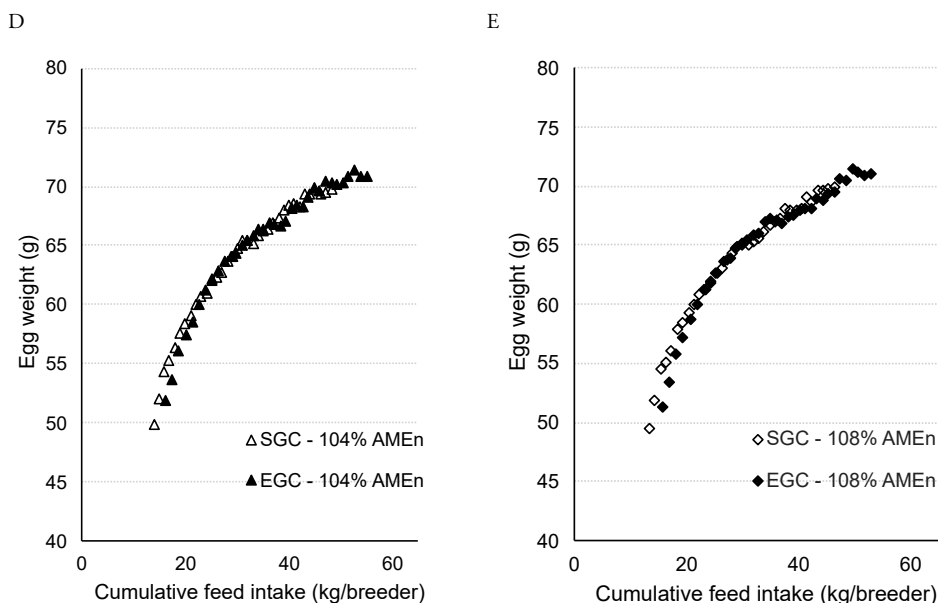


Figure 8. Relationship between cumulative feed intake (A) of the breeder hen and egg weight of broiler breeders between 26 and 60 wk of age, fed at 2 different growth curves (standard growth curve or an elevated growth) and 4 diets, differing in energy-to-protein ratio; (B) 96% AME<sub>n</sub>, (C) 100% AME<sub>n</sub>, (D) 104% AME<sub>n</sub>, or (E) 108% AME<sub>n</sub> from day 0 to 60 wk of age. Each symbol represents an average weekly value of each treatment. Analysis is performed with weekly pen data (n = 24 pens per week).

1 and model 3 (Figure 9). Compared to these studies, the model slightly overestimated egg weight based on cumulative protein intake. This model also immediately implicates that the impact on egg weight of an altered dietary energy-to-protein ratio (and thus protein intake) in a specific phase alone (e.g. rearing or production) will be smaller compared to feeding an altered dietary energy-to-protein ratio during multiple phases, under pair-gain conditions.

Overall, it can be concluded that egg weight is determined by multiple factors, where maternal age and body protein mass within a given age both play an important role. These two important factors can potentially be combined in the parameter cumulative protein intake. Strategies aiming for a higher egg weight should therefore focus on a higher cumulative protein intake. This can either be done by a higher feed allocation or by adjusting the dietary energy-to-protein ratio of the diet.

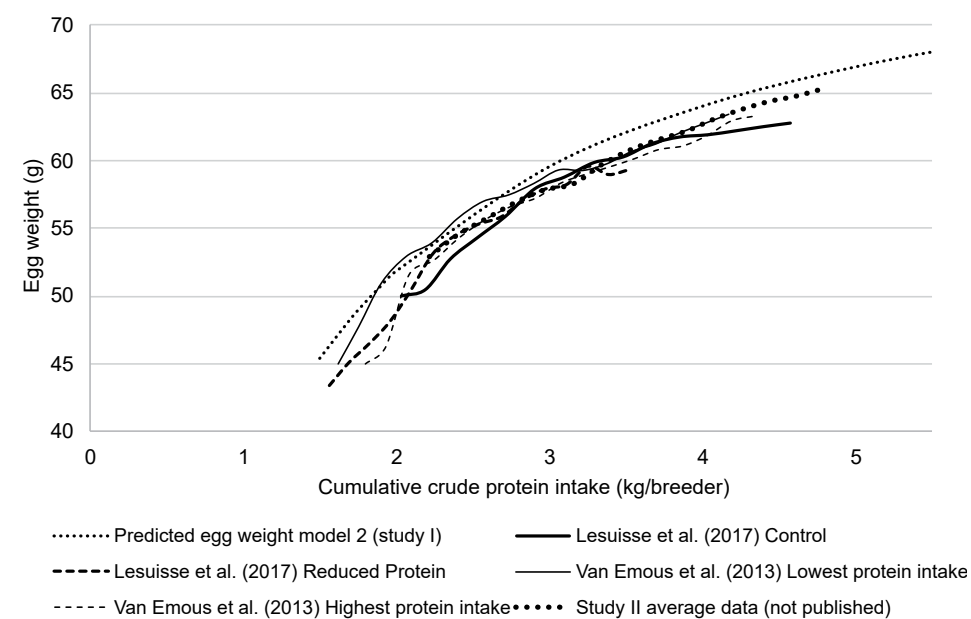


Figure 9. Relationship between cumulative crude protein intake and egg weight between 26 and 40 wk of age. Predicted egg weight is based on the following model: egg weight =  $80.7 - 107.1 / (1 + 0.36 * \text{cum. protein intake})$ . Lesuisse et al. (2017) where breeders were fed on a pair-gain basis a control diet and a reduced protein diet (-25% protein compared to control) from hatch to 40 wk of age. Van Emous et al. (2013) where breeders were fed a low protein diet (-8% protein compared to control) on a standard growth curve (= lowest protein intake) from 2 to 20 wk of age and breeders that were fed a higher protein diet (+8% compared to control) on a higher growth curve from 2 to 20 wk of age (+9% at 20 wk of age compared to standard, after which the growth curve converged to standard growth curve = highest protein intake).

## IMPACT OF BREEDER NUTRITION ON BREEDER PERFORMANCE

In current practice, broiler breeder growth is controlled during rearing and production to achieve a desired body weight at a given age (Cobb Vantress, 2008; Aviagen, 2016). In order to control growth, cumulative feed intake is restricted up to 75% during rearing compared to *ad libitum* fed counterparts (Carney et al., 2022). At one hand, this feed restriction induces welfare issues in breeders, as they experience chronic hunger and stress (De Jong et al., 2002; Decuyper et al., 2010). On the other hand, growth is controlled as *ad libitum* feeding has been shown to impair reproductive performance and health (e.g. metabolic disorders) of the broiler breeder (Bruggeman et al., 1999; Hocking et al., 2002; Heck et al., 2004; Decuyper et al., 2010). This trade-off between hunger, stress and impaired welfare versus reproduction and health of breeders is often referred to as the broiler breeder paradox (Decuyper et al., 2010).



It is questionable, however, whether or not a severe feed restriction is still necessary in modern broiler breeders, as these results are based on studies that were performed in breeders over 2 decades ago.

In the current thesis, a higher breeder growth curve led to similar settable egg production, fertility and hatchability, compared to a standard breeder growth curve (*Chapters 2 and 5*). Furthermore, health issues (including leg problems and prolapse) or mortality did not differ between breeders on the higher growth curve, compared to the standard growth curve (*Chapter 2*). Other studies also observed a comparable reproductive performance, in terms of settable egg production and fertility and hatchability, when breeders had a 7.2 to 22.5% higher body weight during rearing and/or production (Van Emous et al., 2015; Van der Klein et al., 2018; Salas et al., 2019; Zukiwsky et al., 2021) or even *ad libitum* fed breeders (Zukiwsky et al., 2021). Furthermore, no differences in mortality were observed between standard or heavier breeders (Van Emous et al., 2015; Van der Klein et al., 2018; Zukiwsky et al., 2021). All these results suggest that the trade-off between a high breeder body weight versus reproduction and health of breeders might be less severe in modern broiler breeders. Therefore, a relaxation in the degree of feed restriction might be possible in modern broiler breeders without negative effects on reproductive performance or health. However, it remains unclear if a relaxation in the degree of feed restriction also benefits welfare parameters in breeders. Feed allowance was 6.8 to 22.8% higher for breeders on a 7.2 to 22.3% higher growth curve during the rearing period compared to counterparts on a standard breeder recommended growth curve (Van Emous et al., 2015; Van der Klein et al., 2018; *Chapter 2*). It can be speculated that a higher feed allowance may reduce the experience of chronic hunger and stress. However, relative differences in daily feed intake between growth curves are smaller when comparing daily feed intake of a higher breeder growth curve relative to an *ad libitum* fed counterpart (Carney et al., 2022; Figure 10). Feed restriction was on average 34% of *ad libitum* daily feed intake during the rearing period for broilers (Carney et al., 2022) or breeders (*Chapter 2*) on a standard commercial breeder body weight target. Feed restriction was on average still 40% of *ad libitum* daily feed intake during the rearing period when breeders were on a 7.2 to 22.3% higher growth curve (Van Emous et al., 2015; Van der Klein et al., 2018; *Chapter 2*). Therefore, the effect of a higher growth curve, leading to a relaxation in feed restriction, on welfare parameters like hunger and stress are expected to be minimal.

Other strategies to relax the degree of feed restriction, without adjusting the target body weight curve, is adjusting the dietary energy-to-protein ratio (*Chapter 2*) or by diluting the diets (Enting et al., 2007). It was observed that with each percent decrement in dietary energy level, approximately one percent more feed allocation was required to achieve pair-gaining (*Chapter 2*). In line with these results, Enting et

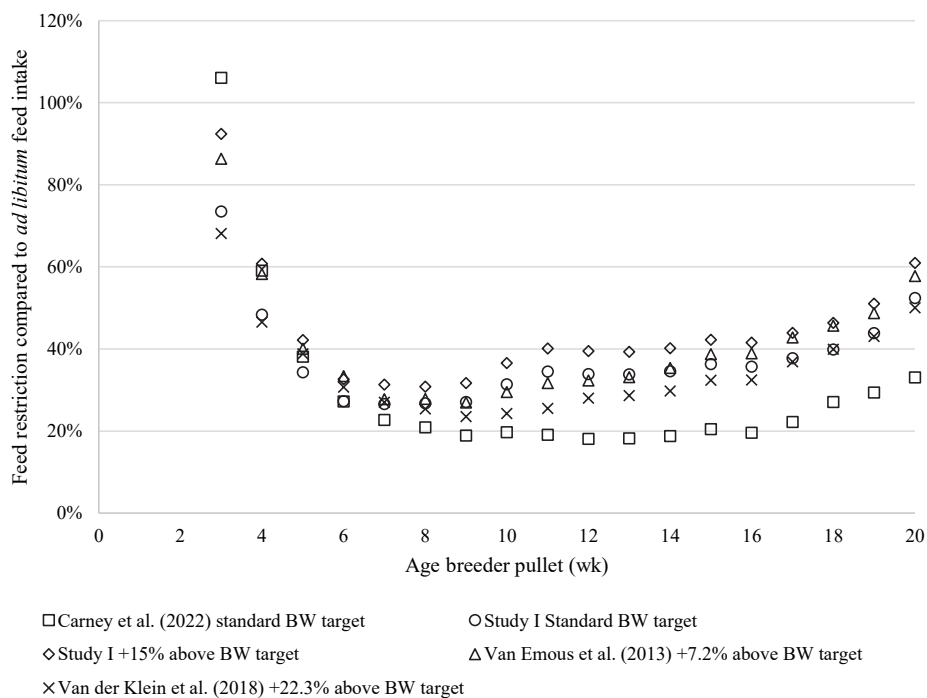


Figure 10. Feed restriction expressed as percentage compared to daily feed intake of an *ad libitum* fed modern broiler strain (Carney et al., 2022). Standard body weight (BW) target were according to commercial breeder recommendations. A higher BW target is expressed as percentage compared to the standard BW target used in the studies.

al. (2007) observed a 12.6% higher feed intake when breeders were fed a diet with 11% lower dietary energy level during rearing and production compared to a control treatment. Furthermore, breeders that were fed a lower dietary energy-to-protein ratio or a diluted diet had a higher reproductive performance, by means of a higher settable egg production (*Chapter 2*) or a higher rate of lay (Enting et al., 2007; *Chapter 3*). As discussed in *Chapter 2*, this might be due to a higher protein intake and these positive effects on reproductive performance are only observed when lower dietary energy-to-protein ratio diets are fed during both rearing and production. Combining the feeding strategy of a higher breeder growth curve with a lower dietary energy-to-protein ratio still resulted in an average estimated feed restriction of 50% of *ad libitum* daily feed intake during the rearing period (Carney et al., 2022; *Chapter 2*). Although still a high level of feed restriction is required, a relaxation from an average 30% feed allowance of *ad libitum* (Carney et al., 2022) to 50% feed allowance of *ad libitum* during rearing, might benefit breeder welfare to a certain extent. However, chronic hunger and stress are still expected as long as feed intake is restricted. It is advised to reduce dietary

energy level in current commercial breeder diets, which improve breeder performance in terms of egg production and which may improve breeder welfare.

There are several points of attention in applying these new feeding strategies in practice. Caution should be taken to translate observed results for fertility and hatchability in younger breeders to older breeders. In the current thesis and in the study of Van Emous et al. (2015), fertility and hatchability was only determined for breeders up to 40 weeks of age. It remains unclear whether or not fertility and hatchability is affected by breeder body weight after 40 weeks of age. Furthermore, an underdeveloped breeder that comes into production too early, in combination with larger eggs, might be more susceptible to prolapse. Breeders on a higher growth curve, fed a low dietary energy-to-protein ratio, sexually matured approximately 5 days earlier and produced 2.6 g heavier eggs at start of production (25 to 28 wk of age) compared to breeders on a standard growth curve, fed a standard energy-to-protein ratio (*Chapters 2 and 3*). In study I, only 4 out of 1,536 breeders (0.3%) were culled due to prolapse over the whole study period (0 to 60 wk of age). This indicates that a higher growth curve is not a risk factor for a higher incidence of prolapse, at least when breeder pullets are photo-stimulated at 21 wk of age. It is unclear whether or not this is also applicable in breeder pullets that are photo-stimulated at an earlier age (e.g. 18 wk of age).

## PRACTICAL IMPLICATIONS

Within the current poultry meat production two supply chain models are used, a non-integrated model and a vertical integrated model. In a non-integrated model, each stage in the supply chain works independent from the other stages and each stage has their own production goals. In an integrated model, two or more stages of the supply chain are under a single ownership and the stages have a combined production goal. The integrated model is designed to increase efficiency, lower expenses and produce additional values. In this thesis, the following stages of the supply chain were included; breeder flock, hatchery, grow-out broiler flocks and slaughter. For practical implications the optimal breeder feeding strategy depends on the type of supply chain model. Therefore, practical implications are split per supply chain model. Furthermore, the optimal breeder feeding strategy depends on which factor, economics (lowest cost per egg or per kilogram of meat), sustainability (lowest use of resources per egg or per kilogram of meat) or welfare, you want to optimize.

### Non-integrated model

Non-integrated models in the poultry meat production chain do not always support optimization of the value chain, as efficient breeder performance (lowest cost per

settable egg) might not concur with optimal broiler performance (highest slaughter yield with lowest feed conversion ratio). From an economic point of view, for optimal breeder performance (highest number of settable eggs) it is advised to lower dietary energy-to-protein ratio with approximately 4% compared to standard breeder recommendations, as this showed to improve breeder performance. It should be calculated whether or not this strategy also results in lower feed cost per settable egg compared to standard breeder recommendations, as these breeders required an approximately 4% higher feed allocation on a standard growth curve. From a sustainability point of view, a higher dietary energy-to-protein ratio is advised compared to standard breeder recommendations, as this resulted in the lowest grams of feed required per settable egg. This indicates that the optimal breeder feeding strategy might depend on which factor, economics or sustainability, you would like to improve. For both economics and sustainability, it is not advised to grow breeders according to a higher growth curve, as this required a higher feed allocation, without benefits for settable egg production (and thus higher feed costs and higher grams of feed per settable egg).

These advices don't take optimal broiler performance or breeder welfare into account. Potentially this can be overcome by inclusion of a bonus system in the non-integrated value chain, which may benefit breeder welfare and broiler performance. For example; breeders on a higher growth curve have a similar performance as counterparts on a standard growth curve, but require a higher feed allocation, leading to a higher investment per settable egg. However, it was shown that broiler performance was higher from breeders on a higher growth curve and potentially this strategy also improves breeder welfare. Therefore, breeder farmers should be paid a bonus for egg weight by the hatchery, rather than being paid for egg numbers, fertility and hatchability alone. With a bonus system, economic disadvantages of a higher investment per settable egg might be overcome. It should be calculated whether or not this strategy also leads to an improved sustainability of the supply chain, as a higher meat yield is obtained.

### **Integrated model**

From an economic point of view, in an integrated model the aim is to produce the highest output (meat yield), with the lowest cost input. To obtain a higher meat yield, it is advised to increase the growth curve for broiler breeders with approximately 15 to 20% compared to standard breeder recommendation. Furthermore, it is advised to increase dietary energy-to-protein ratio of broiler breeder diets with approximately 4% compared to standard breeder recommendations, as this showed to improve broiler performance under standard broiler conditions. It should be calculated whether or not this strategy also results in the highest meat yield per unit of costs. Potentially this strategy goes hand in hand with an improved breeder welfare and sustainability.

## MAIN CONCLUSIONS FROM THIS THESIS

- A higher maternal breeder body weight resulted in higher egg weights, higher chick weight at hatch and a higher post-hatch performance.
- Maternal dietary energy-to-protein ratio affected post-hatch performance in broilers. Exact mechanisms remain unclear, but might depend on transgenerational epigenetic pathways.
- A higher breeder body weight does not penalize nor benefit breeder performance, in terms of settable egg production, fertility or hatchability.
- A lower maternal dietary energy-to-protein ratio is beneficial for breeder performance, in terms of settable egg production, without affecting fertility or hatchability.

## SUGGESTIONS FOR FUTURE RESEARCH

- Chick quality should mainly focus on chick weight at hatch. Furthermore, the post-hatch period should be included in as a criteria for chick quality.
- Future maternal nutrition studies should focus on transgenerational epigenetic pathways:
  - o Gene expression related to protein efficiency and feed intake regulators.
  - o Metabolic hormones related to feed intake.
  - o Determine transgenerational epigenetic pathways in different strains of breeders (e.g. slow- and fast-growing).
  - o Determine which stressor post-hatch interact with transgenerational epigenetic changes induced by breeder nutrition.
- Future maternal nutrition studies should also include older breeders (> 40 wk of age).
- It is unclear whether or not the broiler breeder paradox is still as severe in modern broiler breeders as in broiler breeder over 2 decades ago. Future research should include different relaxation levels of feed restriction (up to *ad libitum*) in breeders to determine whether or not feed restriction is still needed in modern broiler breeders for health and reproductive performance.

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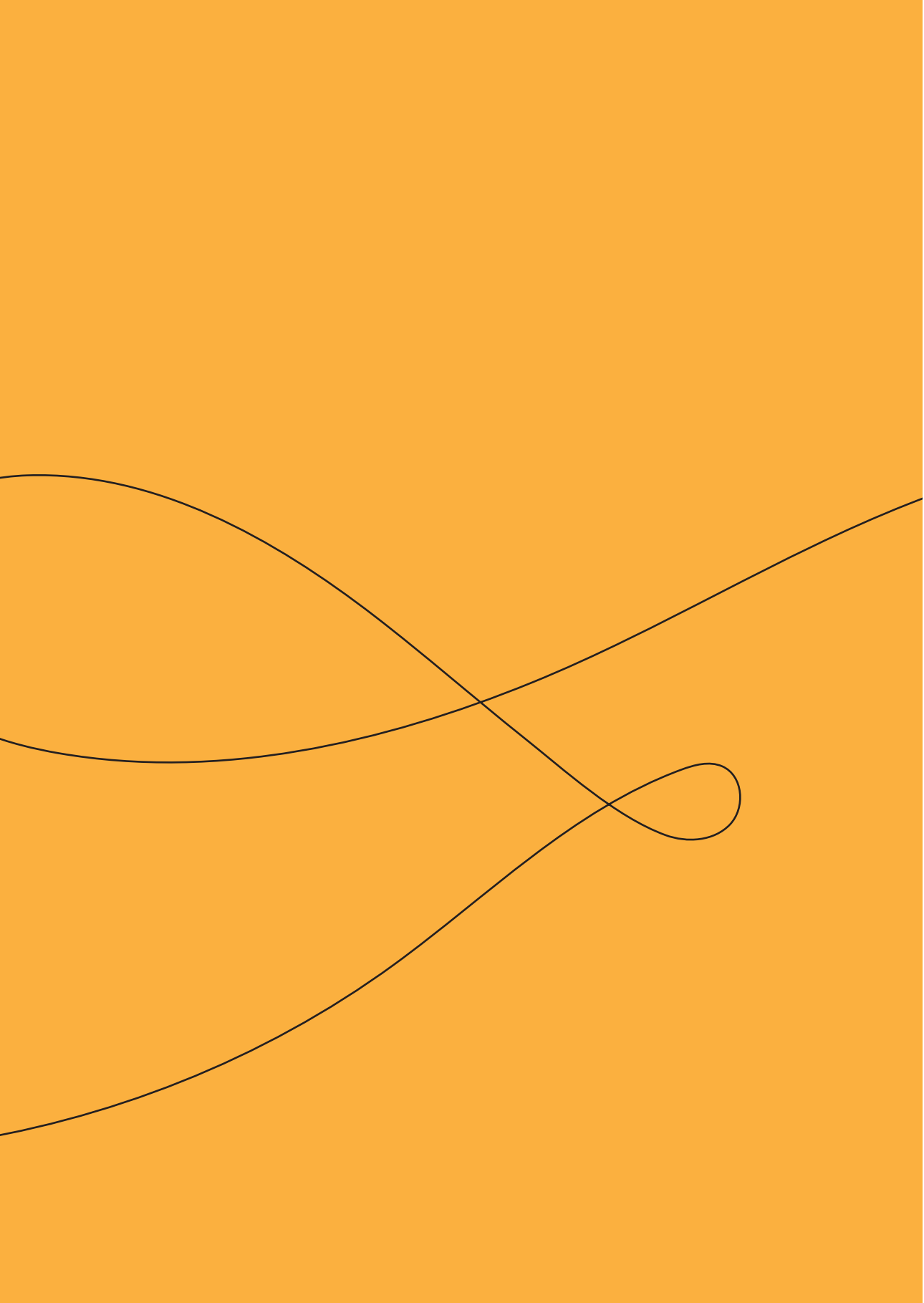
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Summary

Acknowledgements

About the author

Colophon

## SUMMARY

The expected increase in global meat consumption is led mainly by growth in poultry production. This increase in meat production should be achieved in a sustainable and responsible way. An important link in the poultry meat production chain is the quality of day-old chicks. A good day-old chick quality is crucial for performance, health and welfare of broilers. Strategies aiming for an improvement in chick quality might therefore help to enhance sustainability of poultry meat production.

Most research on chick quality has focused on factors post-oviposition and during incubation, for example egg handling, egg storage or incubation temperature. Factors pre-oviposition, such as maternal nutrition, also show potential to improve chick quality and broiler performance. Information, however, on the impact of maternal nutrition on chick quality and broiler performance is scarce and warrant further research. In this thesis, it was hypothesized that a higher maternal body weight results in higher egg weights, which will improve chick quality and broiler performance. Furthermore, it was hypothesized that feeding a higher dietary energy-to-protein ratio during both rearing and laying, by means of a higher dietary energy content, will result in a fatter broiler breeder throughout the breeders' life span. In turn, a fatter broiler breeder can partition more nutrients towards the egg, resulting in better chick quality and broiler performance.

Two studies were conducted to challenge these hypotheses. Within each study, one breeder experiment and two consecutive broiler experiments were conducted. In the first study (*Chapters 2 to 5*), the impact of maternal growth curve (GC), and thus body weight, and a linear increment in dietary energy-to-protein ratio was investigated. In this study, one-day-old pullets ( $n = 1,536$ ) were randomly allotted to 24 pens according to a  $2 \times 4$  factorial arrangement, with 2 GC (standard growth curve = SGC or elevated growth curve = EGC, +15%) and 4 diets, differing in energy-to-protein ratio (96%, 100%, 104%, or 108% AME<sub>n</sub>). All breeder treatments were applied from hatch until 60 wk of age. Feed allocation per treatment was adapted weekly to achieve the targeted growth curve and to achieve pair-gain of breeders fed the different dietary energy-to-protein ratios within each growth curve.

At 28 and 36 wk of age, 60 hatching eggs per maternal pen were selected for incubation and chick quality was assessed at hatch. At each maternal age, 768 day-old broilers were assigned to 32 pens according to maternal treatment and broiler performance was followed until slaughter. Factorial, linear and quadratic contrasts for dietary energy-to-protein ratio for each growth curve were evaluated.

*Chapter 2* describes the findings of this study on productive performance of the breeders. It was observed that EGC breeders required approximately 15% more feed allocation to achieve the targeted growth compared to SGC breeders. Furthermore, it was observed that energy intake mainly determines growth, which means that breeders had a comparable dietary energy intake for each dietary energy-to-protein ratio within each growth curve to achieve pair-gaining. Consequently, dietary protein intake decreased with an increasing dietary energy-to-protein ratio. It was observed that EGC breeders had a 4.1 day earlier sexual maturity and produced 2.3 g larger eggs over the whole production period compared to SGC breeders. Settable egg production did not differ between the two growth curves. An increase in dietary energy-to-protein, at a similar body weight, led to a linear increase in age at sexual maturity ( $\beta = 0.14$  d per % AME<sub>n</sub>) and a linear decrease in egg weight in the first phase of lay (22 to 40 wk of age;  $\beta = -0.06$  g per % AME<sub>n</sub>), regardless of growth curve. Furthermore, an increase in dietary energy-to-protein ratio led to a linear decrease on settable egg production in the first phase of lay, which was more profound in EGC breeders ( $\beta = -0.70$  eggs per % AME<sub>n</sub>) than in SGC breeders ( $\beta = -0.19$  eggs per % AME<sub>n</sub>). In the second phase of lay (41 to 60 wk of age), an interaction between growth curve and dietary energy-to-protein ratio was observed on egg weight. For EGC breeders, an increase in dietary energy-to-protein ratio led to a linear decrease in egg weight ( $\beta = -0.13$  g per % AME<sub>n</sub>), whereas in the SGC, a linear increase in egg weight was observed ( $\beta = 0.03$  g per % AME<sub>n</sub>). No differences between diets were observed on settable egg production in the second phase of lay.

*Chapter 3* describes the findings of the first study on egg quality and egg composition. Egg quality parameters, like albumen height, breaking strength and shell thickness, were barely affected by breeder dietary treatments. Elevated growth curve breeders produced heavier eggs, which was due to 0.8 g more yolk and 1.1 g more albumen, compared to SGC breeders. An interaction between growth curve and dietary energy-to-protein ratio on albumen weight was observed. Dietary energy-to-protein ratio did not affect albumen weight in SGC breeders, but for EGC breeders, a higher dietary energy-to-protein ratio resulted in a 0.9 g linear lower albumen weight from 96% AME<sub>n</sub> to 108% AME<sub>n</sub>. Yolk dry matter content was not affected by breeder dietary treatments. Albumen dry matter content decreased linearly with an increased dietary energy-to-protein ratio and this was more profound in EGC breeders ( $\beta = -0.03$  % per % AME<sub>n</sub>) than in SGC breeders ( $\beta = -0.01$  % per % AME<sub>n</sub>;  $P=0.03$ ).

*Chapter 4* describes the findings of the first study on breeder body composition and energetic efficiency. Body composition was determined at 10 time points from 0 to 60 wk of age. Body protein mass was linearly related to body weight in growing breeders, which can be expressed as  $-6.4 + 0.184 * BW$ , with minimal effects of breeder dietary

treatments. Body fat mass was exponentially related to body weight in growing breeders, which can be expressed as  $-42.2 + 50.8 * 1.0006^{BW}$ , where a higher energy-to-protein ratio resulted in a higher body fat mass at the same body weight. Furthermore, a fat growth spurt was observed towards the end of rearing and start of production. Sexual maturation was related to body protein mass at 21 wk of age, where each 100 g of body protein mass extra advanced sexual maturation by 5.4 days, but it was not related to body fat mass. Calculations were performed to estimate energetic efficiency for growth ( $k_g$ ) and egg production ( $k_e$ ). Both efficiencies varied with age in a quadratic manner between 0.27 – 0.54 for  $k_g$  and between 0.28 – 0.56 for  $k_e$ . The quadratic relationship could be expressed as  $k_g = 0.408 - 0.0319 * Age + 0.00181 * Age^2$  ( $R^2 = 0.72$ ;  $P < 0.001$ ) and  $k_e = -0.211 + 0.034 * Age - 0.00042 * Age^2$  ( $R^2 = 0.46$ ;  $P < 0.001$ ). Treatments had minimal effects on estimated energetic efficiencies in breeders.

*Chapter 5* describes the findings of the first study on chick quality and broiler performance. Fertility and hatchability were not affected by breeder dietary treatments. An increase in breeder dietary energy-to-protein ratio resulted in a linear decrease in embryonic mortality in the first 3 days of incubation ( $\beta = -0.2$  % per % AME<sub>n</sub>). Qualitative chick quality parameters, like activity, navel closure, leg and beak quality were barely affected by maternal dietary treatments. It was observed that broilers from EGC breeders were 1.9 g heavier at hatch and 36 g heavier at slaughter compared to broilers from SGC breeders due to an 1.0 g/d higher growth rate and an 1.5 g/d higher feed intake from hatch to 32 d of age. At hatch, body weight of the broilers decreased with an increasing breeder dietary energy-to-protein ratio ( $\beta = -0.1$  g per % AME<sub>n</sub>), whereas at slaughter age body weight of the broilers increased with an increasing breeder dietary energy-to-protein ratio ( $\beta = 3.2$  g per % AME<sub>n</sub>). This was due to a linear increase in growth rate ( $\beta = 0.1$  g/d per % AME<sub>n</sub>) and feed intake ( $\beta = 0.1$  g/d per % AME<sub>n</sub>). Additionally, an increase in breeder dietary energy-to-protein ratio resulted in a linear decrease in feed conversion ratio ( $\beta = -0.002$  per % AME<sub>n</sub> corrected for body weight differences). Maternal dietary treatments had minor effects on slaughter characteristics, like slaughter yield and meat quality.

Based on the findings of the first study, it was observed that maternal dietary energy-to-protein ratio affects physiology of the offspring, as these broilers had a higher feed conversion ratio and a lower slaughter weight. Potentially due to an altered protein efficiency in the offspring induced by transgenerational epigenetic changes.

In the second study, it was decided to further investigate the interaction between maternal dietary energy-to-protein and offspring dietary protein content. Furthermore, this study aimed to evaluate which breeder period (rearing or laying) has a larger

impact on chick quality and offspring performance. In this second study, one-day-old breeder pullets ( $n = 1,440$ ) were randomly allotted to 24 pens according to a  $2 \times 2$  factorial arrangement, where breeders were fed two dietary energy-to-protein ratios (96% AME<sub>n</sub> and 104% AME<sub>n</sub>) on a pair-gain basis during two periods (rearing (R) and production (P)), according to the EGC of study I. At 29 and 38 wk of age, hatching eggs were selected for incubation per maternal pen and chick quality was assessed at hatch. At each maternal age, 864 broilers were divided over 72 floor pens according to maternal treatment. For each maternal treatment, broiler pens were divided over 3 dietary treatments, which consisted of a low, medium and high (90%, 100% and 110%) digestible lysine (dLys) diets and broiler performance was followed until slaughter.

*Chapter 6* describes the findings of this second study on chick quality and broiler performance. It was observed that maternal dietary energy-to-protein ratio during either rearing or production had no effect on fertility or hatchability and only had minor effects on chick quality parameters. An interaction was observed between maternal dietary energy-to-protein ratio and broiler dietary protein content. Within the 90% dLys treatments, broilers from the 104R-96P breeders had on average a 3.5 g/d lower growth and 115 g lower BW at d 32 compared to the other breeder dietary treatments. Broilers within the 90% dLys treatment from the 104R breeders had on average a 3.9 g/d lower feed intake compared to broilers from the 96R breeders. Within the 100% dLys treatment, no effect of breeder dietary treatment on growth, feed intake, feed conversion ratio or body weight was observed. Within the 110% dLys treatment, broilers from the 104R-96P breeders had a 2.4 g/d higher growth compared to broilers from the 104R-104P breeder diet, with the other 2 breeder dietary treatments in between. Within the 110% dLys, no effect of breeder dietary treatment on feed intake, feed conversion ratio or body weight was observed. These observed effects might be due to epigenetic changes in (metabolic) hormones, which regulate feed intake and only come to expression under a nutritional stressful situation, such as a low protein diet.

In *Chapter 7*, findings from both studies are combined and discussed. It was concluded that a higher maternal body weight resulted in higher egg weights, which is beneficial for chick weight and post-hatch performance. Furthermore, a higher maternal body weight does not penalize nor benefit breeder performance, but might enhance breeder welfare due to a higher feed allocation. A lower maternal dietary energy-to-protein ratio is beneficial for breeder performance. Maternal dietary energy-to-protein ratio affect post-hatch performance in broilers. Exact mechanisms remain unclear, but might depend on transgenerational epigenetic pathways.

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## ABOUT THE AUTHOR

I, Jesse Heijmans, was born on the 25<sup>th</sup> of May 1992 in Veldhoven, The Netherlands. I grew up in Bergeijk, with my parents, my sister and my brother. At home, we had a small hobby farm with all kind of animals. I always liked to help my dad with the animals, especially when one of animals gave birth. After graduation from the Rythovius College in Eersel in 2010, I moved to Wageningen and started my study Animal Sciences at Wageningen University. During my study, I got enthusiastic about cattle and pig reproduction. I conducted my BSc thesis at VIC Sterksel, where I studied the effect of farrowing pen design on parturition length and suckling behaviour in sows. During my MSc, I further specialized in the direction of Adaptation Physiology and Animal Breeding and Genetics. I conducted a minor thesis on genetic parameters for methane emission in Jersey cows at the department of Molecular Biology and Genetics in Foulum, Denmark, which is part of Aarhus University. My major thesis was in cooperation with ForFarmers-Hendrix in Lochem, where I studied the effect of calcium in sow diets on the parturition process and piglet viability. After this thesis, I started my internship in Universidad Nacional de La Plata in Argentina, where I worked in the Animal Reproduction department. There I had the opportunity to assist on cow and sow reproduction research. After successfully completing my MSc Animal Sciences in 2015, I worked for several months for Cargill Animal Nutrition and Coppens Animal Nutrition on a temporal basis to assist their research. Beginning of 2016, I started as a researcher young animals at De Heus Animal Nutrition. In 2018, I had the opportunity to start my PhD in broiler breeder nutrition at De Heus Animal Nutrition of which the results are presented in the current thesis. After my PhD, I will continue my work on poultry research within the R&D department of De Heus Animal Nutrition.



**Colophon**

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