Body composition and reproduction in broiler breeders:

impact of feeding strategies

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ABSTRACT

Nowadays, welfare issues in broiler breeders associated with nutrition and reproductive characteristics, are becoming increasingly challenging. Due to genetic selection on broilers, body composition of breeders has changed dramatically during the last 50 years to less fat and more breast muscle. It is postulated that a certain amount of body fat in broiler breeders at the onset of lay is necessary for maximum performance and offspring quality. Body composition of breeders can be influenced by different feed allowances during rearing and lay, as well as by changes in nutrient composition of the diet. However, little is known about the effects of body composition on reproduction of broiler breeders. In this thesis, we investigated the effects of different feeding strategies during the rearing period on body composition at the end of rearing. Moreover, the effects of differences in body composition at the end of rearing, and feeding strategies during lay were evaluated on breeder performance, incubation traits, offspring performance, behavior and feather cover. From this study, it can be concluded that feeding a low protein diet during rearing decreased breast muscle and increased abdominal fat pad, whereas providing an increased feeding schedule, which resulted in a high growth pattern, only increased abdominal fat pad, at the end of rearing. The higher abdominal fat pad content resulted in an increased hatchability during the first phase of lay and a larger number of eggs during the second phase of lay. For maintaining growth pattern, broiler breeders had to provide a higher amount of feed with an increased energy to protein ratio compared to broiler breeders that were fed a diet with a standard energy to protein ratio. This resulted in an increased eating time and less stereotypic object pecking, which may indicate a reduced hunger and frustration. On the other hand, a low daily protein intake during the rearing and first phase of lay can lead to a poor feather cover. Feeding a high-energy diet during the second phase of lay resulted in increased hatchability, decreased embryonic mortality and more first grade chicks.

Key words: broiler breeder, feeding strategies, body composition, reproduction, behavior

CONTENTS

Chapter 1

General introduction

INTRODUCTION

The basis of the modern poultry meat industry dates back to the late 1950s and nowadays, poultry meat is one of the most important protein sources in human diet. Global poultry meat production in 2000 was 69 million tons and this has been increased to over 97 million tons in 2010 (Windhorst, 2011): an annual production of approximately 70 billion broilers originating from approximately 600 million broiler breeders. These data underline how a relatively small number of parent stock can have a major impact on following links in the poultry meat chain. The impressive growth of the poultry meat industry is supported by improvements in health, nutrition and environmental management (McKay, 2009). However, the major changes in broiler production can be attributed to genetic improvement of the birds as shown by Havenstein et al. (2003a,b). They estimated that the 6 fold increase in carcass yield, measured in a 2001 strain fed a 2001 diet compared to a 1957 strain fed a 1957 diet, was 85-90% due to genetics and 10-15% due to nutrition. This selection on growth efficiency is the result of decades of intensive genetic selection of broilers and consequently also broiler breeders. For example, *ad libitum*-fed standard broiler breeder pullets, from 11 to 24 wk of age, consumed 30% more feed compared to restricted fed pullets, resulting in a dramatic increase (5.4 vs. 2.2 kg) of BW and decreased reproductive performance (Heck et al., 2004). *Ad libitum* feeding compared to a restricted feed intake, can lead to a high mortality, decreased egg quality, lower peak production and lower egg production (Heck et al., 2004). Therefore, feed intake of broiler breeders during rearing is restricted to 25-33% of *ad libitum* intake (Savory et al., 1996; De Jong et al., 2002), but not without adverse effects. Several studies have shown that such a severe feed restriction in broiler breeders lead to behavioral disorders (stereotypic object pecking, overdrinking and pacing) which are indicative of frustration, boredom and hunger. Stereotypic object pecking generally starts after feeding and is mostly performed on the litter, the (empty) drinker, the (empty) feeder, the walls of the pen or to other birds (Kostal et al., 1992; Savory and Maros, 1993; Savory and Kostal, 1996; De Jong et al., 2002; Hocking et al., 2002). To prevent over-drinking, water intake is often restricted in practice (De Jong and Van Krimpen, 2011). Pacing is mainly observed before feed is provided to the birds (Savory and Maros, 1993). Besides undesirable behavior, indicators of chronic stress in birds such as increased plasma corticosterone concentrations (Hocking et al., 1996; Savory and Mann, 1997; De Jong et al., 2002) and increased heterophil to lymphocyte (H/L) ratios (Hocking et al., 1993, 1996; Savory et al., 1993) are observed. This discrepancy between growth capacity, reproduction and welfare is also known as the ‘Broiler Breeder Paradox’ (Decuypere et al., 2010). On the one hand, pullets need to be fed restricted to ensure maintenance of health and reproductive performance, but on the other hand, severe feed restriction leads to decreased welfare caused by behavioral disorders and physiological disturbances.

The main goal in broiler breeder production is to provide fertilized eggs to produce a maximum number of healthy and robust day-old broilers chicks (Zuidhof et al., 2007). Therefore, all aspects of management of modern broiler breeder strains have to be optimized. A national committee, composed of representatives of broiler breeder companies, the feed industry and research associates, identified in 2010 four major issues in broiler breeder production that should require more attention in future research:

1. An observed reduction in fertility and hatchability of eggs.
2. A decreased persistency of egg production.
3. A decline in the quality of day-old chicks.
4. Poor feather cover in ageing breeders.

FERTILITY AND HATCHABILITY OF EGGS

A key problem in broiler breeder production is the decrease in fertility and hatchability of eggs, especially in the second part of the laying period. Fertility of hatching eggs declined from 88.8% in 2000 to 84.7% in 2005 (Van Emous, 2010). This decrease in fertility may be caused by a wide range of factors such as strain, health status of the flock, egg size, egg weight, egg quality, egg storage duration and conditions, egg sanitation, season of the year, and age of the breeders (as reviewed by Yassin et al., 2008). Besides these factors, nutrition played a very important role on fertility and hatchability. A negative effect of a high daily crude protein intake (> 25 g/d) during the laying period on fertility or hatchability of eggs has been reported by Pearson and Herron (1982), Whitehead et al. (1985) and Lopez and Leeson (1995a). A decreased hatchability of fertile eggs could be explained by an increased embryonic mortality as shown by Pearson and Herron (1982) and Whitehead et al. (1985).

Ekmay et al. (2013) showed that increasing levels of dietary lysine and isoleucine at peak production results in a reduction in fertility. An explanation for this effect on fertility was postulated by De Beer (2009), who suggested that an increase in CP intake leads to an increase in nitrogen excretion (De Beer, 2009; Lopez and Leeson, 1995a). This excessive nitrogen excretion may lead to an alkaline environment near the cloaca, where the sperm host tubules are located, with detrimental effects on the semen quality stored in these tubules.

PERSISTENCY OF EGG PRODUCTION

Young broiler breeders divert energy and nutrients to growth or egg production at the start of the laying period. They tend to gain up to 300 g in 10 d during the initial phase (Aviagen, 2006) because the reproductive organs need to be prepared and stimulated for the production of eggs (Renema et al., 2007a). On farm, egg production seems to decrease during the second half of the laying period due to a decreased persistency. In such flocks, a number of birds are found to have started molting spontaneously, coupled by a rapid decrease in fertility of the eggs (Van Emous, 2010).

The current focus during the rearing period is to feed each flock towards a certain target weight at a certain age, without much emphasis on body composition of the young broiler breeder. It can be postulated that genetic selection, focusing on breast meat of the broilers, results in a relatively low body fat content (poor condition) at end of rearing and prior to lay (De Beer, 2009; Decuypere et al., 2010). It is suggested in broiler breeders between 20 and 30 weeks of age that energy intake does not meet their requirements. This was confirmed by calculations on energy requirements carried out by Rabello et al. (2006). Because of this energy imbalance, breeders may metabolize a major part of their body fat reserves. This may lead to a lack of body fat reserves during the second part of the laying period, when breeders have an increased energy requirement due to poor feather cover at that age (Van Emous and De Jong, 2013).

Obesity with associated detrimental effects on reproduction in broiler breeders of 40 wk of age and older was the major problem till approximately 10 years ago (Bornstein et al., 1984; Leclercq et al., 1985; Cahanar et al., 1986; Robinson et al., 1993). The body composition of breeders, however, has changed dramatically during the last five to six decades (Havenstein et al., 2003a; De Beer, 2009). In modern broiler breeders, obesity is not an issue anymore, probably due to the selection of strains with increased breast muscle and decreased fat pad deposition characteristics (Havenstein et al., 2003a). The latter authors reported that a 2001 broiler strain (Ross 308) had a lower percentage of abdominal and carcass fat at 43 d (1.4 and 13.7%, respectively) than a 1957 strain at 85 d (2.0 and 17.9%), when both strains were fed a 2001 diet. Breast meat (% BW) was 20.0 and 12.2% for the 2001 and 1957 strain, respectively. These changes in body composition of broilers did - as a consequence - also affect their parents (broiler breeders). Data from different experiments (Bowmaker and Gous, 1989; Fattori et al., 1993; Renema et al., 2001a; Sun et al., 2006; Robinson et al., 2007; Mba et al., 2010) show the development of abdominal fat pad weight of broiler breeders between 1989 and 2010 (Figure 1). For comparative purposes, only data from studies of breeders at the onset of lay (between wk 20 and 22) and a BW between 1,950 and 2,350 g were used. From this Figure it can be concluded that the abdominal fat pad weight (% BW) at the end of the rearing period has declined significantly in 20 years (from approximately 3% in 1989 to around 0.5% in 2010).

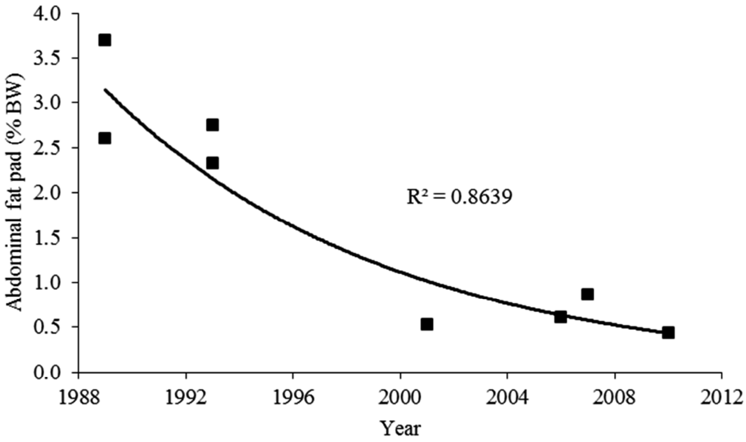

Figure 1. Development of abdominal fat pad weight (% BW) at the end of the rearing period (20 to 22 wk of age) from different experiments (BW between 1,950 and 2,350 g).
Data adopted from: Bowmaker and Gous, 1989; Fattori et al., 1993; Renema et al., 2001a; Sun et al., 2006; Robinson et al., 2007; Mba et al., 2010.

In practice, when breeders consume a certain feed allowance, daily energy intake may be deficient and the farmer will increase the birds feed allowance to achieve the targeted body weight. This higher feed allocation leads to an overfeeding of amino acids and CP resulting in larger breast muscle tissue (Ekmay et al., 2013). To sustain this larger amount of breast muscle tissue, additional daily energy is necessary and this may decrease the amount of feed energy that can be allocated to egg production (Ekmay et al., 2013). An increasing energy to protein ratio during the rearing as well as the laying period may have positive effects on body fat reserves of the broiler breeders and this may positively influence persistency of lay of the flock. In accordance, Sun and Coon (2005) concluded that feeding a higher fat diet during the laying period result in more body weight gain, larger eggs and more carcass fat. It is, therefore, suggested by some researchers (Sun and Coon, 2005; De Beer, 2009; Decuypere et al., 2010) that a certain proportion of body fat in breeders at the onset of lay is necessary for maximum egg production.

QUALITY OF DAY-OLD CHICKS

The importance to decrease mortality of broilers in broiler production in the EU has increased as chick mortality is used as an important indicator of welfare (European Union, 2007). Total mortality in a broiler flock is directly related to the quality of day-old chicks. The quality of day-old chicks depends on a wide range of factors such as breeder strain, breeder age, egg weight, egg storage and condition, and incubation conditions (as reviewed by Yassin et al., 2008). At the onset of lay, nutrients absorbed by young broiler breeders are used for growth and egg production. The balance between pubertal growth and the onset of lay will have an influence on the transfer of energy, nutrients, minerals and vitamins of the broiler breeder towards the egg. A decreased utilization of specific lipids for egg production can affect hatchability but can also affect the offspring (Noble et al., 1986; Latour et al., 1996). Pearson and Herron (1982) reported that a high daily intake of protein compared to a low intake of protein (27.0 and 21.3 g/d, respectively) during lay, resulted in increased mortality and malformation of embryos. Similarly, Whitehead et al. (1985) reported a significant increase in saleable chicks per breeder when the breeder diet contained 13.7 instead of 16.8% protein. On the other hand, no dietary effects (energy and protein) on embryonic mortality were found by Spratt and Leeson (1987). The latter indicates that a correct energy to protein ratio is important for pre-peak egg production. They showed that before 30 wk of age an energy to crude protein ratio of around 17.5 kcal ME/g CP produces heavier chicks. Any other energy to crude protein ratio resulted in lower chick weights. A significantly low dietary crude protein content (10%) will lead to lower egg and chick weights (Lopez and Leeson, 1995b).

FEATHER COVER

Feather coverage of broiler breeders has decreased over the last decade (Van Emous and De Jong, 2013). The cause for this poor plumage condition is not yet clear. Nevertheless, a farm inventory of Van Emous (unpublished data) showed that factors such as feeding space and behavior of males and females during feeding time seem to be highly relevant for feather condition. In the literature, only a few studies have been conducted into the effects of dietary energy and protein on plumage condition. Twinning et al. (1976) showed that dietary protein content above 16% should be sufficient to ensure proper plumage development at an early age. This could be explained by the fact that feathers contain high concentrations of protein and amino acids (Stilborn et al., 1997). Moreover, amino acids involved in the synthesis of feather keratin are the sulfur-containing amino acids methionine and cysteine (Leeson and Summers, 2005). It is, therefore, suggested that dietary protein and amino acid levels are very important during the initial rearing period to develop a sustainable feather cover.

SCOPE OF THE STUDY

Body composition of broiler breeders at the end of the rearing period has changed during the last five to six decades (i.e. more breast meat and less body fat). It is hypothesized that this change in body composition may negatively affect breeder performance, incubation traits and offspring performance. Because tissue growth is directly affected by dietary nutrient composition, a nutritional approach to this topic is highly relevant.

Besides the indirect effect of body composition on the different traits during lay, it is hypothesized that during the laying period, different feeding strategies (e.g. a low daily protein intake) could also directly affect body composition reproduction, incubation traits, offspring performance, behavior and feather cover. The possible direct and indirect effects of different feeding strategies during the rearing and laying period are shown in Figure 2.

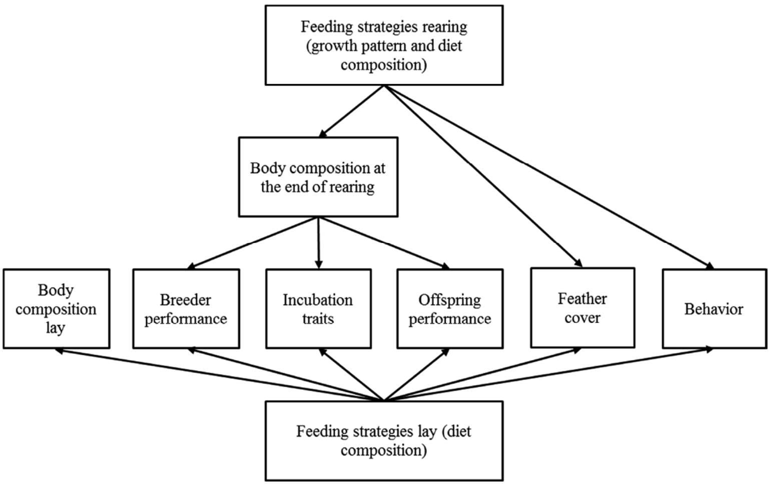

Figure 2. Possible effects of different feeding strategies during the rearing and laying period.

The objectives of the present study are:

1. To investigate the effects of different feeding strategies during the rearing period (growth pattern and low protein diets) on body composition at end of rearing.
2. To evaluate the effects of differences in body composition at the end of the rearing period (and thus the carryover effects of feeding strategies during rearing) on breeder performance, incubation traits, and offspring performance.
3. To determine the direct effects of different feeding strategies during the rearing period on behavior and feather cover.
4. To determine the direct effects of different feeding strategies during the laying period on body composition, breeder performance, incubation traits, offspring performance, behavior, and feather cover.

The overall practical objective of the present study is to develop new feeding strategies during the rearing and laying period for broiler breeders in order to alter body composition with positive effects on reproduction, offspring and welfare, for a more sustainable approach of broiler breeder production.

OUTLINE OF THE THESIS

This thesis describes the results of two broiler breeder experiments. Both experiments were carried out with Ross 308 broiler breeders. The first experiment was carried out with one-day-old chicks till 40 wk of age while the second experiment was carried out with one-day-old chicks till 60 wk of age. In general, Chapters 2, 3 and 4 report the results of the first experiment while the Chapters 5 and 6 report the result of the second experiment.

In Chapter 2, the effects of growth patterns and dietary crude protein levels during the rearing period on body composition of female broiler breeders at the end of the rearing period as well as carryover effects on egg production were investigated.

Chapter 3 focuses on the effects of the different feeding strategies during the rearing period on behavioral traits and feather cover in broiler breeder females during the rearing and laying period.

The effects of different feeding strategies during the rearing period on incubation traits and offspring performance are discussed in Chapter 4.

Based on the results of the first experiment (Chapters 2 to 4), in the second experiment, a different dietary protein level was chosen as treatment during the rearing period while during the laying period a different dietary energy level was used.

Chapter 5 describes the effects of different dietary protein levels during the rearing period and different dietary energy levels during the laying period on body composition, breeder performance and incubations traits.

In Chapter 6 the results of the observations on behavior and feather cover during the rearing and laying period are presented.

The results reported in Chapters 2 to 6 are discussed and evaluated in the General Discussion (Chapter 7). The results of the current thesis are compared with data from other experiments and explanations for differences between treatments are given. Practical and economic implications for new feeding strategies aimed at improving broiler breeder reproduction and welfare during the rearing and laying period are provided. Also suggestions for further research are formulated in this Chapter.

Chapter 2

Effects of growth patterns and dietary protein levels during rearing on body composition and performance in broiler breeder females during the rearing and laying period

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ABSTRACT

The combined effects of growth pattern (GP) and dietary CP level during rearing (2 to 22 wk of age) on body composition and performance were investigated in broiler breeder females from 0 to 40 wk of age. One-day-old pullets (n = 768) were randomly allotted to 48 pens according to 2 growth patterns (standard = SGP and high = HGP) and fed 1 of 3 dietary CP levels (high = CPh, medium = CPm, and low = CPl). From 19 to 22 wk of age, feeding level was gradually adjusted to obtain a similar target BW for all birds, and then until 40 wk of age, all birds received similar amounts of a standard breeder diet. During the rearing period, the HGP pullets were fed a higher feed intake level (6.5%) than SGP pullets. To meet BW targets at 22 wk of age, feed intake from d 14 onward had to be increased for the CPm (4.6%) and CPl (10.0%) treatments. Breast muscle percentages of HGP and SGP pullets were similar at any age, although abdominal fat pad at 20 wk was 0.18% higher for HGP pullets. Pullets fed the CPl diet had a lower breast muscle percentage compared with pullets fed the CPm and CPh diets (0.46 and 0.85% at wk 10, 0.81 and 1.45% at wk 20, respectively). Abdominal fat pad in CPl pullets were 0.18 and 0.22% (wk 10), and 0.24 and 0.42% (wk 20) higher compared with CPm and CPh pullets, respectively. At 40 wk of age, no effects on breast muscle and abdominal fat pad were found among all treatments. Egg production, sexual maturation, and egg weight were not affected by GP and CP levels during rearing. It was concluded that a low CP diet during rearing decreased breast muscle and increased abdominal fat pad, whereas a high GP only increased abdominal fat pad, at the end of the rearing period. Decreasing dietary CP level seems to be more effective in increasing abdominal fat pad than increasing GP.

Key words: broiler breeder, feed strategy, rearing, body composition, performance

INTRODUCTION

Modern-day broilers have approximately 9% more breast muscle, whereas the total fat percentage is approximately 7% lower than broilers 30 yr ago (De Beer, 2009). The results of selection changed body composition of broilers and led to major changes in the growth potential of these birds (Havenstein et al., 2003a,b; Renema et al., 2007b). Not only have feed conversion ratio, growth rate, and body composition of broilers changed, but also of broiler breeders. At the onset of lay, modern broiler breeders have less fat and more breast muscle than a few decades ago, resulting in a delay of maturity (Decuypere et al., 2010). More breast muscle has resulted in an increased energy requirement to maintain this metabolically active tissue (De Beer, 2009). Some researchers suggest that a certain percentage of body fat in broiler breeders at the onset of lay is necessary for an adequate reproductive performance (Bornstein et al., 1984; Sun and Coon, 2005; De Beer, 2009; Mba et al., 2010). Yu et al. (1992a,b) hypothesized that a sufficient feed allowance and a minimum body fat content during the prebreeding period are important to promote sexual maturity in broiler breeders. Body composition can be affected by the use of different feed allowances during rearing (Fattori et al., 1993; Renema et al., 2001a; Robinson et al., 2007) and laying (Bornstein et al., 1984; Bowmaker and Gous, 1989; Renema et al., 2001b). On the other hand, body composition can also be influenced by differences in diet composition. Different energy or protein levels may affect the fat content of the breeder during rearing (Miles et al., 1997; Hudson et al., 2000) or laying (Pearson and Herron, 1981; Spratt and Leeson, 1987). Recently, Mba et al. (2010) showed that a low dietary protein level during rearing increased abdominal fat and decreased breast muscle of pullets at the onset of lay. However, the best method for influencing body composition before the onset of lay is not yet clear.

This study investigated the combined effects of 2 different growth patterns and 3 different dietary protein levels, during rearing of broiler breeder females, on body composition during rearing and mature bird performance. It was hypothesized that an increased abdominal fat content at the end of the rearing period will improve reproductive performance of modern broiler breeders during the laying period.

MATERIALS AND METHODS

The protocol for the experiment conformed to the standards for animal experiments and was approved by the Ethical Committee of Wageningen UR, the Netherlands. Animal care guidelines were used according to the Euro guide recommendations for animal use for experimental and other scientific purposes (Forbes et al., 2007).

Birds, housing and management

A total of 768 one-day-old Ross 308 female broiler breeder chickens were housed in 2 identical climate-controlled rooms. All chickens were individually identified by steel wing tags fitted in wk 1. Within each room, 24 floor pens (0.90 × 1.50 m) were used, each containing 16 pullets at the start of the experiment. The number of pullets per pen was gradually reduced to 15 (wk 4), 12 (wk 10), 9 (wk 15), and 6 (wk 20), due to dissection procedures (2 per pen at wk 10, 15, and 20), sex errors, and if no mortality had occurred some outlier birds were removed additionally. Stocking density was reduced from 11.9 pullets per m^2 in wk 1 to 4.4 pullets at 20 wk of age. Each pen contained 2 perches, 2 feeding troughs (total length of 100 cm), and 4 nipple drinkers, with wood shavings used as litter. Throughout the experiment, litter quality was maintained by adding new wood shavings every 6 wk. At wk 20, a laying nest was placed outside each pen while one of the feeding troughs was removed. During the first 2 d, temperature in the housing was maintained at 33°C and from d 3 onward, temperature was gradually decreased to reach 20°C at 5 wk of age and maintained thereafter. Light was on 24 h per day for the first 2 d, with a gradual reduction to 8 h per day by wk 3, which was maintained until wk 21. Birds were photo-stimulated with 11 h of light at wk 21, and day length was extended by 1 h (later 0.5 h) per wk to a 15L:9D light schedule at 27 wk of age. This was maintained until the end of the experiment at 40 wk of age, with lights on from 0400 to 1900 h. During rearing, a light intensity of 20 lx at the bird level was applied; during laying this was increased to 60 lx. Pullets were vaccinated according a standard vaccination program of the management guide of this breed, and beaks were trimmed at d 3. Feed was provided *ad libitum* from d 0 to 2 wk of age with a maximum of 40 g of feed per pullet toward the end of this period. Pullets were restricted in their amount of feed, from wk 3 onward. During the experiment birds were fed diets in a mash form daily. Water availability was restricted during the rearing period by closing the nipples 2 h after all feed had been eaten to prevent overconsumption of water. Health status of the hens was monitored daily.

Experimental design

From wk 0 to 2, all pullets followed the same growth pattern and received the same standard starter-1 diet. At d 14, birds were randomly allotted to 1 of 6 dietary treatments according to a 2 × 3 factorial design. Factors were 2 growth patterns (standard = SGP and high = HGP) and 3 dietary protein levels (high protein = CPh, medium protein = CPm, and low protein = CPl). A starter-2 diet was fed from 2 to 6 wk of age, a grower diet from 6 to 15 wk of age, and a prebreeder diet (in the transition period) from 15 to 22 wk of age. From 22 to 40 wk of age, a standard breeder diet was provided to all birds. Compositions and calculated contents of the diets are presented in Table 1. Growth patterns were set to reach differences of 200 g in BW at 20 wk of age: 2,400 g (HGP) vs. 2,200 g (SGP). The SGP was the recommended breeder growth pattern (Aviagen, 2006). Body weight targets were directive, and the daily feed allocation was adjusted weekly per pen to reach the predetermined BW target of that week. From 19 to 22 wk of age, feeding level was gradually adjusted to obtain a similar target BW for all birds at onset of lay as soon as possible. Within each phase, all diets (from 2 to 22 wk of age) had similar energy levels. Digestible amino acid levels were lowered by 8 and 16% for the CPm and CPl diets, respectively, compared with the CPh diet. Differences between CPh, CPm, and CPl diets were obtained by changing specific ingredients. Amino acid contents relative to digestible lysine, however, were similar for all diets.

Observations

Feed intake, BW, and uniformity. Feed intake (g/bird per d) was recorded weekly and adjusted to reach the target BW. To monitor BW and BW gain, all hens per pen were weighed weekly in the morning before feeding from 0 to 27 wk of age. From 28 wk onward, birds per pen were weighed at a 2-weekly interval 6 h after feeding to prevent any disturbance of oviposition. Body weight development (g/bird per wk) was used to determine the amount of feed for the next week for the different treatments. Individual BW of all hens was recorded at 5, 10, 15, and 20 wk of age. Body weight uniformity (CV%) was determined by calculating the SD of BW divided by the average BW for each pen. Mortality was calculated excluding culled and dissected birds.

Body composition. On wk 10, 15, 20 and 40, 2 randomly selected birds per pen were killed by cervical dislocation and weighed. The pectoralis major, pectoralis minor, and abdominal fat pad were dissected from the carcass and weighed. Total breast weight was calculated as the sum of the weights of the pectoralis major and minor. The weights of the breast muscle and abdominal fat pad were calculated as a percentage of BW.

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

	Starter1	Starter2 (15-42 d)			Grower (43-105 d)			Pre-breeder (106-154 d)			Breeder1
Item	(0-14 d)	CPh[1]	CPm	CPl	CPh	CPm	CPl	CPh	CPm	CPl	(155-280 d)
Ingredients											
Maize	450.0	440.0	440.0	440.0	400.0	400.0	400.0	400.0	400.0	400.0	425.0
Wheat	189.6	200.0	200.0	200.0	190.0	190.0	190.0	248.9	249.4	250.0	200.0
Soybean meal	216.8	148.2	99.1	50.0	43.0	26.5	10.0	101.8	64.5	27.3	104.0
Sunflower meal	49.5	-	-	-	-	-	-	-	-	-	-
Maize gluten feed	-	125.0	125.0	125.0	104.1	104.1	104.1	-	39.0	78.0	-
Rapeseed meal	35.0	35.0	35.0	35.0	25.0	17.5	10.0	35.0	35.0	35.0	50.0
Wheat middlings	-	-	33.2	66.3	150.0	158.2	166.3	150.0	150.0	150.0	99.2
Peas	-	-	8.2	16.4	-	-	-	-	-	-	-
Maize gluten meal	10.0	1.2	8.0	14.7	-	-	-	-	-	-	-
Maize starch	-	-	-	-	-	6.7	13.3	-	-	-	-
Alfalfa meal	-	-	-	-	52.1	61.1	70.0	15.9	12.5	9.0	-
Soya oil	7.2	9.3	9.4	9.5	2.0	2.4	2.7	2.0	2.9	3.9	27.6
Chalk	16.5	17.2	17.5	17.7	15.5	15.3	15.0	24.6	25.0	25.4	18.0
Limestone	-	-	-	-	-	-	-	-	-	-	54.0
Monocalcium phosph.	10.2	9.6	9.6	9.7	4.0	4.2	4.4	5.0	4.9	4.7	5.9
Salt	1.5	0.8	0.6	0.4	0.4	0.3	0.2	1.5	1.2	1.0	1.8
Sodium carbonate	4.2	3.7	3.9	4.2	4.3	4.5	4.6	4.1	4.1	4.0	3.8
Premix rearing[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	-	-	-	--
Premix laying[3]	-	-	-	-	-	-	-	10.0	10.0	10.0	10.0
Choline Chloride-50%	2.8	2.8	2.8	2.8	2.0	2.0	2.0	-	-	-	-
Natuphos	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	-	-	-
Rovabio Excel AP	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	-	-	-
L-Lysine	0.8	1.2	1.8	2.3	1.8	1.9	1.9	0.5	1.0	1.5	0.2
DL-Methionine	1.0	0.7	0.5	0.3	0.4	0.3	0.2	0.6	0.5	0.4	0.6
L-Threonine	-	0.2	0.3	0.4	0.3	0.3	0.2	-	-	-	-
Analyzed content[4]											
DM	879.3	872.3	872.6	872.9	876.9	875.9	873.3	869.0	870.3	871.2	882.1
Ash	57.8	58.1	55.8	56.1	59.9	60.8	60.6	60.0	61.4	60.8	96.0
Fat	42.4	45.5	47.0	48.6	37.9	40.7	39.5	34.7	36.9	38.2	59.1
Crude fiber	37.5	33.2	34.8	36.5	52.7	54.3	56.0	39.9	40.6	41.2	32.5
Crude protein	207.4	192.1	179.6	145.9	141.3	129.6	122.7	148.8	139.6	127.9	144.8
Starch	375.3	405.7	413.8	418.2	365.9	381.1	395.5	401.4	405.6	385.6	391.3
Reducing sugars[5]	38.6	33.6	31.5	29.0	34.4	32.9	31.6	35.2	33.2	31.5	31.9
NSP[6]	160.1	178.6	183.7	188.7	226.7	229.2	231.7	185.9	193.4	200.8	158.7
Calculated content											
AME_n (kcal/kg)	2,795	2,800	2,800	2,800	2,600	2,600	2,600	2,700	2,700	2,700	2,780
AME_n:CP ratio[7]	13.5	14.6	15.6	19.2	18.4	20.1	21.2	18.1	19.3	21.1	19.0
Digestible lysine	8.6	7.2	6.6	6.0	5.4	4.9	4.5	5.6	5.2	4.7	5.3
Digestible M+C	6.7	5.7	5.3	4.8	4.5	4.2	3.8	5.0	4.7	4.4	4.8
Digestible thr.	6.0	5.2	4.8	4.4	4.0	3.6	3.3	4.1	3.8	3.4	4.0
Digestible tryp.	2.0	1.5	1.4	1.2	1.2	1.1	1.0	1.5	1.3	1.1	1.4
Calcium	10.0	10.0	10.0	10.0	9.0	9.0	9.0	12.0	12.0	12.0	30.0
Total phosphorus	6.5	6.5	6.5	6.5	5.8	5.8	5.8	5.7	5.8	5.8	5.4
Av. phosphorus	4.1	4.1	4.1	4.1	3.2	3.2	3.2	3.2	3.2	3.2	2.9
Physical characteristic											
Particle size (mm)	0.34	0.38	0.38	0.38	0.34	0.34	0.36	0.31	0.31	0.31	0.58

[1]Dietary protein level. CPh = high dietary crude protein; CPm = medium dietary crude protein; CPl = low dietary crude protein.
[2]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 30 mg; vitamin K3, 1.5 mg; vitamin B1, 2.0 mg; vitamin B2, 7.5 mg; vitamin B6, 3.5 mg; vitamin B12, 0.02 mg; niacinamide, 35 mg; D-pantothenic acid, 10 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 12 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 85 mg (as MnO_2); zinc, 60 mg (as $ZnSO_4$); cobalt, 0.4 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 0.8 mg (as KI); selenium, 0.1 mg (as $Na_2SeO_3 \cdot 5H_2O$).
[3]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin K3, 5.0 mg; vitamin B1, 3.0 mg; vitamin B2, 12.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.03 mg; niacinamide, 55 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.4 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 10 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 120 mg (as MnO_2); zinc, 100 mg (as $ZnSO_4$); cobalt, 0.25 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 2.0 mg (as KI); selenium, 0.3 mg (as $Na_2SeO_3 \cdot 5H_2O$); choline chloride 50%, 2.0 g; Natuphos, 0.1 g; Rovabio Excel AP, 50 mg.
[4]Based on 2 analysis in duplicate per diet.
[5]Mono- and disaccharides as glucose units.
[6]Calculated by subtracting the crude protein, fat, starch, reducing sugars, and ash content from the DM content.
[7]AME_n:CP ratio = kcal AME_n/g CP.

Egg production. Oviposition started at 23 wk of age. All eggs were collected daily and recorded every week for the determination of weekly and total egg production. All cracked, soft-shelled, double-yolked, dirty, and small eggs (under 50 g) were recorded and defined as unsettable. A clean egg (above 50 g) with an intact shell and a single yolk, was defined as a settable egg. Egg production was recorded from the day the first egg in the pen was collected to the last day of the experiment (40 wk of age). Egg weights of all hatching eggs (settable and small eggs) were recorded on a weekly base. Age at 50% production (d) was determined by linear interpolation of the week (in days) where birds past 50% rate of lay. Peak egg production was determined as a 3-wk rolling average.

Statistical analysis

Data were analyzed as a randomized block design with general ANOVA (GenStat 14 Committee, 2011). The effects of room and pen were added to the random term of the model. Pen was the experimental unit and parameters were tested for normal distributions before analyzes. Data were presented as means ± SEM. All statements of significance are based on testing at $P \leq 0.05$.

RESULTS

Mortality

The average mortality during the rearing (from 2 to 22 wk of age) and laying (from 22 to 40 wk of age) periods was 0.4 and 1.4%, respectively. No differences were observed in mortality between treatments during both periods (data not presented).

Feed intake, BW, and uniformity

Feed and nutrient intakes between 2 and 22 wk of age are shown in Table 2. To meet BW targets at 22 wk of age, feed and energy intake between 2 and 22 wk of age were increased in SGP-CPm, and SGP-CPl birds by 4.8 and 9.5%, respectively, compared to the SGP-CPh treatment. Birds fed the HGP-CPm and HGP-CPl diets received a 4.4 and 10.5% higher feed amount and as a consequence also energy amount to meet BW targets at 22 wk of age compared to the HGP-CPh birds, respectively. Protein intake was decreased by 3.3 and 5.9% in SGP-CPm and SGP-CPl birds, compared to the SGP-CPh birds, whereas HGP-CPm and HGP-CPl birds had a 3.6 and 5.0% lower protein intake. Differences in digestible lysine and methionine + cysteine intake were similar as in protein intake.

Table 2. Effects of growth pattern (GP), dietary protein level (CP) and their interaction on total feed intake, energy intake, CP intake, dig. Lys intake, and dig. Met + Cys intake in broiler breeders from 2 to 22 wk of age.

Item[1]	Feed intake (kg/pullet)	AME_n intake (kcal/pullet)	CP intake (g/pullet)	Dig. Lys intake (g/pullet)	Dig. Met + Cys intake (g/pullet)
Treatment					
SGP					
CPh	9.67^{f}	$25,836^{f}$	$1,464.5^{d}$	55.06^{d}	47.29^{d}
CPm	10.13^{e}	$27,057^{e}$	$1,415.5^{e}$	53.06^{e}	45.98^{e}
CPl	10.59^{c}	$28,285^{c}$	$1,378.1^{f}$	50.69^{f}	44.39^{f}
HGP					
CPh	10.28^{d}	$27,448^{d}$	$1,555.7^{a}$	58.48^{a}	50.21^{a}
CPm	10.74^{b}	$28,668^{b}$	$1,499.6^{b}$	56.21^{b}	48.71^{b}
CPl	11.36^{a}	$30,338^{a}$	$1,477.6^{c}$	54.32^{e}	47.56^{c}
SEM	0.02	45	2.4	0.09	0.08
Main effect GP					
SGP	10.13^{B}	$27,059^{B}$	$1,419.3^{B}$	52.94^{B}	45.89^{B}
HGP	10.79^{A}	$28,818^{A}$	$1,511.0^{A}$	56.34^{A}	48.83^{A}
SEM	0.01	26	1.4	0.05	0.04
Main effect CP					
CPh	9.98^{C}	$26,642^{C}$	$1,510.1^{A}$	56.77^{A}	48.75^{A}
CPm	10.43^{B}	$27,862^{B}$	$1,457.6^{B}$	54.64^{B}	47.35^{B}
CPl	10.98^{A}	$29,312^{A}$	$1,427.8^{C}$	52.50^{C}	45.98^{C}
SEM	0.01	32	1.7	0.06	0.05
P-value					
GP	<0.001	<0.001	<0.001	<0.001	<0.001
CP	<0.001	<0.001	<0.001	<0.001	<0.001
GP × CP	<0.001	<0.001	0.009	0.028	0.023

[a-f]Treatments means within a column and factor without a common superscript differ significantly ($P \leq 0.05$).
[A-C]Differences within the main effects without a common superscript differ significantly ($P \leq 0.05$).
[1]Each value represents the mean of 8 replicate pens. SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

Body weights and CV at different ages are shown in Table 3. At 5, 10, 15, and 20 wk of age, the HGP pullets were 12, 70, 123, and 163 g heavier than the SGP pullets, respectively. Pullets on the different dietary protein levels followed the same growth pattern (SGP or HGP). Although daily feed allocations were adjusted for the different protein groups to reach the predetermined BW target of certain week, the results showed a small difference in BW between the protein groups of maximal 25 and 22 g at 5 and 10 wk of age, respectively. These differences disappeared at 15 and 20 wk of age. No differences in CV among treatments at 5, 10 and 15 wk of age were found. However, at 20 wk of age pullets fed the CPl diet showed, on average, a 3.3% lower CV compared with the pullets fed the CPm and CPh diets.

Table 3. Effects of growth pattern (GP), dietary protein level (CP) and their interaction on BW and CV in broiler breeders at 5, 10, 15, and 20 wk of age.

	5 wk of age		10 wk of age		15 wk of age		20 wk of age	
Item[1]	BW (g)	CV (%)	BW (g)	CV (%)	BW (g)	CV (%)	BW (g)	CV (%)
Treatment								
SGP								
CPh	603	17.8	1,068	15.5	1,580	16.6	2,200	12.3
CPm	581	16.8	1,077	14.9	1,576	13.6	2,181	10.2
CPl	580	17.6	1,092	16.6	1,572	12.7	2,168	7.5
HGP								
CPh	613	16.6	1,142	12.7	1,697	12.8	2,349	10.1
CPm	601	15.8	1,145	16.0	1,696	14.2	2,344	11.4
CPl	586	15.3	1,161	13.8	1,705	12.2	2,345	8.3
SEM	3	1.1	3	1.2	7	1.4	10	1.2
Main effect GP								
SGP	588[b]	17.4	1,079[b]	15.7	1,576[b]	14.3	2,183[b]	10.0
HGP	600[a]	15.9	1,149[a]	14.2	1,699[a]	13.1	2,346[a]	9.9
SEM	2	0.7	2	0.7	4	0.8	6	0.7
Main effect CP								
CPh	608[a]	17.2	1,105[b]	14.1	1,638	14.7	2,275	11.2[a]
CPm	591[b]	16.3	1,111[b]	15.4	1,636	13.9	2,263	10.8[a]
CPl	583[c]	16.4	1,127[a]	15.2	1,639	12.4	2,257	7.9[b]
SEM	2	0.8	2	0.9	5	1.0	7	0.8
P-value								
GP	<0.001	0.113	<0.001	0.141	<0.001	0.303	<0.001	0.927
CP	<0.001	0.708	<0.001	0.516	0.907	0.297	0.168	0.013
GP × CP	0.124	0.822	0.666	0.193	0.515	0.277	0.336	0.278

[a-c]Differences within the main effects without a common superscript differ significantly ($P \leq 0.05$).

[1]Each value represents the mean of 8 replicate pens. SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

From 23 wk onward, all groups were provided the same daily amount of feed to allow convergence of the 2 growth patterns (Table 4). At 25 wk of age, HGP hens had a 80 g higher BW than SGP hens, but from 30 wk of age onward, BW were not significantly different between treatments (data not shown).

Table 4. Feed allowances (g/bird per d) from 0 to 40 wk of age for Ross 308 broiler breeders reared at different dietary treatments.

	Treatment[1,2]					
Age (wk)	SGP-CPh	SGP-CPm	SGP-CPl	HGP-CPh	HGP-CPm	HGP-CPl
1	16.9 (0.7)	16.4 (0.6)	16.8 (0.6)	16.7 (0.7)	17.1 (0.7)	16.8 (0.6)
2	28.9 (0.9)	28.4 (1.1)	28.7 (1.5)	28.9 (0.6)	29.0 (1.1)	28.4 (0.9)
3	37.0 (0.0)	37.0 (0.0)	37.0 (0.0)	38.0 (0.0)	38.0 (0.0)	38.0 (0.0)
4	42.4 (0.5)	43.1 (0.4)	43.5 (0.5)	44.5 (0.5)	44.9 (0.4)	45.6 (0.5)
5	45.3 (0.7)	45.4 (0.7)	47.4 (1.1)	46.8 (0.7)	48.1 (1.1)	49.7 (1.2)
6	47.2 (0.4)	52.1 (0.9)	57.3 (0.9)	51.7 (0.6)	56.4 (0.8)	60.8 (0.4)
7	48.9 (0.7)	52.9 (0.3)	56.5 (0.6)	52.6 (0.4)	56.4 (0.7)	60.3 (0.7)
8	53.5 (0.5)	57.5 (0.4)	61.3 (0.3)	57.3 (0.3)	61.2 (0.1)	66.6 (0.7)
9	58.9 (0.5)	62.5 (0.5)	65.4 (0.1)	63.6 (0.5)	65.5 (0.3)	72.2 (0.1)
10	62.2 (0.7)	65.7 (0.6)	68.4 (0.4)	67.2 (0.6)	69.3 (0.3)	74.2 (0.4)
11	64.3 (0.5)	67.1 (0.5)	69.0 (0.1)	69.1 (0.5)	71.4 (0.3)	75.8 (0.1)
12	67.1 (0.7)	69.8 (0.4)	71.4 (0.6)	72.2 (0.6)	74.0 (0.4)	78.9 (0.6)
13	70.4 (0.7)	72.9 (0.5)	74.6 (1.1)	75.7 (0.9)	76.6 (0.1)	81.9 (0.7)
14	73.1 (0.7)	75.2 (0.1)	77.4 (0.7)	78.5 (0.9)	81.0 (0.6)	85.9 (0.1)
15	76.0 (0.7)	77.6 (0.6)	80.4 (0.9)	82.9 (0.8)	86.0 (0.9)	89.9 (0.0)
16	78.9 (0.7)	81.3 (0.8)	84.2 (0.9)	85.7 (0.9)	88.8 (0.7)	93.4 (0.7)
17	82.3 (0.6)	85.4 (1.3)	88.4 (1.1)	89.1 (1.1)	93.3 (1.1)	97.5 (1.3)
18	81.3 (1.0)	86.5 (1.1)	92.0 (0.5)	89.5 (1.8)	95.5 (0.6)	103.1 (1.2)
19	85.5 (1.4)	90.8 (2.1)	96.5 (0.9)	92.4 (1.9)	99.4 (0.3)	103.5 (2.2)
20	96.3 (0.2)	102.2 (1.4)	107.4 (1.1)	99.8 (1.1)	105.2 (1.2)	110.9 (1.7)
21	103.1 (0.0)	108.5 (0.2)	115.0 (0.2)	103.6 (0.2)	108.8 (0.2)	115.4 (0.2)
22	108.3 (0.0)	113.7 (0.0)	119.8 (0.0)	108.3 (0.0)	113.7 (0.0)	119.8 (0.0)
23	114.0 (0.0)					
24	120.0 (0.0)					
25	133.0 (0.0)					
26	147.0 (0.0)					
27	160.0 (0.0)					
28-31	165.0 (0.0)					
32	163.0 (0.0)					
33	160.0 (0.0)					
34	158.0 (0.0)					
35	155.0 (0.0)					
36-40	157.0 (0.0)					

[1]Each value represents the mean (+SE) of 8 replicate pens. Starter-1 between 0 and 2 wk, starter-2 between 2 and 6 wk, grower between 6 and 15 wk, prebreeder between 15 and 22 wk, and breeder 1 between 22 and 40 wk of age.
[2]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

Body composition

At 10 and 20 wk of age, HGP pullets had 0.07 and 0.16% more abdominal fat pad than SGP pullets, respectively (Table 5). At 15 wk of age no differences in abdominal fat pad were observed between SGP and HGP. A decrease in dietary protein level resulted in a linear reduction in breast muscle content and a linear increase in abdominal fat pad at 10 and 20 wk of age. At 15 wk of age, body composition was not affected by dietary protein level. No carryover effects of growth pattern and dietary protein level during rearing were found on body composition at 40 wk of age.

Table 5. Effects of growth pattern (GP), dietary protein level (CP) and their interaction on breast muscle (BM) and abdominal fat (AF) pad weight of Ross 308 broiler breeders at 10, 15, 20, and 40 wk of age.

	10 wk of age		15 wk of age		20 wk of age		40 wk of age	
Item[1]	BM (%)[2]	AF (%)[2]	BM (%)	AF (%)	BM (%)	AF (%)	BM (%)	AF (%)
Treatment								
SGP								
CPh	14.38	0.03	14.99	0.00	17.73	0.28	17.48	2.51
CPm	14.09	0.06	14.88	0.00	17.05	0.29	18.04	2.14
CPl	13.68	0.19	14.89	0.01	16.77	0.52	17.66	2.42
HGP								
CPh	14.38	0.05	15.50	0.00	18.05	0.21	17.57	2.05
CPm	13.90	0.09	15.27	0.02	17.46	0.55	17.71	2.43
CPl	13.37	0.34	14.72	0.03	16.12	0.80	17.48	2.13
SEM	0.25	0.05	0.27	0.01	0.31	0.08	0.29	0.20
Main effect GP								
SGP	14.05	0.09	14.92	0.00	17.18	0.36[b]	17.73	2.35
HGP	13.88	0.16	15.17	0.02	17.21	0.52[a]	17.59	2.20
SEM	0.14	0.03	0.15	0.01	0.18	0.05	0.17	0.11
Main effect CP								
CPh	14.38[a]	0.04[b]	15.25	0.00	17.89[a]	0.24[c]	17.53	2.28
CPm	13.99[ab]	0.08[b]	15.08	0.01	17.25[b]	0.42[b]	17.88	2.28
CPl	13.53[b]	0.26[a]	14.81	0.02	16.44[c]	0.66[a]	17.57	2.27
SEM	0.17	0.03	0.19	0.01	0.22	0.06	0.21	0.14
P-value								
GP	0.410	0.065	0.265	0.118	0.911	0.029	0.568	0.349
CP	0.005	<0.001	0.260	0.158	<0.001	<0.001	0.440	0.999
GP × CP	0.823	0.292	0.398	0.531	0.180	0.071	0.782	0.145

[a-c]Differences within the main effects without a common superscript differ significantly ($P \leq 0.05$).
[1]Each value represents the mean of 8 replicate pens. SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.
[2]Percentage of BW.

Egg production

Applying different growth patterns and dietary protein levels during the rearing period did not affect total eggs/hen, total settable eggs/hen, total unsettable eggs/hen, egg weight, age at sexual maturity (defined as age at 50% production), peak egg production, and age at peak egg production (Table 6).

Table 6. Effects of growth pattern (GP), dietary protein level (CP) and their interaction on performance of Ross 308 broiler breeders from 23 to 40 wk of age.

Item[1]	Total eggs/hen	Total settable eggs/hen[2]	Total unsettable eggs/hen[3]	Egg weight (g)[4]	ASM (d)[5]	Peak egg production (%)	Age at peak egg prod. (d)
Treatment							
SGP							
CPh	88.9	80.7	8.2	57.4	184.0	96.1	224.0
CPm	89.9	82.3	7.6	57.8	184.5	97.6	214.4
CPl	89.6	82.0	7.6	57.5	184.7	96.9	222.2
HGP							
CPh	88.7	80.5	8.2	57.1	185.5	97.3	210.9
CPm	91.9	83.5	8.5	57.2	183.0	97.9	224.0
CPl	88.9	80.6	8.3	57.4	184.7	95.8	215.2
SEM	1.5	1.5	0.8	0.4	0.9	1.1	5.7
Main effect GP							
SGP	89.5	81.7	7.8	57.6	184.4	96.9	220.2
HGP	89.8	81.5	8.3	57.2	184.4	97.0	216.7
SEM	0.8	0.9	0.5	0.2	0.5	0.6	3.3
Main effect CP							
CPh	88.8	80.6	8.2	57.2	184.7	96.7	217.4
CPm	90.9	82.8	8.1	57.5	183.7	97.8	219.2
CPl	89.2	81.3	7.9	57.5	184.7	96.3	218.8
SEM	1.0	1.1	0.6	0.3	0.6	0.8	4.1
P-value							
GP	0.765	0.901	0.454	0.260	0.985	0.866	0.459
CP	0.317	0.306	0.951	0.735	0.409	0.406	0.951
GP × CP	0.603	0.680	0.860	0.841	0.234	0.574	0.135

[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.
[2]Number of eggs weighing above 50 g, not including soft shell, crack, double yolk, or dirty eggs, and each value represents the mean of 8 replicates over 18 wk (settable eggs).
[3]Number of eggs weighing under 50 g, soft shell, crack, double yolk, or dirty eggs, and each value represents the mean of 8 replicates over 18 wk (unsettable eggs).
[4]Egg weight is determined for all hatching eggs (settable and small eggs), and each value represents the mean of 8 replicates over 18 wk determined in a 1-wk interval.
[5]ASM = age of sexual maturity, defined as age at 50% production.

DISCUSSION

The aim of this study was to evaluate the effects of different growth patterns and dietary protein levels on body composition and performance during the rearing and laying period of broiler breeder hens.

Effect of growth pattern

The increased feed intake to reach the 8% higher BW targets for the HGP hens at end of rearing was according to expectations. For each 100 g of BW increase at 20 wk of age, 0.40 kg of extra feed intake was required. This finding is in close agreement with those of Renema et al. (2001a) and Hocking et al. (2002) who found 0.35 (20 wk of age) and 0.46 (24 wk of age) kg of feed per 100 g increase of BW, respectively. The value in the current study is somewhat lower than the 0.55 kg per 100 g increase of BW found by Gous and Cherry (2004), probably because half of their experimental groups were subjected to different convex growth patterns. These birds reached an approximately 55% higher BW at 12 wk of age, resulting in a relative higher proportion of feed necessary for maintenance. Hens in that study raised, according to a linear growth curve, a value of 0.44 kg per 100 g BW, which is similar to the results in the current study. The absence of an effect of a higher BW target at the end of the rearing period on CV was also shown in an experiment of Hocking et al. (2001).

Up to 15 wk of age, pullets managed to have a standard and high growth pattern showed similar relative breast muscle and abdominal fat pad weights. At 20 wk of age, however, abdominal fat pad was slightly increased for the HGP compared to the SGP pullets, whereas breast muscle weight was not affected by the 8% higher BW at the end of the rearing period. In contrast, Fattori et al. (1993) and Renema et al. (2001a) did not find an effect of a 15 or 8% higher BW at 20 wk of age on abdominal fat pad content, respectively. Differences in abdominal fat pad appeared in these studies when, at the end of the rearing period, BW differed 23 and 21%, respectively. Sun and Coon (2005), on the other hand, reported a decreased protein content of the body and no differences in fat content of the birds when BW was increased 13% at 20 wk of age. It was surprising that the severely restricted broiler breeder pullets in the current study showed a slightly increased relative abdominal fat pad weight when fed 6.5% more feed (but still far below *ad libitum* feed intake level) to an 8% higher BW at 20 wk of age. Differences in fat pad content between the birds of the 2 growth patterns at 20 wk of age fully disappeared in the hens at 40 wk of age. It was, therefore, suggested that if hens with a standard versus a high BW at the end of the rearing period were fed similar amounts of feed during the laying period, hens with the high BW were relatively more restricted than their standard BW counterparts, forcing them to use their body reserves. Sun and Coon (2005), also applied the principle of convergent BW curves during lay and found no differences in abdominal fat pad weight at the end of the laying period. On the other hand, Renema et al. (2001b), who maintained BW differences during the laying period, still had a higher abdominal fat pad weight for the heavier birds at the end of lay.

The relation between BW and sexual maturity was mentioned by several authors (Renema et al., 2001a,b; Hocking, 2004; Ekmay et al., 2012) who reported that as BW increases at the end of rearing period, age at which sexual maturity occurs decreases. Contrary to those findings, a higher BW at the end of the rearing period did not advance sexual maturity in the current study and is as found by Zuidhof et al. (2007). Gous and Cherry (2004) found that every extra 100 g BW at 20 wk of age advanced sexual maturity by 1.5 d. This is in close agreement with Sun and

Coon (2005) and R. A. van Emous (unpublished data) who found 50% hen-day egg production advance by 0.9 and 1.0 d per 100 g extra BW, respectively. Renema et al. (2001a) even found an advancement of 3.0 d per 100 g extra BW. The difference between the above-mentioned studies and the current one could be due to the fact that in these studies growth patterns remained to be distinct from each other, whereas our growth patterns converged toward onset of lay. In the current study, the HGP hens were fed relative restricted from wk 20 onward compared with the higher BW, to achieve a similar BW as the SGP hens at the onset of lay.

An 8% (163 g) heavier BW for the HGP hens at the end of the rearing period in the current study did not increase average egg weight. This is in agreement with the results of Fattori et al. (1991) and Hocking et al. (2001, 2002), who did not find an effect of an 8% (158 g at 20 wk of age) and 20% (365 g at 18 wk of age) higher BW target on average egg weight. Renema et al. (2001a,b), Sun and Coon (2005), and R. A. van Emous (unpublished data) attained larger differences in BW [21% (338 g), 13% (229 g), and 21% (427 g)] at the end of the rearing period (20 wk of age), respectively, resulting in a 1.1, 0.9, and 1.0 g higher egg weight. The reason for the absence of an effect on egg weight in the current study for the HGP hens and some of the studies mentioned above maybe due to the fact that from the end of the rearing period onward, the HGP hens were more severely restricted than the SGP hens because they were fed a similar amount of feed to converge BW. Another reason could be that the differences in BW at the end of the rearing period were not sufficiently large to affect initial or average egg weight. This hypothesis seems to be confirmed by other researchers who did not find a difference in average (Gous and Cherry, 2004; Ekmay et al., 2012) or initial (Robinson et al., 2007) egg weight even when birds showed a 16% (370 g), 20% (approximately 430 g), and 29% (588 g) higher BW at the end of the rearing period, respectively. These researchers also used a feeding schedule during the initial laying period to converge BW.

Total and settable eggs were not influenced by HGP. These results are similar to those of Fattori et al. (1991), Hocking et al. (2002), Gous and Cherry (2004), Sun and Coon (2005) and Zuidhof et al. (2007). Ekmay et al. (2012), however, reported an increased number of eggs per hen housed as a result of a 20% higher BW at the end of the rearing period. It was suggested that this was due to an earlier sexual maturity and higher peak production. Renema et al. (2001b) found the lowest total egg production for standard BW compared with lighter and heavier hens, which was caused by a higher number of defective eggs.

In conclusion, subjecting hens to an 8% higher target BW at the end of the rearing period by increasing feed intake resulted in a minor increase in abdominal fat content of the body but no effect on breast muscle at the end of the rearing period nor on body composition and production performance during the laying period.

Effect of dietary protein level

In line with our expectations, this study showed that reducing dietary protein level resulted in an increased feed intake to meet the same BW target at the end of the rearing period. These results are similar to those of Lilburn and Myers-Miller (1990), Miles et al. (1997), Hudson et al. (2000) and Hocking et al. (2002), who found that providing low-protein diets with a similar energy content required more feed to reach the same target BW. In the present study, birds on CPm and CPl diets with an 8 and 16% decreased amino acid level required only 4.6 and 10% more feed to reach a similar BW, respectively. It seems that the CPh diet is limiting in energy, and therefore these birds used amino acids as an energy source, inducing lower growth efficiency (less water accretion).

In the current study, no effects on CV were found between dietary protein levels at 5, 10, and 15 wk of age (Table 3). Contrary to our results, Hudson et al. (2000) found a lower CV at 6 wk of age when pullets were fed a high dietary protein level (20 vs. 12% CP). The contrast in protein in that study, however, was much higher than in the current study, resulting in a larger difference in feed intake. In the present study, a lower CV was found at 20 wk of age for CPl birds compared with CPh and CPm birds. In contrast, in a study of Hocking et al. (2001), CV at 24 wk of age was no longer affected by dietary protein level during the rearing period, whereas CV was lower at 12 and 18 wk of age when pullets were fed a high dietary protein level. It is hypothesized that the lower CV in the current study at 20 wk of age was due to the 10% increased feed intake of this diet during the rearing period, resulting in less competitive feeding behavior. The 4.6% higher feed intake of the birds on the CPm diet was probably not sufficient for achieving a lower CV.

Hens fed the CPm and CPl diets deposited more fat and less protein when fed different amounts of feed to achieve the same target BW at 10 and 20 wk of age (Table 5), probably caused by an increased hepatic lipogenesis as suggested by De Beer and Coon (2007). A nutritional alternation of lipogenesis by the feed is mainly attained by an altered energy to protein ratio in the diet (Yeh and Leveille, 1969) or by fasting and refeeding (Rosebrough, 2000). In the current study, the energy to protein ratio (kcal of ME per g of CP) between 15 and 22 wk of age obviously differed in the CPm (19.3) and CPl (21.1) diets, relative to the CPh diet (18.1) which might have been resulted in an increased conversion of dietary carbohydrates to lipid deposition in the body.

Breast muscle weight of the hens fed the CPl diet as compared with the CPh diet was decreased at 10 wk of age (13.5 vs. 14.4%) and 20 wk of age (16.4 vs. 17.9%). Abdominal fat pad weight of the hens fed the CPl diet compared with the CPh diet was increased at 10 wk of age (0.26 vs. 0.04%) and 20 wk of age (0.66 vs. 0.24%). Breast muscle and abdominal fat pad weights of the hens fed the CPm diets were mostly intermediate between hens fed the CPh and CPl diets. These results are in close agreement with those of Mba et al. (2010), who found that feeding a 14% CP diet compared with a 16% CP diet resulted at 12 and 25 wk of age resulted in a 0.8 and 2.0% lower breast muscle weight and a 0.09 and 0.40% higher abdominal fat pad weight, respectively. Also Miles et al. (1997) and Hudson et al. (2000) reported similar effects of changes in body composition due to changes in dietary protein level.

Surprisingly, dietary protein level did not affect abdominal fat pad at 15 wk of age (Table 5). This pattern in development of abdominal fat pad weight was previously reported by Bennett and Leeson (1990), who found a decreased abdominal fat pad weight (% BW) between 2 and 14 wk of age followed by an increased fat pad weight between 14 and 24 wk of age. This phenomenon might be explained by the severe feed restriction level (about 25-33% of *ad libitum* intake) of the hens between 7 and 16 wk of age (Savory et al., 1996; De Jong et al., 2002; Mench, 2002; De Jong and Jones, 2006). This severe feed restriction during midterm of rearing probably forced the hens to prioritize feed nutrients to major processes in the body. At the end of the rearing period (20 wk of age), feed allowances are gradually increased to allow the birds to deposit abdominal fat again.

Egg production was not affected by dietary protein level. Miles et al. (1997) and Pishnamazi et al. (2011) also found similar results. On the contrary, Hocking et al. (2002) observed a decreased egg production when hens were fed low-protein diets during rearing. In their study, however, the low-protein treatment had an extremely low protein content of 10% between 15 and 18 wk of age, where our low-protein diet only decreased to 12.8% in that period.

In conclusion, feeding low dietary protein levels during rearing resulted in decreased breast muscle weights, increased abdominal fat pad weights, and increased feed intake during the rearing period to obtain similar BW curves. When hens were fed subsequently a standard breeder diet with increasing amounts of feed, differences in body composition disappeared and no effects were found on egg production during the laying period.

CONCLUSION

The overall conclusion is that under the restrictions of the current study, differences in dietary protein during the rearing period were more effective than modifying the growth pattern in changing body composition at the end of the rearing period. The hypothesis, that an increased abdominal fat content of the body at the end of the rearing period may improve reproductive performance during the laying period, needs to be rejected. That is, no effect of any of the dietary treatments on egg production could be detected. This study, therefore, revealed that our nutritional interventions that changed body composition toward the end of the rearing period were not severe enough to cause permanent differences in egg production. It seems that the broiler breeders in our study showed a large amount of resilience.

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Chapter 3

Effects of growth patterns and dietary protein levels during rearing on feed intake, eating time, eating rate, behavior, plasma corticosterone concentration, and feather cover in broiler breeder females during the rearing and laying period

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ABSTRACT

An experiment was conducted to evaluate the effect of growth patterns (GP) and dietary crude protein levels (CP) during rearing (2 to 22 wk of age) on feed intake, eating time, eating rate, behavior, plasma corticosterone concentration, and feather cover in broiler breeder females during the rearing and laying period. A total of 768 day-old Ross 308 broiler breeder chicks, of which 288 hens were followed during the laying period, were allotted to 6 different treatments during the rearing period according to a 2 × 3 factorial design, with 8 replicates (pens) per treatment. Two growth patterns were followed by a restricted feeding regime up to a target body weight (BW) at 20 wk of age of 2,200 g (standard growth pattern = SGP) and 2,400 g (high growth pattern = HGP) and 3 dietary protein levels (high = CPh, medium = CPm, and low = CPl). During lay, all birds were fed a standard breeder diet and followed a standard growth pattern. During rearing, HGP birds were fed on average 6.5% more feed than SGP birds. In HGP birds, eating time (min/d) during the rearing period increased by 17%, whereas eating rate (g feed/min) decreased by 8%, compared to SGP birds. This prolonged feeding behavior of HGP birds, but stereotypic object pecking and animal pecking was not reduced. Feather cover was not affected by growth pattern during the rearing and laying period. Only at 16 wk of age a lower plasma corticosterone concentration was found for the HGP birds. HGP birds showed more feeding and sitting behavior, but less foraging behavior during the rearing period, while during the laying period only more walking behavior was observed. In order to maintain target weights, feed intake levels of CPm and CPl during rearing were set 4.6 and 10.0% higher than CPh, whereas eating time was increased by 22 and 63% and eating rate was decreased by 9 and 26%, respectively. A prolonged eating time during rearing for CPm and CPl birds resulted in more time spent on feeding and resting and less stereotypic object pecking and animal pecking compared to CPh birds during rearing. In contrast to the rearing period, eating time and eating rate disappeared during the laying period. Plasma corticosterone concentrations were not affected by dietary protein level during the rearing and laying period. Feather cover was inferior by lowering the dietary protein level, in particularly during the first 11 wk of rearing. It is concluded that dietary protein levels positively affected some behavioral traits during the rearing period, whereas these traits were only slightly affected by growth patterns. However, the physiological parameter (plasma corticosterone concentration) was not affected.

Key words: broiler breeder, dietary protein level, growth pattern, behavior, feather cover

INTRODUCTION

Over the past 30 years, growth potential of broiler breeders has increased drastically, due to selection on growth and feed efficiency of the progeny (Renema et al., 2007b). *Ad libitum* feeding of such broiler breeder females during the rearing period resulted in a high BW prior to the laying period, resulting in excessive mortality (Heck et al., 2004) and decreased reproductive performance (Yu et al., 1992b; Hocking et al., 2002). Therefore, feed intake of broiler breeders during rearing is restricted to 25-33% of *ad libitum* intake (Savory et al., 1996; De Jong et al., 2002). The most severe restriction usually occurs between 7-8 and 15-16 wk of age (De Jong and Jones, 2006). Feed restricted broiler breeders show behavioral disorders that are indicative of hunger and frustration, such as stereotypic object pecking and over-drinking (i.e., Hocking et al., 1996, 2001; Savory and Kostal, 1996; De Jong et al., 2002). In addition, indicators of chronic stress in birds such as increased plasma corticosterone concentrations (Hocking et al., 1996; Savory and Mann, 1997; De Jong et al., 2002) and increased heterophil to lymphocyte (H/L) ratios (Hocking et al., 1993, 1996; Savory et al., 1993) are observed.

The best method for improving welfare in parent stock of so-called fast growing broilers is not yet elucidated. One of the methods to reduce the negative effects of feed restriction on bird welfare could be the application of alternative feeding strategies that may enhance eating time during the day (De Jong and Van Krimpen, 2011). De Jong et al. (2005a) applied scattered feeding and feeding twice a day during rearing, thereby increasing eating time, but they did not find any effect on physiological indicators of stress and hunger. Nielsen et al. (2011) found that high levels of dietary insoluble fiber in the rearing period, in combination with scattered feeding, may improve the welfare of broiler breeders. Diluting the feed also increased the time spent eating, which is noted as a promising method for improving bird welfare (Hocking et al., 2004; De Jong et al., 2005b). In some studies, dietary dilution (by adding fiber) reduced stereotypic object pecking (De Jong et al., 2005b; Hocking et al., 2004), although these effects were not observed in other studies (Hocking, 2006; Jones et al., 2004). It could be argued that feeding a lower dietary protein level while maintaining the same growth rate demands a higher feed intake level and prolonged feeding behavior which may decrease stereotypic pecking behavior (Hocking et al., 2004; Mason et al., 2006). To the authors knowledge, no studies until now have been conducted in which dietary protein levels and growth patterns are investigated together. The current study aimed to evaluate the effects of different growth patterns and dietary protein levels on feed intake, eating time, behavioral traits, plasma corticosterone concentration, and feather cover in broiler breeder females. It was hypothesized that a high growth pattern (a 200 g higher BW at 20 wk of age) and a low dietary protein level would increase eating time and decrease stereotypic behavior, thereby improving broiler breeder welfare.

MATERIALS AND METHODS

Housing, birds, and management

A total of 768 day-old Ross 308 female broiler breeder chicks were housed in two identical climate-controlled rooms. All chickens were individually identified by wing tags and beaks were trimmed at d 3. Each room contained 24 floor pens (0.90 m × 1.50 m), and each pen contained 16 birds at the start of the experiment. This number was gradually reduced to 6 pullets at 20 wk of age due to grading (removing the smallest pullets) and dissection procedures (Chapter 2). So, initial stocking density of 11.9 pullets per m^2 at day-old was reduced to 4.4 pullets per m^2 at 20 wk of age. The sidewalls of the pens were built of wire mash so that pullets could see birds in other pens. Each pen contained 2 perches (1.8 m total length), 2 feeding troughs (1.0 m total length), and 4 nipple drinkers (nipple line was also used as perch), whereas wood shavings were used as litter. During the first 2 d, temperature in the house was maintained at 33°C. From d 3 onward, the temperature was gradually reduced to 20°C at 5 wk of age. Light was set at 24 h per day for the first 2 d, with a gradual reduction to 8 h at d 21, which was maintained until d 147 (wk 21). Birds were photostimulated with 11 h of light in wk 22, and day length was gradually extended by 1 h (later 0.5 h) per wk to a L:D schedule of 15 h of light and 9 h of dark at 27 wk of age. The photoperiod lasted from 0400 to 1900 h. This schedule was maintained until the end of the experiment at 40 wk of age. During rearing and laying, a light intensity of 20 and 60 lx, respectively, was applied at bird level.

Feed was provided *ad libitum* during the first two weeks of the rearing period. Thereafter birds were fed restricted to keep the respective target body weight (BW). Birds were fed every day and all diets were provided in mash form. Water intake was restricted during the rearing and laying period by limiting the water supply till 2 h after feeding time, in order to prevent over-drinking. Throughout the experiment, litter quality was maintained by adding new wood shavings every 6 wk. At 20 wk of age, an accessible laying nest was placed at the front side of each pen, while one of the feeding troughs was removed. Pullets were vaccinated according to a standard vaccination program (Aviagen-EPI, Roermond, The Netherlands) and health status of the hens was monitored daily.

The protocol for the experiment agreed with standards for animal experiments and was approved by the Ethical Committee of Wageningen UR, Lelystad, The Netherlands.

Experimental design

From 0 to 2 wk of age, all birds received *ad libitum* a standard starter-1 diet. At d 14, birds were randomly allotted to 6 different treatments according to a 2 × 3 factorial design. The factors were two growth patterns (standard growth pattern = SGP and high growth pattern = HGP) and three dietary protein levels (high crude protein = CPh, medium crude protein = CPm, and low crude protein = CPl). During the rearing period, birds followed a phase feeding system. The starter-1 diet was fed from 0 to 2, the starter-2 from 2 to 6, the grower from 6 to 15, and the pre-breeder from 15 to 22 wk of age. From 22 to 40 wk of age, a standard breeder diet was provided to all birds. Beginning at 2 wk of age, half of the pens followed the breeder recommended growth pattern (SGP = 2,200 g; Aviagen-EPI, 2007) and the other half of the birds followed an elevated growth pattern (HGP = 2,400 g) to reach a difference of 200 g in BW at 20 wk of age. From 20 to 22 wk of age, feeding level was gradually adjusted to obtain a similar target BW for all birds at onset of lay as soon as possible.

The nitrogen corrected apparent metabolizable energy levels were similar for all diets per phase during the rearing period, but crude protein and amino acids levels were decreased for the CPm and CPl diets. Amino acid levels for the CPm and CPl diets were reduced by 8 and 16% compared to the CPh diet, respectively. Ingredient composition, and analyzed and calculated nutrient contents of the diets are presented in Table 1.

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

Item	Starter1 (0-14 d)	Starter2 (15-42 d)			Grower (43-105 d)			Pre-breeder (106-154 d)			Breeder1 (155-280 d)
		CPh[1]	CPm	CPl	CPh	CPm	CPl	CPh	CPm	CPl	
ngredients											
Maize	450.0	440.0	440.0	440.0	400.0	400.0	400.0	400.0	400.0	400.0	425.0
Wheat	189.6	200.0	200.0	200.0	190.0	190.0	190.0	248.9	249.4	250.0	200.0
Soybean meal	216.8	148.2	99.1	50.0	43.0	26.5	10.0	101.8	64.5	27.3	104.0
Sunflower meal	49.5	-	-	-	-	-	-	-	-	-	-
Maize gluten feed	-	125.0	125.0	125.0	104.1	104.1	104.1	-	39.0	78.0	-
Rapeseed meal	35.0	35.0	35.0	35.0	25.0	17.5	10.0	35.0	35.0	35.0	50.0
Wheat middlings	-	-	33.2	66.3	150.0	158.2	166.3	150.0	150.0	150.0	99.2
Peas	-	-	8.2	16.4	-	-	-	-	-	-	-
Maize gluten meal	10.0	1.2	8.0	14.7	-	-	-	-	-	-	-
Maize starch	-	-	-	-	-	6.7	13.3	-	-	-	-
Alfalfa meal	-	-	-	-	52.1	61.1	70.0	15.9	12.5	9.0	-
Soya oil	7.2	9.3	9.4	9.5	2.0	2.4	2.7	2.0	2.9	3.9	27.6
Chalk	16.5	17.2	17.5	17.7	15.5	15.3	15.0	24.6	25.0	25.4	18.0
Limestone	-	-	-	-	-	-	-	-	-	-	54.0
Monocalcium phosph.	10.2	9.6	9.6	9.7	4.0	4.2	4.4	5.0	4.9	4.7	5.9
Salt	1.5	0.8	0.6	0.4	0.4	0.3	0.2	1.5	1.2	1.0	1.8
Sodium carbonate	4.2	3.7	3.9	4.2	4.3	4.5	4.6	4.1	4.1	4.0	3.8
Premix rearing[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	-	-	-	--
Premix laying[3]	-	-	-	-	-	-	-	10.0	10.0	10.0	10.0
Choline Chloride-50%	2.8	2.8	2.8	2.8	2.0	2.0	2.0	-	-	-	-
Natuphos	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	-	-	-
Rovabio Excel AP	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	-	-	-
L-Lysine	0.8	1.2	1.8	2.3	1.8	1.9	1.9	0.5	1.0	1.5	0.2
DL-Methionine	1.0	0.7	0.5	0.3	0.4	0.3	0.2	0.6	0.5	0.4	0.6
L-Threonine	-	0.2	0.3	0.4	0.3	0.3	0.2	-	-	-	-
Analyzed content[4]											
DM	879.3	872.3	872.6	872.9	876.9	875.9	873.3	869.0	870.3	871.2	882.1
Ash	57.8	58.1	55.8	56.1	59.9	60.8	60.6	60.0	61.4	60.8	96.0
Fat	42.4	45.5	47.0	48.6	37.9	40.7	39.5	34.7	36.9	38.2	59.1
Crude fiber	37.5	33.2	34.8	36.5	52.7	54.3	56.0	39.9	40.6	41.2	32.5
Crude protein	207.4	192.1	179.6	145.9	141.3	129.6	122.7	148.8	139.6	127.9	144.8
Starch	375.3	405.7	413.8	418.2	365.9	381.1	395.5	401.4	405.6	385.6	391.3
Reducing sugars[5]	38.6	33.6	31.5	29.0	34.4	32.9	31.6	35.2	33.2	31.5	31.9
NSP[6]	160.1	178.6	183.7	188.7	226.7	229.2	231.7	185.9	193.4	200.8	158.7
Calculated content											
AME_n (kcal/kg)	2,795	2,800	2,800	2,800	2,600	2,600	2,600	2,700	2,700	2,700	2,780
AME_n:CP ratio[7]	13.5	14.6	15.6	19.2	18.4	20.1	21.2	18.1	19.3	21.1	19.0
Digestible lysine	8.6	7.2	6.6	6.0	5.4	4.9	4.5	5.6	5.2	4.7	5.3
Digestible M+C	6.7	5.7	5.3	4.8	4.5	4.2	3.8	5.0	4.7	4.4	4.8
Digestible thr.	6.0	5.2	4.8	4.4	4.0	3.6	3.3	4.1	3.8	3.4	4.0
Digestible tryp.	2.0	1.5	1.4	1.2	1.2	1.1	1.0	1.5	1.3	1.1	1.4
Calcium	10.0	10.0	10.0	10.0	9.0	9.0	9.0	12.0	12.0	12.0	30.0
Total phosphorus	6.5	6.5	6.5	6.5	5.8	5.8	5.8	5.7	5.8	5.8	5.4
Av. phosphorus	4.1	4.1	4.1	4.1	3.2	3.2	3.2	3.2	3.2	3.2	2.9
Physical characteristic											
Particle size (mm)	0.34	0.38	0.38	0.38	0.34	0.34	0.36	0.31	0.31	0.31	0.58

[1]Dietary protein level. CPh = high dietary crude protein; CPm = medium dietary crude protein; CPl = low dietary crude protein.

[2]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 30 mg; vitamin K3, 1.5 mg; vitamin B1, 2.0 mg; vitamin B2, 7.5 mg; vitamin B6, 3.5 mg; vitamin B12, 0.02 mg; niacinamide, 35 mg; D-pantothenic acid, 10 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 12 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 85 mg (as MnO_2); zinc, 60 mg (as $ZnSO_4$); cobalt, 0.4 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 0.8 mg (as KI); selenium, 0.1 mg (as $Na_2SeO_3 \cdot 5H_2O$).

[3]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin K3, 5.0 mg; vitamin B1, 3.0 mg; vitamin B2, 12.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.03 mg; niacinamide, 55 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.4 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 10 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 120 mg (as MnO_2); zinc, 100 mg (as $ZnSO_4$); cobalt, 0.25 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 2.0 mg (as KI); selenium, 0.3 mg (as $Na_2SeO_3 \cdot 5H_2O$); choline chloride 50%, 2.0 g; Natuphos, 0.1 g; Rovabio Excel AP, 50 mg.

[4]Based on 2 analysis in duplicate per diet.

[5]Mono- and disaccharides as glucose units.

[6]Calculated by subtracting the crude protein, fat, starch, reducing sugars, and ash content from the DM content.

[7]AME_n:CP ratio = kcal AME_n/g CP.

Observations

BW and feed intake. During rearing, pullets were weighed on a weekly basis to obtain weekly BW and calculate BW gain. The amount of feed for each week was proportioned to obtain the target BW gain of that week.

Eating time and eating rate. At 7, 12, 17, 22, and 27 wk of age, eating time, which was defined as the time interval between provision of the feed and the moment that the feed troughs were empty, was determined by visual inspection per pen. Eating rate (g feed/min) was calculated as feed intake (g/bird per d) divided by the number of eating min per d.

Behavioral observations. Behavior of the birds was observed by scan sampling each pen at 7, 12, 17, 22, and 27 wk of age. Behavior was scored by counting the number of birds in the pen exhibiting different activities according to an ethogram (Table 2). Behavioral observations started 1 h after the start of feeding (between 0800 and 0815 h) at seven sequential hours between 0900 and 1500 h on two consecutive days. Pens were observed 14 times in total per age period. Feeding and drinking behavior was only recorded when feed and water was available. During the feeding and drinking period, stereotypic object pecking was defined as pecking at the side walls of the pens. When feed troughs were empty and drinking nipples were closed, pecking at these objects was scored as stereotypic object pecking behavior as well. Sitting on perch was defined as sitting on the perch or nipple line.

Table 2. Ethogram showing the behavioral measurements, according to De Jong et al. (2005a).

Behavior	Definition
Feeding	Pecking at feed at the feeding troughs
Drinking	Pecking at water at the drinking nipples
Foraging	Pecking, scratching the litter
Perching	Sitting on the perches
Walking	Walking or running without performing other behavior
Standing	Standing without performing other behavior
Sitting	Sitting without performing other behavior
Object pecking	Pecking at parts of pen, wall, empty feeding troughs or empty drinking nipples
Animal pecking	All pecking at other birds

Plasma corticosterone concentration. At 11, 16, 21, and 26 wk of age, blood was collected from the wing vein by venipuncture of 2 birds per pen between 1000 and 1100 h. Hens were quietly approached and quickly picked up, and blood was sampled (2.5 ml) within 1 min after handling the bird in order to avoid elevations of plasma corticosterone concentrations. To determine plasma corticosterone concentrations, blood was stored in heparinized tubes on ice and within 1 h after sampling centrifuged at 3,000 rpm at 4°C. Plasma was stored at -20°C in 96, 1.2 ml well masterblocks (Griener) until analysis of corticosterone concentrations. Plasma corticosterone concentrations were analyzed using a commercially available double antibody RIA kit (ImmuChemTM, MP Biomedicals, Illkirch Cedex, France) which had an intra-assay CV of 3.9%.

Feather cover. At 6, 11, 16, 20, 25, 30, 35, and 40 wk of age, feather cover per individual bird was scored according to the method described by Bilcik and Keeling (1999). Scores, varying from 0 (intact feathers) to 5 (completely denuded area), were given for each of seven body parts (neck, breast, belly, back, wings, tail, and legs). The average of these seven scores was also used for analysis.

Statistical analysis

The experiment was set up as a 2 × 3 factorial design with two growth patterns and three dietary protein levels as the main effects. From the beginning until the end of the experiment, pen was treated as the experimental unit. The data were analyzed by the General Analysis of Variance ANOVA (GenStat 14.1, GenStat Committee, 2011). Growth pattern, dietary protein level, observation week, and their interactions were used as factors in the statistical model of the data. Room was considered to be the random term. Parameters were tested for normal distributions before analysis. After inspection of diagnostic plots of residuals, it was decided to analyze the behavioral variables with a logistic regression model (data expressed as percentages). Data are presented as mean ± SEM. All statements of significance are based on testing at $P < 0.05$.

RESULTS

Feed intake

To meet BW targets at 22 wk of age, feed intake between 2 and 22 wk of age was increased in HGP pullets by 6.5% compared to the SGP treatment. The pullets fed the CPm and CPl diets required a 4.6 and 10.0% higher feed intake to meet BW targets at 22 wk of age, respectively, compared to pullets fed the CPh diet, as previously reported in Chapter 2.

Eating time and eating rate

Eating time was not affected by growth pattern in birds fed the CPh and CPm diets during the rearing period, however, CPl showed a prolonged eating time when following the HGP at 12 and 17 wk of age compared to SGP (Table 3).

As a consequence of the higher feed intake of the HGP pullets, eating time was prolonged by 17%, whereas eating rate decreased by 8% during the rearing period compared to the SGP pullets (Table 3).

The decrease in dietary protein level resulted in a higher feed intake, prolonged eating time and in a reduction in eating rate during the rearing period. Birds fed the CPm diet showed a prolonged eating time of 22% (33 and 11% at 7 and 17 wk of age, respectively) and a decreased eating rate of 9% (range: 17-5%) compared to the CPh birds. Eating time of birds fed the CPl diet increased with age by 63% (range: 83-36%), whereas eating rate decreased by 26% (range: 37-19%) compared to birds fed the CPh diet. Eating time was affected by feed intake and changed during the rearing period (Figure 1).

Table 3. Feed intake (g/bird per d), eating time (min/d), and eating rate (g feed/min) in Ross 308 broiler breeders during the rearing period.

	7			12			17		
Item[1]	Feed intake	Eating time	Eating rate	Feed intake	Eating time	Eating rate	Feed intake	Eating time	Eating rate
Treatment									
SGP									
CPh	48.9	114	0.43	67.1^{e}	81^{d}	0.86	82.3^{e}	66^{de}	1.29
CPm	52.9	151	0.36	69.8^{d}	99^{c}	0.72	85.4^{d}	73^{cd}	1.22
CPl	56.5	215	0.27	71.4^{cd}	116^{b}	0.63	88.4^{cd}	82^{b}	1.13
HGP									
CPh	52.6	137	0.39	72.2^{c}	94^{cd}	0.78	89.1^{c}	73^{cd}	1.24
CPm	56.4	182	0.32	74.0^{b}	101^{c}	0.75	93.3^{b}	80^{bc}	1.19
CPl	60.3	245	0.25	78.9^{a}	156^{a}	0.51	97.5^{a}	107^{a}	0.92
SEM	0.6	9	0.02	0.6	5	0.03	1.1	3	0.05
Main effect GP									
SGP	52.8^{B}	160^{B}	0.35^{A}	69.4^{B}	99^{B}	0.74^{A}	85.3^{B}	74^{B}	1.21^{A}
HGP	56.5^{A}	188^{A}	0.32^{B}	75.0^{A}	117^{A}	0.68^{B}	93.3^{A}	86^{A}	1.12^{B}
SEM	0.4	5	0.01	0.3	3	0.02	0.7	2	0.03
Main effect CP									
CPh	50.8^{C}	125^{C}	0.41^{A}	69.6^{C}	87^{C}	0.82^{A}	85.7^{C}	69^{C}	1.27^{A}
CPm	54.7^{B}	167^{B}	0.34^{B}	71.9^{B}	100^{B}	0.73^{B}	89.3^{B}	77^{B}	1.21^{A}
CPl	58.4^{A}	230^{A}	0.26^{C}	75.1^{A}	136^{A}	0.57^{C}	92.9^{A}	94^{A}	1.02^{B}
SEM	0.4	6	0.01	0.4	3	0.02	0.8	2	0.04
P-values									
GP	<0.001	<0.001	0.027	<0.001	<0.001	0.049	<0.001	<0.001	0.030
CP	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GP × CP	0.799	0.866	0.920	<0.001	0.001	0.068	0.021	0.003	0.166

[a-e]Means within columns and treatment with different superscript differ significantly ($P < 0.05$).
[A-C]Means within a main effect with a different superscript differ significantly ($P < 0.05$).
[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

No significant carryover effects of treatments on eating time during the laying period were observed (Table 4). Birds fed the CPm and CPl diet during the rearing period showed an 8 and 16% increased eating rate at 22 wk of age, respectively, compared to the birds fed the CPh diet.

Table 4. Feed intake (g/bird per d), eating time (min/d), and eating rate (g feed/min) in Ross 308 broiler breeders during the laying period.

	22			27		
Item[1]	Feed intake	Eating time	Eating rate	Feed intake	Eating time	Eating rate
Treatment						
SGP						
CPh	108.3	64	1.71	160.0	139	1.18
CPm	113.7	63	1.85	160.0	143	1.16
CPl	119.8	60	2.02	160.0	132	1.26
HGP						
CPh	108.3	67	1.63	160.0	150	1.09
CPm	113.7	65	1.77	160.0	131	1.25
CPl	119.8	66	1.84	160.0	127	1.30
SEM		3	0.08		9	0.08
Main effect GP						
SGP	113.9	62	1.86	160.0	138	1.20
HGP	113.9	66	1.75	160.0	136	1.21
SEM		2	0.04		5	0.05
Main effect CP						
CPh	108.3	66	1.67^{B}	160.0	145	1.14
CPm	113.7	64	1.81^{AB}	160.0	137	1.20
CPl	119.8	63	1.93^{A}	160.0	129	1.28
SEM		2	0.05		6	0.06
P-values						
GP	-	0.103	0.076	-	0.782	0.868
CP	-	0.594	0.005	-	0.232	0.210
GP × CP	-	0.782	0.758	-	0.424	0.508

$^{A-C}$Means within a main effect with a different superscript differ significantly ($P < 0.05$).
[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

Behavioral observations

Feeding behavior was not affected by growth pattern in birds fed the CPm diet during the rearing period, however, birds fed the CPh and CPl diets spent more time feeding when following HGP compared to SGP (Table 5).

Birds fed the HGP diet spent 8% more time feeding, 40% more sitting and 33% less time foraging during the rearing period compared to the SGP birds. Birds fed the CPl diet showed 44% more feeding behavior, 28% more perching, 46% more standing, 143% more sitting, 48% less stereotypic object pecking, and 58% less animal pecking compared to the birds fed the CPh diet. Birds fed the CPm diet showed on average, intermediate levels in between the birds fed the CPh and CPl diets during the rearing period. During the laying period, birds fed the HGP diet spent 32% more time walking than the SGP reared birds (data not shown), although GP did not affect walking time during rearing. Birds fed the CPl diet spent 57% more time on standing behavior than the CPh birds during the laying period, which was a carryover effect from the rearing period. Birds fed the CPl diet tended ($P = 0.067$) to show 18% less time (28.9 vs. 23.7%) stereotypic object pecking during the laying period than the CPm and CPh birds (data not shown). Birds in all treatments showed an age effect on all behavioral traits during the rearing period and most traits during the laying period.

Table 5. Behavioral traits (% of time) observed using a scan sampling technique in Ross 308 broiler breeders during the rearing period.

Item[1]	Feed[2]	Drink	Forage	Perch	Walk	Stand	Sit	Obj	Anim
Treatment									
SGP									
CPh	20.3^{e}	4.5	3.0	15.1	1.3	14.4	1.7^{bc}	32.9	7.0
CPm	25.1^{c}	2.8	2.9	18.6	1.5	16.5	2.0^{bc}	26.1	4.5
CPl	28.6^{b}	2.8	2.3	17.3	1.4	20.0	2.4^{bc}	19.9	5.4
HGP									
CPh	21.9^{d}	3.5	1.5	14.5	1.2	13.7	1.2^{c}	33.4	9.3
CPm	25.8^{c}	3.8	2.5	17.1	1.5	17.9	2.8^{b}	24.3	4.3
CPl	32.1^{a}	2.9	1.3	20.6	1.6	20.8	4.4^{a}	14.9	1.4
SEM	0.4	0.5	0.4	1.5	0.2	1.5	0.4	1.8	1.5
Main effect GP									
SGP	24.7^{B}	3.3	2.7^{A}	17.0	1.4	17.0	2.0^{B}	26.3	5.6
HGP	26.6^{A}	3.4	1.8^{B}	17.4	1.4	17.4	2.8^{A}	24.2	5.0
SEM	0.3	0.3	0.3	0.8	0.1	0.9	0.3	1.0	0.9
Main effect CP									
CPh	21.1^{C}	4.0	2.2	14.8^{C}	1.2	14.0^{C}	1.4^{C}	33.2^{A}	8.1^{A}
CPm	25.4^{B}	3.3	2.7	17.8^{B}	1.5	17.2^{B}	2.4^{B}	25.2^{B}	4.4^{B}
CPl	30.4^{A}	2.8	1.8	19.0^{A}	1.5	20.4^{A}	3.4^{A}	17.4^{C}	3.4^{B}
SEM	0.3	0.4	0.3	1.0	0.2	1.1	0.3	1.3	1.1
P-values									
GP	<0.001	0.889	0.016	0.710	0.904	0.654	0.031	0.065	0.467
CP	<0.001	0.134	0.142	0.024	0.254	<0.001	<0.001	<0.001	0.006
Age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GP × CP	0.005	0.255	0.243	0.247	0.870	0.719	0.024	0.223	0.211
GP × Age	<0.001	0.841	0.056	0.025	0.759	0.836	0.349	0.042	0.855
CP × Age	<0.001	0.811	0.133	0.646	0.687	0.002	0.032	0.039	0.710
GP × CP × Age	0.021	0.184	0.694	0.164	0.338	0.194	0.280	0.028	0.058

[a-e]Means within columns and treatment with different superscript differ significantly ($P < 0.05$).
[A-C]Means within a main effect with a different superscript differ significantly ($P < 0.05$).
[1]Each value represents the mean of 3 replicates over 10 wk, determined in a 5-wk interval (7, 12 and 17 wk of age). SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level. Age of observation was included in the model but not showed here.
[2]Feed = pecking at the feed at the feeding troughs; drink = pecking at the drinking nipples; forag = foraging; perch = sitting on perch; walk = walking; stand = standing; sit = sitting; obj = object pecking; anim = pecking at other animals.

Plasma corticosterone concentrations

No treatment effects ($P > 0.05$) were found for plasma corticosterone concentrations at the different ages, except for a small significant effect ($P < 0.049$) of growth pattern at 16 wk of age (Table 6).

Table 6. Plasma corticosterone concentrations (ng/mL) of Ross 308 broiler breeders at 11, 16, 21, and 26 wk of age.

Item[1]	11 wk of age	16 wk of age	21 wk of age	26 wk of age
Treatment				
SGP				
CPh	1.83	2.83	2.06	3.56
CPm	2.20	2.87	2.25	3.15
CPl	2.90	2.65	1.63	3.68
HGP				
CPh	2.06	2.58	1.55	2.51
CPm	2.18	2.25	1.84	3.56
CPl	3.28	1.74	1.95	3.13
SEM	0.52	0.36	0.19	0.40
Main effect GP				
SGP	2.31	2.78[a]	1.98	3.46
HGP	2.50	2.19[b]	1.78	3.07
SEM	0.30	0.21	0.11	0.23
Main effect CP				
CPh	1.95	2.71	1.81	3.03
CPm	2.19	2.56	2.05	3.35
CPl	3.09	2.20	1.79	3.41
SEM	0.36	0.25	0.13	0.28
P-values				
GP	0.647	0.049	0.201	0.226
CP	0.076	0.349	0.324	0.593
GP × CP	0.925	0.660	0.065	0.184

[a-b]Effect means within columns and treatment with no common superscript differ significantly ($P < 0.05$).
[1]Each value represents the mean of 4 replicates over 15 wk, determined in a 5-wk interval (11, 16, 21, and 26 wk of age). SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

Feather cover

The HGP birds showed a lower feather cover score on the neck compared to the SGP birds (Table 7). Growth pattern did not affect the feather cover of the other body parts, both during the rearing and laying period. During the rearing period, feather cover on the breast, back, wings, tail, and legs showed a decreasing trend when dietary CP content was reduced.

An interaction between dietary protein level and age was found on average feather cover during rearing (Figure 2). At 6 and 11 wk of age, the CPl-birds showed an inferior feather cover as compared to the other treatments. From 16 to 40 wk of age, however, no significant differences were found in feather cover among the dietary protein levels.

Table 7. Feather cover score of Ross 308 broiler breeders during the rearing period.[1]

Item[2]	Neck	Breast	Belly	Back	Wings	Tail	Legs	Average
Treatment								
SGP								
CPh	2.28	2.19	2.24	0.31	1.32	1.09	2.56	1.71
CPm	2.26	2.32	2.20	0.36	1.36	1.13	2.68	1.76
CPl	2.32	2.44	2.34	0.38	1.62	1.26	2.85	1.89
HGP								
CPh	2.17	2.16	2.24	0.31	1.26	1.09	2.45	1.67
CPm	2.20	2.27	2.23	0.33	1.48	1.11	2.62	1.75
CPl	2.18	2.37	2.33	0.40	1.60	1.18	2.79	1.83
SEM	0.05	0.05	0.05	0.03	0.04	0.04	0.06	0.03
Main effect GP								
SGP	2.29^{A}	2.32	2.26	0.35	1.43	1.16	2.70	1.79
HGP	2.18^{B}	2.27	2.26	0.35	1.45	1.13	2.62	1.75
SEM	0.03	0.03	0.03	0.02	0.02	0.03	0.04	0.02
Main effect CP								
CPh	2.23	2.17^{C}	2.24	0.31^{C}	1.29^{C}	1.09^{B}	2.50^{C}	1.69^{C}
CPm	2.23	2.30^{B}	2.21	0.35^{B}	1.42^{B}	1.12^{B}	2.65^{B}	1.75^{B}
CPl	2.25	2.41^{A}	2.33	0.39^{A}	1.61^{A}	1.22^{A}	2.82^{A}	1.86^{A}
SEM	0.04	0.04	0.04	0.02	0.03	0.03	0.04	0.02
P-values								
GP	0.014	0.208	0.930	0.924	0.668	0.354	0.127	0.137
CP	0.876	<0.001	0.061	0.017	<0.001	0.010	<0.001	<0.001
Age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GP × CP	0.678	0.935	0.909	0.770	0.042	0.645	0.910	0.733
GP × Age	0.607	0.485	0.580	0.577	0.668	0.372	0.954	0.673
CP × Age	0.264	0.053	0.035	<0.001	0.096	0.209	0.617	0.003
GP × CP × Age	0.639	0.978	0.714	0.742	<0.001	0.882	0.995	0.793

[A-C]Differences within the main effects without a common superscript differ significantly ($P < 0.05$).
[1]Feather cover score ranges from 0 (intact feathers) to 5 (completely denuded area).
[2]Each value represents the mean of 4 replicates over 14 wk, determined in an approximately 5-wk interval (6, 11, 15, and 20 wk of age). SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level. Age of observation was included in the model but not showed here.

DISCUSSION

The present experiment was designed to study the effects of different feeding strategies during rearing on feed intake, eating time, eating rate, behavior, plasma corticosterone concentrations, and feather cover and on carryover effects of these strategies on behavior and feather cover during the laying period of broiler breeder females. Some growth pattern and dietary protein level interactions were found on eating time and feeding behavior during the rearing period. These interactions were not very relevant because they were not crossing but showed the same direction although somewhat diverging and therefore we focus in this paper on the main treatments.

Feed intake, eating time, and eating rate

Birds that followed the HGP were provided, on average, 6.5% more feed compared to the SGP birds, resulting in a prolonged eating time of 17% during the rearing period. The relative prolonged eating time could be explained by the lower eating rate of these birds. The lower eating rate of HGP birds might have been caused by the larger daily feed allowance. De Jong et al. (2005b) found an average eating rate of 0.8, 1.5, and 1.5 g feed/min at 6, 13, and 16 wk of age, respectively. In our experiment lower average eating rates of 0.3, 0.7, and 1.2 g feed/min at 7, 12, and 17 wk of age, respectively were found. This was unexpected, it might be assumed that due to selection, modern broiler breeders will have a higher eating rate than broiler breeders of 10 years ago. The lower eating rate in the current experiment as compared to those reported by De Jong et al. (2005b) could have been caused by the smaller particle sizes in the experimental diets (0.35 mm) versus those in the diets used by De Jong et al. (2005b) (0.95 mm; Enting, pers. comm.). A reduced eating rate as a consequence of feeding fine particles was also reported by Picard et al. (2000) in experiments with broilers. The lower eating rate of the HGP birds during rearing disappeared during the laying period, suggesting that this acquired behavior was not programmed.

An increased eating time and a decreased eating rate during rearing were also observed when birds were fed CPm and CPl diets in contrast to the CPh diet. A higher feed intake resulted in a linear prolonged eating time and a reduction in eating rate. As expected, the differences in eating time and eating rate between the treatments were larger for dietary protein level than for growth pattern. This may suggest a glucostatic satiety inducing a lower eating rate due to the higher energy intake in the low protein diets.

The greatest effect of feed intake on eating time was found at 7 wk of age, while these differences became less when birds grew older (Figure 1). This phenomenon was previously reported by De Jong et al. (2005b) who fed rearing pullets four different diluted diets. While ageing, pullets in the current study increased their feed intake - though increasingly restricted - because they were trained to consume the same amount of feed in a shorter time interval. Additionally, in the beginning of the rearing period (7 wk of age) birds maybe hampered in higher eating rate if feed intake increases due to physical gut/crop fill.

During the laying period, no carryover effects from the rearing period on eating time and eating rate were found, probably due to the fact that all birds were fed similar amounts of feed with an equal dietary composition from 20 wk of age onward.

In contrast to the rearing period, there was a remarkable opposite effect on eating time and eating rate at 22 and 27 wk of age for the CPm and CPl birds, compared to the CPh birds. Birds fed the CPm and CPl diets at these ages had a higher eating rate, probably due to an accustomed larger feed allowance during earlier ages.

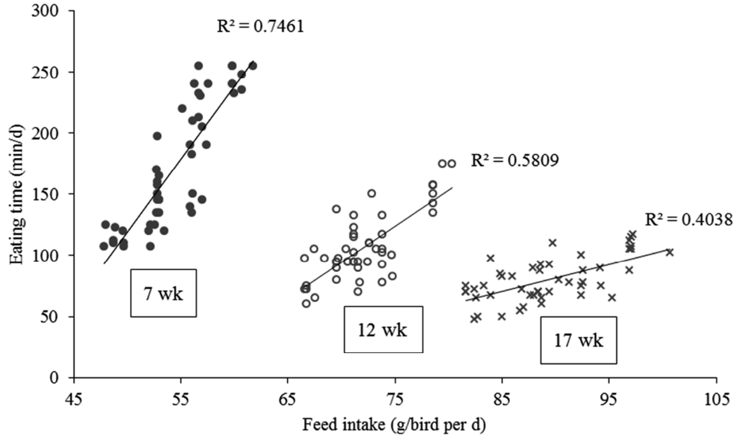

Figure 1. Relation between feed intake (g/bird per d) and eating time (min/d) at different ages during rearing of broiler breeder pullets.

Behavioral traits

Contrary to our expectations, an increased feed intake (6.5%) and a prolonged eating time of the HGP birds did not decrease stereotypic object pecking and animal pecking. Hocking et al. (2004) found that a higher feed intake the welfare of the birds might improve due to the extended eating time resulting in a reduction in pecking behavior. Additionally, Mason et al. (2006) postulated that birds spent more time eating, leave less time available for stereotypic object pecking behavior. In the current study, differences in stereotypic object pecking due to differences in feed intake seem to be masked by the severe feed restriction of modern broiler breeders compared to breeds some decades ago (Renema et al., 2007b).

A higher feed allowance of 4.6 and 10.0% for the CPm and CPl birds, respectively, compared to CPh birds, resulted in a linear increase in time spent eating, perching, and standing, and reduced stereotypic object pecking and animal pecking during the rearing period. This may indicate that their hunger or frustration of the eating motivation of the CPm and CPl birds was reduced because stereotypic object pecking is an important indicator of hunger and frustration of the feeding behavior in broiler breeders (Savory et al., 1996; Savory and Kostal, 1996; De Jong et al., 2005b). Reduced activity may also indicate that hunger and frustration are reduced (Savory and Maros, 1993; Hocking et al., 1996; Savory et al., 1996; De Jong et al., 2005b). In the current study, CPm and CPl birds were more resting (more perching, standing, and sitting) which supports the suggestion that these birds experienced less hunger and frustration than the CPh birds. The current results are in agreement with Hocking et al. (2001) who found that birds fed a high dietary protein level spent less time resting and more time spot-pecking than birds fed a low dietary protein level.

The positive effects on behavior in the current study were first of all caused by the differences in feed intake (and thus feeding behavior) whereby more or less time was available to express other behavior (Hocking et al., 2004; Mason et al., 2006). Second, the effects on behavior were due to a different crude fiber and NSP content of the diets (Table 1). Crude fiber and NSP content of the CPm and CPl diets were, compared to the CPh diet, 3 and 6% higher, respectively. In combination with the higher feed intake for the CPm and CPl diets, daily intake of crude fiber and NSP was 7 and 16% higher, respectively. This phenomenon was also observed by Nielsen et al. (2011) who found that birds fed a diet with a high content of insoluble fibers showed no stereotypic pecking behavior whereas this was frequently seen in birds fed the control diet.

Plasma corticosterone concentrations

Restricted fed broiler breeders usually show higher plasma corticosterone concentrations compared to unrestricted fed birds (Hocking et al., 1996; Savory and Mann, 1997; De Jong et al., 2003). However, it is not clear whether these elevated plasma corticosterone concentrations reflect psychological stress or metabolic effects resulting from feed restriction, or both (De Jong et al., 2003). In the current study, however, the treatments had almost no effect on plasma corticosterone concentrations at the different ages. It seems that the modern broiler breeder with its higher plasma corticosterone concentrations at all ages is less responsive to differences in feed allowance than the broiler breeder a decade ago. Probably due to an increased growth potential of broiler breeders during the last 30 years (Renema et al., 2007b) feed restriction became more severe which resulted in higher plasma corticosterone concentrations. Moreover, it seems that the average level of plasma corticosterone concentrations in the current study was about five times higher halfway the rearing period than in previous studies (De Jong et al., 2003; 2005b).

Feather cover

Feathers contain a high concentration of protein and amino acids (Stilborn et al., 1997) and the dietary concentration of these nutrients can affect feather development and quality (e.g. Leeson and Walsh, 2004). The major amino acids involved in the synthesis of feather keratin are the sulfur-containing amino acids methionine and cysteine (Van Krimpen et al., 2005). No effect of growth pattern was found on feather cover due to the same diet composition for both treatments. The broiler breeder pullets fed the low protein diets had a poorer feather cover during the first half of the rearing period (Figure 2). Twining et al. (1976) showed that dietary protein levels over 16% increased the amount of feathers in the litter, indicating a faster feather growth and molting. It may be suggested that low protein diets repartition amino acid usage towards breast muscle development at the expense of feather development. Initial differences in feather cover between the different dietary protein levels disappeared in the second part of the rearing period. From this moment onward, feather development and molt has completed and only the CPm and CPl birds show compensatory feather growth resulting in no differences between the treatment groups after 11 wk of age.

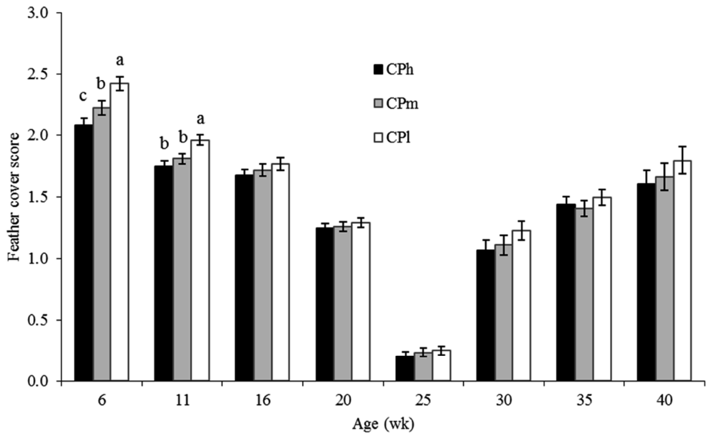

Figure 2. Feather cover score (mean ± SEM) from 6 to 40 wk of age for broiler breeder pullets reared on a high (CPh), medium (CPm), and low (CPl) dietary crude protein (CP) level.
[a,c]Different letters indicate significant differences among treatments ($P < 0.05$) within age.
Feather cover was scored from 0 (intact) to 5 (bold) for each of 7 different body parts and averaged to a total feather cover score per bird (Bilcik and Keeling, 1999).

CONCLUSION

Low dietary protein levels improved broiler breeder welfare during rearing, as shown by a decrease of stereotypic object pecking, which is considered to be a behavioral indicator of reduced hunger and frustration. A high growth pattern also increased feed intake and eating time, but did not result in significant effects on stereotypic object pecking. Differences in behavior in broiler breeders disappeared during the laying period when birds were fed similar amounts of the same diet. A less restricted feed intake level induced by a low dietary protein level seems to be more effective in changing behavior of broiler breeders than inducing a higher growth pattern during rearing. This change, however, was not supported by a decrease in plasma corticosterone concentrations.

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Chapter 4

Effects of growth patterns and dietary protein levels during rearing of broiler breeders on fertility, hatchability, embryonic mortality, and offspring performance

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ABSTRACT

The objective of this study was to determine the effects of different growth patterns and dietary CP levels during rearing in broiler breeder female on fertility, hatchability, embryonic mortality, and offspring performance. A 2 × 3 factorial arrangement of treatments was used, with 2 growth patterns to reach a target body weight (BW) at 20 wk of age of 2,200 g (standard = SGP) or 2,400 g (high = HGP), and 3 dietary protein levels (high = CPh, medium = CPm, and low = CPl). Fresh egg composition and organ development in hatchlings were determined. Offspring of the different groups were reared till an age of 34 d and feed intake, BW gain, mortality, and carcass composition were determined. In 29 wk old HGP compared to SGP breeders, fertility and hatchability of set eggs were increased, embryonic mortality between d 1 to 9 was decreased whereas hatchability of fertile eggs was not affected. Breeders fed the CPm diet showed a decreased hatchability of fertile eggs caused by an increased embryonic mortality between d 18 and 21 compared to breeders fed the CPh and CPl diets. Offspring of 29-wk old HGP breeders tended ($P = 0.059$) to have a higher BW at d 34 than offspring of SGP breeders, which was achieved by a tendency for a higher BW gain ($P = 0.057$). Offspring of breeders fed the CPm and CPl diet showed a higher feed intake between d 18 to 27 and during the total growth period, compared to offspring of CPh breeders. Male broilers of CPl breeders had higher breast meat yield than male broilers of CPh breeders, while breast meat yield of female broilers was not affected by dietary protein levels. This experiment showed that a higher growth pattern during the rearing period increased fertility, decreased embryonic mortality, and improved offspring performance in young breeders, whereas decreased dietary protein level had less pronounced effects on these traits.

Key words: broiler breeder, feeding strategy, rearing, hatchability, offspring

INTRODUCTION

Broiler breeder production is an important link in the poultry meat chain. The main goal in broiler breeder production is to provide fertilized eggs for the production of healthy and robust day-old chicks (Zuidhof et al., 2007). During the last decades, a major problem in broiler breeder production is the decrease in fertility and hatchability of eggs, especially during the second part of the laying period. In the Netherlands, fertility of hatching eggs declined from about 89% in 2000 to 85% in 2005 (Van Emous, 2010). This decrease in fertility may be caused by a wide range of management factors, such as breeder strain, health of breeder flock, egg quality, egg storage duration and conditions, egg sanitation, season, and age of the breeders (as reviewed by Yassin et al., 2008). Besides these factors, nutrition can play a major role in fertility and hatchability of eggs. For instance, a number of authors reported negative effects of a high compared to a low daily dietary CP intake (27 vs. 23, 26 vs. 21, and 25 vs. 16 g/d) during the laying period on fertility and hatchability of eggs, respectively (Pearson and Herron, 1982; Whitehead et al., 1985; Lopez and Leeson, 1995a). Moreover, Pearson and Herron (1982) reported that a high compared to a low daily protein intake (27 vs. 23 g/d) during the laying period, resulted in an increased mortality and malformation of embryos. In a more recent study, Ekmay et al. (2013) showed in a dose-response study that higher levels of dietary lysine and isoleucine at peak production resulted in a reduction in fertility. Besides the effects on incubation traits, other studies have described the relationship of broiler breeder nutrition on performance (Lopez and Leeson, 1995b; Enting et al., 2007) and processing yields of their offspring (Spratt and Leeson, 1987; Rao et al., 2009; Zhu et al., 2012). Body weight at the end of the rearing period is also mentioned as a potential factor that might affect fertility and hatchability of hatching eggs. Renema et al. (2001a) demonstrated that a higher BW during the rearing and laying period increased fertility and hatchability and decreased embryonic mortality, although these effects were not observed in other studies (Fattori et al., 1991; Hocking et al., 2002; De Beer and Coon, 2007; Zuidhof et al., 2007; Ekmay et al., 2012). De Beer and Coon (2007) noted the importance of lean protein mass as threshold for the onset of sexual maturity. Besides, it is also suggested by some researchers that a certain proportion of body fat in broiler breeders at the onset of lay is necessary for an adequate reproductive performance (Sun and Coon, 2005; De Beer, 2009; Mba et al., 2010). Body composition can be adjusted by different feed allowances during rearing and laying (e.g. Fattori et al., 1993; Renema et al., 2001a,b; Chapter 2) but also by differences in diet composition (Mba et al., 2010; Chapter 2). To our knowledge, there are relatively few studies on the effect of different feeding strategies during the rearing period of broiler breeders on fertility, hatchability, and performance of the offspring. Therefore, the current trial was carried out to determine the effects of different growth patterns and dietary protein levels of broiler breeders on fertility, hatchability, embryonic mortality, and offspring performance.

MATERIALS AND METHODS

The experimental protocol conformed to the standards for animal experiments and was approved by the Ethical Committee of Wageningen UR, the Netherlands. Animal care guidelines were used as provided by the Euro guide recommendations for animal use for experimental and other scientific purposes (Forbes et al., 2007).

Stocks and management broiler breeders

A total of 768, 1-d-old Ross 308 female broiler breeder chicks were housed in 2 identical climate-controlled rooms (24 pens per room; 48 pens in total) between 0 and 40 wk of age. At the start of the rearing period, 16 pullets were housed per pen and this was gradually reduced to 15 (wk 4), 12 (wk 10), 9 (wk 15), and 6 (wk 20) per pen, due to dissection procedures, sex errors, mortality or outlier birds. A more detailed description of this experiment is reported in Chapter 2. Males for semen production, were reared elsewhere and introduced to the experiment when the hens were 20 wk of age. They were individual housed in a separated room in cages containing a perch, a feed trough and 3 nipple drinkers. Males received 8 h per day light at 20 wk of age and this was increased to 11 h per day at 21 wk of age and then extended by 1 h (later 0.5 h) per week to a 15Light (L):9Dark (D) lighting program at 27 wk of age. This schedule was maintained until the end of the experiment at 40 wk of age. Males were kept at a light intensity of 60 lx and a temperature of 20°C during the experimental period. Furthermore, all males received a similar quantity of feed, approximately 100 to 120 g daily, adjusted for their body weight gain.

Breeders were artificially inseminated twice a week at 27 wk of age and once a week throughout the remaining of the experimental period, until 38 wk of age with 100 µL pooled diluted semen collected from the males using the abdominal massage method, as described by Burrows and Quinn (1937). Semen was diluted using Beltsville Poultry Semen Extender (Continental Plastic Corp., Delavan, WI) according to Sexton (1977).

Experimental design breeder trial

From wk 0 to 2, all broiler breeder pullets followed the same growth pattern and received the same standard starter-1 diet. At d 14, pullets were randomly allotted to 1 of 6 dietary treatments according to a 2 × 3 factorial design as reported in Chapter 2. In brief, factors were 2 growth patterns (standard = SGP and high = HGP) and 3 dietary protein levels (high protein = CPh, medium protein = CPm, and low protein = CPl). Growth patterns were set to reach differences of 200 g in BW at 20 wk of age: 2,400 g (HGP) vs. 2,200 g (SGP). The higher target BW was achieved by following a more rapid weekly BW gain during the entire rearing period. A starter-2 diet was fed from 2 to 6 wk of age, a grower diet from 6 to 15 wk of age, and a pre-breeder diet from 15 to 22 wk of age. From 22 to 40 wk of age, a standard breeder diet was provided to all breeders. Digestible amino acid levels were lowered within each phase during the rearing by 8 and 16% for the CPm and CPl diets, respectively, compared to the CPh diet. The major calculated and analyzed nutrients of the breeder diets are presented in Table 1. A more detailed description of these diets is reported in Chapter 2.

Body weight targets were directive for feed allocation and therefore all birds per pen were weighed at a weekly (to wk 27) or 2-weekly (from wk 27 onward) interval. Daily feed allocation was adjusted weekly per pen to reach the predetermined BW target of that week. From 19 to 22 wk of age, feeding level was gradually adjusted to obtain a similar target BW for all breeders at onset of lay. Within each phase, all diets (from 2 to 22 wk of age) had similar energy levels.

Table 1. Calculated and analyzed nutrients of the breeder diets (g/kg, as-fed basis).

	Starter-2 (15-42 d)			Grower (43-105 d)			Pre-breeder (106-154 d)		
Item	CPh[1]	CPm	CPl	CPh	CPm	CPl	CPh	CPm	CPl
Calculated content									
AME_n (kcal/kg)	2,800	2,800	2,800	2,600	2,600	2,600	2,700	2,700	2,700
CP	172.9	160.4	147.9	140.7	132.8	125.0	150.0	140.0	130.0
Dig. lysine	7.2	6.6	6.0	5.4	4.9	4.5	5.6	5.2	4.7
Dig. M + C	5.7	5.3	4.8	4.5	4.2	3.8	5.0	4.7	4.4
Analyzed content[2]									
DM	872.3	872.6	872.9	876.9	875.9	873.3	869.0	870.3	871.2
Crude ash	58.1	55.8	56.1	59.9	60.8	60.6	60.0	61.4	60.8
CP	192.1	179.6	145.9	141.3	129.6	122.7	148.8	139.6	127.9
Crude fat	45.5	47.0	48.6	37.9	40.7	39.5	34.7	36.9	38.2
Starch	405.7	413.8	418.2	365.9	381.1	395.5	401.4	405.6	385.6
Reducing sugars[3]	33.6	31.5	29.0	34.4	32.9	31.6	35.2	33.2	31.5

[1]Dietary protein level: CPh = high; CPm = medium; CPl = low.
[2]Based on 2 analysis in duplicate per diet.
[3]Mono- and disaccharides as glucose units.

Stocks and management offspring

Day-old-chicks from the hatching eggs of 29 wk old breeders were used for a growth trial between 0 and 34 d of age. After feather sexing, male and female chicks were randomly allotted to 96 floor pens (1.0 × 0.75 m), with 10 chicks (female or male) per pen, with wood shavings as bedding material. Stocking density was 13.3 chicks per m^2 at the start of the growth trial. Temperature inside the house was 34°C at the start of the experiment and was gradually decreased to 20°C at 28 d, where after it remain constant. The lighting program was 23L:1D during the first 2 d and after 2 d of age it was in a 24 h cycle 4L:4D+4x(3L:1D) onward, at a light intensity of 20 lx. Each pen contained one feed through and one drinking nipple. A commercial available four phase pelleted broiler diet program was provided to all chicks (Table 2). A starter diet was fed from 0 to 10 d, a grower-1 diet from 11 to 17 d, a grower-2 diet from 18 to 27 d, and a finisher diet from 28 to 34 d.

Observations

Characteristics of hatching eggs. To determine egg composition, 4 hatching eggs per breeder pen of a daily production were collected from 30, 34, and 38 wk old breeders. Within 5 h after collection, 3 eggs per pen were used to examine the albumen height. The eggs were broken on a flat surface and the height of the albumen was measured halfway between the yolk and the edge of the inner thick albumen using an albumen height gauge (Ames, Waltham, MA, USA). The albumen was removed from the shells and shells plus membranes were weighed. One egg per pen was stored for 3 to 5 d at 18°C. To separate the albumen and yolk easily, the eggs were boiled for 10 min. After cooling for 5 min in running water, the shell (plus membranes), yolk and albumen were separated and weighed. Shell thickness (without inner and outer shell membranes; membranes were removed manually) was measured at three places (top, middle, bottom), using a micrometer (Mitutoyo, Miyazaki, Japan). The weight of the albumen was calculated as the difference between the egg weight and the weight of the shell and yolk.

Fertility, hatchability, embryonic mortality, and chick quality. At 29, 33 and 37 wk of age, eggs from each breeder pen were daily collected during 7 d of production. Afterwards, eggs were weighed individually, and sorted in settable and non-settable eggs. Settable eggs were defined as eggs weighing > 50g, not including soft shells, cracks, dirty, or double yolks. Per pen, 30 settable eggs were set in an incubator (HatchTech, Veenendaal, the Netherlands). Eggs from two pens were set on one tray. Eggshell temperature was maintained at 37.8°C throughout incubation (Lourens et al., 2005) and relative humidity (RH) was maintained between 40 and 50% throughout incubation. Carbon dioxide level was maintained below 0.35%. Eggs were turned over 90° every hour until d 18 of incubation. At d 18 of incubation, eggs were candled and clear eggs were opened to determine infertility or stage of embryonic mortality. Eggs containing living embryos were transferred to hatching baskets and placed in the same incubator.

Table 2. Composition and calculated contents of the broiler diets (g/kg, as-fed basis).

Item	Starter (0 to 10 d)	Grower-1 (11 to 17 d)	Grower-2 (18 to 27 d)	Finisher (28 to 34 d)
Ingredients				
Wheat	399.8	390.7	406.2	549.2
Soybean meal	277.0	248.7	235.0	228.5
Maize	200.0	204.3	208.3	90.0
Rapeseed expeller	-	39.1	37.5	23.1
Fat	-	34.5	38.8	44.3
Rapeseed meal	30.0	30.4	37.5	27.7
Soya oil	18.0	14.2	5.7	6.1
Fish meal	15.0	-	-	-
Chalk	12.5	9.7	7.8	7.8
Palm oil	10.4	-	2.2	4.1
Broiler premix A[1]	6.8	7.0	-	7.4
Broiler premix B[2]	7.5	7.6	-	-
Broiler premix C[3]	-	-	9.4	-
Broiler premix D[4]	6.0	-	-	-
Monocalcium phosphate	9.7	5.8	3.6	2.4
Salt	1.1	1.4	1.3	1.5
L-Lysine	2.9	3.3	3.5	4.3
DL-Methionine	2.3	2.2	2.1	2.1
L-Threonine	0.9	0.8	0.8	1.1
Hostazym® P10000 Liquid[5]	-	0.2	0.2	0.2
Phytase	0.1	0.1	0.1	0.2
Calculated nutrient composition[6]				
AME_n (kcal/kg)	2,820	2,930	2,935	2,990
CP	220.8	202.1	196.8	198.9
Crude fat	57.6	78.7	75.7	75.9
Starch	369.6	369.6	381.7	391.5
Calcium	9.5	7.2	6.2	5.5
Total phosphorus	6.2	5.1	4.6	4.2
Available phosphorus	4.4	3.4	3.0	2.9
Digestible lysine	11.6	10.6	10.3	10.1
Digestible methionine	5.4	4.9	4.7	4.6
Digestible M + C	8.5	7.8	7.6	7.6

[1]Provided per kg complete diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E, 50 IU; vitamin K3, 3.0 mg; vitamin B1, 0.9 mg; vitamin B2, 7.5 mg; niacin, 45.0 mg; pantothenic acid, 12.0 mg; vitamin B6, 5.0 mg; biotin, 0.091 mg; folic acid, 0.9 mg; vitamin B12, 0.025 mg; betaine, 500.0 mg; sodium, 0.6 g; iron, 60.0 mg; copper, 13.6 mg; zinc, 60.0 mg; manganese, 80.0 mg; iodine, 2.0 mg; selenium, 0.3 mg; Digestarom 1317 (phytogenic feed additive; essential oils, natural herbs and spices), 149.6 mg; BHT/BHA, 3.1 mg; propyl gallate, 1.1 mg; ethoxyquin, 0.4 mg; citric acid, 4.5 mg.
[2]Provided per kg complete diet: vitamin A, 2,000 IU; vitamin E, 50 IU; vitamin B2, 1.0 mg; niacin, 15.0 mg; pantothenic acid, 1.0 mg; biotin, 0.075 mg; folic acid, 0.3 mg; betaine, 500.0 mg; Y-MOS (aromatic yeast autolysate), 1,000 mg; nicarbazin, 50 mg; narasin, 50 mg.
[3]Provided per kg complete diet: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 100 IU; vitamin K3, 4.0 mg; vitamin B1, 0.9 mg; vitamin B2, 8.9 mg; niacin, 59.6 mg; pantothenic acid, 14.9 mg; vitamin B6, 5.0 mg; biotin, 0.199 mg; folic acid, 0.9 mg; vitamin B12, 0.025 mg; betaine, 744.5 mg; sodium, 0.8 g; chloride, 0.1 g; iron, 59.6 mg; copper, 18.0 mg; zinc, 79.4 mg; manganese, 81.0 mg; iodine, 2.0 mg; selenium, 0.3 mg; Digestarom 1317, 148.5 mg; BHT/BHA, 3.1 mg; propyl gallate, 1.1 mg; ethoxyquin, 0.4 mg; citric acid, 4.5 mg; Y-MOS, 496.8; salinomycin, 69.5 mg.
[4]Provided per kg complete diet: Natugrain® TS (feed enzyme; glucanase and xylanase), 113 mg.
[5]Huvepharma, Sofia, Bulgaria (xylanase).
[6]Calculated according to CVB, 2009.

At d 21.5 of incubation, unhatched eggs were opened to determine stage of embryonic mortality. At d 18 and 21.5 of incubation, the following stages of embryonic mortality were used to classify the dead embryos: d 1 to 9 (white membrane over the yolk till feather follicle visible), d 10 to 17 (small embryo with feathers), d 18 to 21.5 (full grown embryo). Fertility was calculated as percentage of set eggs. Hatchability was calculated as percentage of set and of fertile eggs. Embryonic mortality was calculated as a percentage of fertile eggs. At d 21.5, all chicks were weighed and graded as first and second grade chicks. A chick was classified as a first grade chick when the chick was clean and dry, free of deformities, and when eyes were bright (Tona et al., 2004). The other chicks were classified as second grade chicks. Second grade chicks were calculated as a percentage of total hatched chicks.

Characteristics of hatchlings. Day-old-chicks (3 per pen) of 33 and 37 wk old breeders were weighed and euthanized by cervical dislocation to determine the absolute weights of residual yolk, intestines, liver, stomach, and heart. The relative weights of the different parts were calculated as a percentage of yolk free body mass (YFBM).

Offspring performance and processing yields. Day-old-chicks from 29 wk old breeders were sexed and used to determine offspring performance. Body weight of the broilers in each pen was determined at d 0, 10, 17, 27, and 34. Feed intake, BW gain, and feed conversion ratio (FCR) in each pen were recorded and calculated for the periods d 0 to 10, 11 to 17, 18 to 27, and 0 to 34. Mortality and health were recorded daily (including probable causes of any illness or deaths). At d 34, 2 random broilers per pen were identified by steel wing tags (one tag per wing) and BW was measured. Broilers were transported and slaughtered at Plukon Poultry BV (Wezep, the Netherlands). The fasting and water withdrawal period before slaughter was 10 h. Carcasses were cut up by trained personnel approximately 15 h postmortem, after reaching an internal breast temperature of maximum 2ºC. Weights of the whole carcass, wings, legs (thighs + drums), remaining carcass, and breast meat were recorded. Carcass yield was calculated as the whole carcass divided by BW. Processing yields were calculated as the individual parts divided by whole carcass weight.

Statistical analysis

Pen was treated as the experimental unit. The data were analyzed per breeder age on a completely randomized design using 2-way ANOVA (GenStat 14.1, GenStat Committee, 2011). For the incubation trials, a group of 30 eggs was used as experimental unit. Growth pattern, dietary protein level, and their interactions were used as factors in the statistical model. The statistical model for offspring performance and yields included sex and the interactions with sex. Room was considered to be the random term. Parameters were tested for normal distributions before analyzes. Data are presented as mean ± SEM. Unless otherwise noted, all statements of significance are based on testing at $P < 0.05$. When the ANOVA indicated a significant treatment effect, means were compared by the LSD.

RESULTS

Characteristics of hatching eggs

No effects of growth pattern and dietary crude protein levels of the broiler breeders on egg yolk weight, albumen weight, albumen yolk ratio, shell weight, shell thickness and albumen height were found at different breeder ages (data not shown).

Fertility, hatchability, embryonic mortality, and chick quality

Results of the incubation trial of 29 wk old breeders are shown in Table 3. Results of the incubation trials of 33 and 37 wk old breeders did not show any difference on fertility, hatchability, and embryonic mortality (data not shown). In 29 wk old breeders, fertility and hatchability of settable eggs was 3.5 and 5.3% higher for the HGP compared to SGP breeders, respectively. Embryonic mortality between d 1 and 9 of incubation was 2.1% lower in hatching eggs of HGP breeders compared to eggs of SGP breeders. No difference was found between SGP and HGP regarding hatchability of fertile eggs, second grade chicks, and chick weight.

Table 3. Fertility, hatchability, embryonic mortality, second grade chicks, and chick weight of hatching eggs obtained from 29 wk of age Ross 308 broiler breeders reared on different growth patterns (GP) and dietary protein levels (CP).

Item[1]	Fertility (%)	Hatch. of set eggs (%)	Hatch. of fertile eggs (%)	Embryonic mortality of fertile eggs			2^{nd} grade chicks (%)	Chick weight (g)
				1 to 9 d (%)	10 to 17 d (%)	18 to 21.5 d (%)		
Treatment (n=8)								
SGP								
CPh	92.1	83.6	90.8	6.1	0.0	3.1	0.4	36.5
CPm	94.2	81.8	86.8	6.3	0.0	6.9	0.0	36.6
CPl	86.7	79.2	91.3	6.0	0.0	2.8	0.0	37.1
HGP								
CPh	96.8	90.6	93.7	2.3	0.0	4.1	0.9	36.9
CPm	92.9	82.8	88.9	5.3	0.0	5.7	0.0	36.5
CPl	93.7	87.0	93.0	4.0	0.4	2.7	0.5	36.7
SEM	1.9	2.5	1.7	1.3	0.2	1.3	0.3	0.4
Main effect GP (n=24)								
SGP	91.0^{b}	81.5^{b}	89.6	6.1^{a}	0.0	4.3	0.1	36.7
HGP	94.5^{a}	86.8^{a}	91.8	3.9^{b}	0.1	4.2	0.5	36.6
SEM	1.1	1.5	1.0	0.7	0.1	0.7	0.2	0.2
Main effect CP (n=16)								
CPh	94.4	87.1	92.2^{a}	4.2	0.0	3.6^{b}	0.6	36.6
CPm	93.5	82.3	87.9^{b}	5.8	0.0	6.3^{a}	0.0	36.6
CPl	90.2	83.1	92.1^{a}	5.0	0.2	2.7^{b}	0.3	36.9
SEM	1.3	1.8	1.2	0.9	0.1	0.9	0.2	0.3
P-values								
GP	0.030	0.015	0.119	0.038	0.341	0.916	0.226	0.806
CP	0.074	0.143	0.022	0.443	0.377	0.021	0.174	0.690
GP × CP	0.093	0.359	0.945	0.542	0.377	0.724	0.686	0.727

[a-b]Means within a column and within an item lacking a common superscript differ ($P \leq 0.05$).
[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

When broiler breeder pullets were fed the CPl diet during the rearing period, a tendency (Δ = 3.7; P = 0.074) for lower fertility of hatching eggs was observed in comparison with the other treatments. Breeders fed the CPm diet showed, on average, a lower hatchability of fertile eggs (4.3%) and a higher embryonic mortality (3.2%) between d 18 to 21 compared to the CPh and CPl diet.

Characteristics of hatchlings

A significant ($P < 0.001$) effect of growth pattern of the broiler breeders was observed on residual yolk weight of the offspring. Residual yolk weight of hatchlings of 33 wk old SGP broiler breeders was 16% higher compared to the residual yolk weight of hatchlings of HGP breeders. No other effects were found regarding characteristics of hatchlings of breeders at 33 wk of age. No effects were found on hatchling characteristics of 37 wk old breeders (data not shown).

Offspring performance and processing yields

Body weight and BW gain of the offspring of 29 wk old breeders are shown in Table 4, feed intake and FCR in Table 5, mortality in Table 6, and processing yields in Table 7. Broilers of HGP breeders gained more weight (2.4 g/d) than broilers of SGP breeders from d 28 to 34 (P = 0.035). In combination with a tendency to higher BW gain (Δ = 1.0 g/d; P = 0.061) between d 11 and 17, this resulted in a tendency to higher (Δ = 0.8 g/d; P = 0.057; Table 4) average daily BW gain over the entire growth period (d 0 to 34). The tendency to improved daily BW gain also resulted in a tendency to higher BW at the end of the growth period (2,087 vs. 2,058 g; P = 0.059). No effects of dietary protein level were found on BW and BW gain at different ages and at the end of the growth period (d 34).

Table 4. BW at d 10, 17, 27, and 34 and partial and total BW gains of male and female broilers of hatching eggs obtained from 29 wk of age Ross 308 broiler breeders reared on different growth patterns (GP) and dietary protein levels (CP).

	BW (g)				BW gain (g/d)				
Item[1]	10 d	17 d	27 d	34 d	0 to 10 d	11 to 17 d	18 to 27 d	28 to 34 d	Total
Treatment (n=16)									
SGP									
CPh	280	624	1,456	2,048	24.4	49.2	83.2	98.5	60.9
CPm	279	625	1,460	2,056	24.3	49.3	83.5	99.4	61.2
CPl	282	630	1,471	2,072	24.5	49.8	84.0	100.2	61.7
HGP									
CPh	280	624	1,448	2,059	24.3	49.1	82.4	101.8	61.3
CPm	282	637	1,490	2,101	24.5	50.7	85.4	101.7	62.5
CPl	286	645	1,490	2,101	24.9	51.3	84.5	101.8	62.5
SEM	3	6	14	18	0.2	0.6	0.9	1.4	0.5
Main effect GP (n=48)									
SGP	280	626	1,462	2,058	24.4	49.4	83.6	99.4[b]	61.3
HGP	283	635	1,476	2,087	24.6	50.4	84.1	101.8[a]	62.1
SEM	2	4	8	11	0.1	0.3	0.5	0.8	0.3
Main effect CP (n=32)									
CPh	280	624	1,452	2,053	24.4	49.1	82.8	100.2	61.1
CPm	281	631	1,475	2,078	24.4	50.0	84.4	100.5	61.9
CPl	284	638	1,480	2,086	24.7	50.6	84.3	101.0	62.1
SEM	2	4	10	13	0.2	0.4	0.7	1.0	0.4
Main effect SEX (n=48)									
Male	286[a]	655[a]	1,561[a]	2,222[a]	24.9[a]	52.7[a]	90.7[a]	110.2[a]	66.2[a]
Female	277[b]	607[b]	1,377[b]	1,923[b]	24.1[b]	47.1[b]	77.0[b]	90.9[b]	57.2[b]
SEM	2	4	8	11	0.1	0.3	0.5	0.8	0.3
P-values									
GP	0.270	0.080	0.210	0.059	0.242	0.061	0.505	0.035	0.057
CP	0.293	0.096	0.102	0.180	0.297	0.067	0.175	0.833	0.176
SEX	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GP × CP	0.702	0.431	0.346	0.662	0.523	0.354	0.376	0.846	0.662
GP × SEX	0.861	0.638	0.627	0.977	0.881	0.433	0.693	0.387	0.989
CP × SEX	0.872	0.762	0.812	0.744	0.877	0.523	0.841	0.606	0.738
GP × CP × SEX	0.901	0.402	0.605	0.907	0.823	0.154	0.853	0.236	0.909

[a-b]Means within a column and within an item lacking a common superscript differ ($P \leq 0.05$).
[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

Despite a tendency towards a higher feed intake for the HGP broilers between d 11 and 17, no other effects on feed intake and FCR were found for the broilers of HGP and SGP breeders (Table 5).

Offspring of breeders fed the CPm and CPl diet showed a higher feed intake (2.7 and 3.5 g/d, respectively) between d 18 to 27 compared to broilers of CPh breeders. This resulted in a higher feed intake (1.6 and 2.1 g/d, respectively) for the broilers of breeders fed CPm and CPl diets during the entire growth period, compared to broilers of CPh fed breeders. No effect on FCR was found for the different dietary protein levels. Mortality from d 28 to 34 tended ($P = 0.052$) to be higher for the broilers of HGP breeders compared to the broilers of SGP breeders, although growth pattern did not affect mortality over the entire growing period (Table 6). From d 0 to 10, mortality of the female broilers was lower ($\Delta = 1.1\%$) compared to the male broilers, but toward the end of the growing period mortality was not affected.

Table 5. BW at d 10, 17, 27, and 34 and partial and total BW gains of male and female broilers of hatching eggs obtained from 29 wk of age Ross 308 broiler breeders reared on different growth patterns (GP) and dietary protein levels (CP).

	Feed intake (g/d)					FCR (kg of feed/kg of BW gain)				
Item[1]	0 to 10 d	11 to 17 d	18 to 27 d	28 to 34 d	Total	0 to 10 d	11 to 17 d	18 to 27 d	28 to 34 d	Total
Treatment (n=16)										
SGP										
CPh	29.0	70.4	118.4	162.2	89.0	1.188	1.434	1.425	1.650	1.463
CPm	29.0	71.4	120.3	163.1	90.0	1.195	1.450	1.442	1.646	1.473
CPl	29.3	71.9	121.6	164.6	90.9	1.198	1.444	1.450	1.651	1.476
HGP										
CPh	29.1	71.8	118.2	162.5	89.4	1.197	1.466	1.437	1.603	1.462
CPm	29.3	72.9	121.7	166.6	91.5	1.196	1.440	1.429	1.642	1.465
CPl	29.7	72.2	122.1	166.8	91.6	1.190	1.411	1.448	1.640	1.467
SEM	0.3	0.8	1.2	1.8	0.7	0.009	0.016	0.010	0.016	0.006
Main effect GP (n=48)										
SGP	29.1	71.2	120.1	163.3	90.0	1.194	1.443	1.439	1.649	1.471
HGP	29.4	72.3	120.7	165.3	90.9	1.195	1.439	1.438	1.628	1.464
SEM	0.2	0.5	0.7	1.0	0.4	0.005	0.009	0.006	0.009	0.004
Main effect CP (n=32)										
CPh	29.0	71.1	118.3[b]	162.3	89.2[b]	1.193	1.450	1.431	1.626	1.462
CPm	29.2	72.2	121.0[a]	164.8	90.8[a]	1.196	1.445	1.435	1.644	1.469
CPl	29.5	72.0	121.8[a]	165.7	91.3[a]	1.194	1.427	1.449	1.645	1.471
SEM	0.2	0.6	0.9	1.3	0.5	0.006	0.011	0.007	0.011	0.004
Main effect SEX (n=48)										
Male	29.6[a]	74.7[a]	128.1[a]	177.2[a]	95.9[a]	1.189	1.419[b]	1.414[b]	1.609[b]	1.448[b]
Female	28.9[b]	68.8[b]	112.6[b]	151.4[b]	85.0[b]	1.200	1.462a	1.463[a]	1.668[a]	1.488a
SEM	0.2	0.5	0.7	1.0	0.4	0.005	0.009	0.006	0.009	0.004
P-values										
GP	0.228	0.087	0.565	0.178	0.159	0.926	0.790	0.937	0.106	0.211
CP	0.319	0.340	0.013	0.171	0.022	0.948	0.321	0.174	0.411	0.310
SEX	0.008	<0.001	<0.001	<0.001	<0.001	0.123	0.001	<0.001	<0.001	<0.001
GP × CP	0.916	0.668	0.815	0.696	0.751	0.611	0.111	0.434	0.346	0.777
GP × SEX	0.711	0.775	0.744	0.965	0.766	0.675	0.286	0.273	0.198	0.704
CP × SEX	0.314	0.237	0.564	0.370	0.526	0.352	0.867	0.565	0.974	0.719
GP × CP × SEX	0.703	0.737	0.382	0.802	0.657	0.142	0.432	0.237	0.253	0.657

[a-b]Means within a column and within an item lacking a common superscript differ ($P \leq 0.05$).

[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

Table 6. Mortality of male and female broilers of hatching eggs obtained from 29 wk of age Ross 308 broiler breeders reared on different growth patterns (GP) and dietary protein levels (CP).

	Mortality (%)				
Item[1]	0 to 10 d	11 to 17 d	18 to 27 d	28 to 34 d	Total
Treatment (n=16)					
SGP					
CPh	1.3	0.0	0.6	0.6	2.5
CPm	2.0	0.0	0.6	0.0	2.7
CPl	0.0	0.0	0.7	0.0	0.7
HGP					
CPh	0.0	0.0	1.9	2.5	4.4
CPm	0.6	1.3	1.3	0.6	3.8
CPl	0.6	0.6	0.0	0.6	1.9
SEM	0.7	0.4	0.7	0.6	1.2
Main effect GP (n=48)					
SGP	1.1	0.0	0.7	0.2	2.0
HGP	0.4	0.6	1.0	1.3	3.3
SEM	0.4	0.3	0.4	0.4	0.7
Main effect CP (n=32)					
CPh	0.6	0.0	1.3	1.6	3.4
CPm	1.3	0.6	0.9	0.3	3.2
CPl	0.3	0.3	0.4	0.3	1.3
SEM	0.5	0.3	0.5	0.5	0.8
Main effect SEX (n=48)					
Male	1.3^{a}	0.4	1.1	0.6	3.4
Female	0.2^{b}	0.2	0.6	0.8	1.9
SEM	0.4	0.3	0.4	0.4	0.7
P-values					
GP	0.220	0.087	0.497	0.052	0.158
CP	0.306	0.372	0.434	0.089	0.145
SEX	0.049	0.565	0.448	0.694	0.118
GP × CP	0.248	0.372	0.377	0.539	0.937
GP × SEX	0.636	0.565	0.164	0.240	0.891
CP × SEX	0.828	0.717	0.864	0.539	0.969
GP × CP × SEX	0.378	0.717	0.172	1.000	0.763

[a-b]Means within a column and within an item lacking a common superscript differ ($P \leq 0.05$).
[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

Despite a small difference on wing yields for the different dietary protein levels, no effects of GP and dietary CP level treatments were found on processing yields (Table 7). An interaction effect on breast meat yield between sex of the offspring and dietary protein level fed to the breeder pullets was found. Male broilers of CPl fed breeders had higher breast meat yield than male broilers of CPh fed breeders, while breast meat yield of female broilers was not affected by dietary protein levels (Figure 1).

Table 7. Processing yields of male and female broilers of hatching eggs obtained from 29 wk of age Ross 308 broiler breeders reared on different growth patterns (GP) and dietary protein levels (CP).

Item[1]	BW (g)	Carcass yield (% BW)	Wings (% carcass)	Legs (% carcass)	Back (% carcass)	Breast meat (% carcass)
Treatment (n=16)						
SGP						
CPh	2,278	66.5	11.1	34.2	15.4	31.8
CPm	2,249	66.4	11.2	34.5	15.6	31.2
CPl	2,282	66.4	11.0	34.5	15.5	31.5
HGP						
CPh	2,271	66.2	11.3	34.7	15.6	31.0
CPm	2,345	66.4	11.0	34.3	15.4	31.8
CPl	2,340	67.0	10.9	34.5	15.4	31.6
SEM	33	0.3	0.1	0.2	0.2	0.3
Main effect GP (n=48)						
SGP	2,270	66.5	11.1	34.4	15.5	31.5
HGP	2,319	66.5	11.0	34.5	15.5	31.5
SEM	19	0.2	0.1	0.1	0.1	0.2
Main effect CP (n=32)						
CPh	2,275	66.4	11.2[a]	34.4	15.5	31.4
CPm	2,297	66.4	11.1[ab]	34.4	15.5	31.5
CPl	2,311	66.7	11.0[b]	34.5	15.5	31.6
SEM	23	0.2	0.1	0.2	0.1	0.2
Main effect SEX (n=48)						
Male	2,476[a]	66.3	11.1	34.8[a]	15.4	31.4
Female	2,112[b]	66.7	11.1	34.1[b]	15.6	31.6
SEM	19	0.2	0.1	0.1	0.1	0.2
P-values						
GP	0.074	0.717	0.312	0.435	0.839	0.860
CP	0.544	0.487	0.045	0.817	0.959	0.790
SEX	<0.001	0.188	0.346	<0.001	0.218	0.342
GP × CP	0.291	0.370	0.163	0.214	0.520	0.103
GP × SEX	0.513	0.924	0.656	0.368	0.287	0.937
CP × SEX	0.063	0.549	0.593	0.045	0.380	0.027
GP × CP × SEX	0.931	0.323	0.621	0.245	0.225	0.466

[a-b]Means within a column and within an item with no common superscript differ significantly ($P < 0.05$).
[1]SGP = standard growth pattern; HGP = high growth pattern; CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.

DISCUSSION

Effect of growth pattern

The present results indicated that a higher BW target of broiler breeders at the end of the rearing period improved fertility, hatchability of set eggs, and reduced embryonic mortality during early egg production (29 wk of age). It is suggested that these positive results were caused by a 7.5% higher BW (2,346 vs. 2,183 g) in combination with a higher abdominal fat pad content (0.52 vs. 0.36%) of the HGP breeders at the end of the rearing period compared to the SGP breeders (Chapter 2). The relationship between a higher BW and earlier sexual maturity was reported by several authors (Renema et al., 2001a,b; Hocking, 2004; Ekmay et al., 2012). Although from 20 wk of age onward the same amount of feed was provided to breeders raised on different growth patterns, BW between the two groups still differed at 29 wk of age (HGP: 3,713 vs. SGP: 3,653 g) (Chapter 2). Another possibility for an increased fertility in the HGP breeders could be due to a different egg composition, as shown by Ekmay et al. (2013, 2014), although this was not proved to be significantly different in the current study.

In contrast to our results, Renema et al. (2001a,b) did not find an effect on fertility when pullet BW at 20 wk of age was increased by 8%. This might be explained by the different breeds (Shaver Starbro by Renema et al., 2001a,b; Ross 308 in Chapter 2), or moreover by the different properties due to the over 10 years of advances in selection and breeding.

Besides the potential effect of an increased BW on incubation traits, some researchers suggested that a certain minimum percentage of body fat in broiler breeders at the end of the rearing period is necessary for an adequate reproductive performance (Sun and Coon, 2005; De Beer, 2009; Mba et al., 2010). In the current study abdominal fat pad at 20 wk of age was, on average, 0.44%. This is much lower than the, on average, 2.8% what was found by Bowmaker and Gous (1989) and Fattori et al. (1993). This could be an indication that nowadays broiler breeders strains are much leaner than previous ones. It can also not be entirely excluded that differences in breeds could affect the incubation results.

The results of the current study are in agreement with the results of Gous and Cherry (2004), Zuidhof et al. (2007) and Ekmay et al. (2012), who did not find an effect of a 16, 25, and 20% higher BW at 20 wk of age, respectively, on hatchability of fertile eggs. Moreover, Renema et al. (2001a,b) found no effect on fertility and a negative effect on hatchability of fertile and set eggs when pullet BW at 20 wk of age was increased by 154 g (+8%). Renema et al. (2001a,b) and Zuidhof et al. (2007), however, found no effects on body fat. No information regarding body fat content was recorded in the studies of Gous and Cherry (2004) and Ekmay et al. (2012).

The increased fertility at 29 wk of age for the HGP breeders had disappeared at 33 and 37 wk of age. This disappearance of the effect was probably caused by smaller differences in BW and body fat between breeders on both growth patterns when they became older. At 33 and 37 wk of age, the difference in BW between the two treatments in the current study was halved compared to the difference at 29 wk of age. Moreover, the difference in the weight of the abdominal fat pad had totally disappeared at 40 wk of age (Chapter 2).

The slightly higher BW gain during the 34 d growing period of the offspring of the HGP breeders, resulted in a slightly higher BW at d 34. It can be hypothesized that body composition of the HGP breeders at end of rearing (slightly more fat deposition) might have had a positive effect on the offspring. This is underlined by Van der Waaij et al. (2011) who found that offspring of feed restricted breeders are significantly lighter and have relatively more abdominal fat pad compared to offspring of breeders fed *ad libitum*.

In conclusion, the higher target BW as well as the higher body fat content of the HGP breeders at the end of the rearing period was associated with higher fertility and a decreased early embryonic mortality of early hatching egg production. Offspring of young broiler breeders with a higher target BW at the end of the rearing period tended to have a higher growth rate.

Effect of dietary protein level

In the current study, broiler breeder pullets that were fed rearing diets with decreased dietary protein levels showed a decreased fertility of early hatching eggs. This was in line with previous studies of Walsh and Brake (1997) who found that a low dietary crude protein level (11 or 14 vs. 17%) during the rearing period decreased fertility of hatching eggs during the entire laying period. Contrary to the current study, Hocking et al. (2002) did not find an effect of different dietary protein levels (13 vs. 15.5% weighted average) during the rearing period on fertility. Based on four different studies, it was suggested by Walsh and Brake (1997) that when pullets consumed less than 1,180 g of CP between 0 and 20 wk of age, the fertility during the entire laying period would be reduced. In the current study, total CP intake (averaged over growth pattern treatments) between 0 and 20 wk of age was 1,350, 1,300, and 1,280 g for CPh, CPm, and CPl, respectively. In the current study, the highest fertility was found when the total CP intake was above 1,300 g between 0 and 20 wk of age. This is 10% higher than the minimum level suggested by Walsh and Brake (1997). The differences between the two studies on the minimum level of total CP intake, is probably due to the variation in BW between the two studies as a result of genetic selection on CP deposition over time. In the study of Walsh and Brake (1997) and in the current study average BW at 20 wk of age were 2,000 and 2,265 g, respectively. The ratio between cumulative CP intake and BW at 20 wk of age, however was similar (0.59 and 0.57, respectively) for the Walsh and Brake (1997) and the current study.

Broiler breeders fed the CPm diet had a reduced hatchability of fertile eggs, which was caused by an increased embryonic mortality between 18 and 21 d of incubation. An increased embryonic mortality during that stage of the incubation period could be due to respiratory failure, poor resorption of the yolk sac, embryo malposition or abnormalities, characteristics that were not recorded in the current study. The underlying mechanisms for these problems are mostly a too high temperature during that stage of the incubation process (Lourens et al., 2005; Molenaar et al., 2011). The clear increase of embryonic mortality only in the CPm breeders in the current study is difficult to explain. Contrary to the results from the current study, Miles et al. (1997) and Hocking et al. (2002) did not find any effect on hatchability of fertile eggs when pullets were fed different dietary protein levels during the rearing period.

When breeder pullets were fed the CPh diet, resulting in a 4.3% lower feed intake compared to the CPm breeders (Chapter 2), daily feed intake between 18 and 27 d of age of the offspring was also decreased. It can be speculated - as an epigenetic effect - that the decreased feed intake of the offspring of CPh breeder pullets was probably due to the lower feed intake of their mothers. Differences in maternal feed intake may have trigged inducing reprogramming of genes that are responsible for feed intake at a lower level of their offspring (Van der Waaij et al., 2011). The difference in daily feed intake did not affect BW, BW gain, and FCR.

Despite a small effect on wing yields, no effect on processing yields of the offspring were found due to feeding different dietary protein levels to breeder pullets. Surprisingly, an interaction effect between sex of the offspring and dietary protein level fed to the broiler breeder pullets on breast meat yield of the offspring was found (Figure 1). Male broilers of CPl breeders showed a higher breast meat yield compared to male broilers of CPh breeders, while breast meat yield of female broilers was not affected by dietary protein levels of the breeder pullets. Spratt and Leeson (1987) also showed that male and female offspring responded differently to breeder diets, which may be related to epigenetic sex-specific genes that affect body composition in the offspring (Van der Waaij et al., 2011). Broilers in the current study from CPl breeders were fed a standard diet (containing a high CP level) which in turn resulted in a higher breast meat deposition for the male broilers.

Moraes et al. (2011) fed broiler breeder pullets 3 different energy levels (2,950, 2,800, and 2,650 ME kcal/kg) combined with 2 CP levels (14 and 16%) and found that the low energy to high protein ratio diet gave a higher pectoralis major yield of the offspring than the medium energy to low protein ratio diet (16.3 vs. 15.7% of live weight). Both treatments, however, had lower carcass yields than the high energy to high protein ratio diet. These findings, as well as the results of the current study, illustrate that dietary protein and energy level in broiler breeder nutrition can alter offspring processing yield. As postulated above, the underlying mechanisms behind these interactions are still unknown but may be related to metabolic adjustment by changes in gene expression (Rao et al., 2009; Choi and Friso, 2010). For example, as shown by Heijmans et al. (2008) and Rao et al. (2009), strong epigenetic effects on the offspring were found when humans or breeders, respectively, where exposed to suboptimal nutrition.

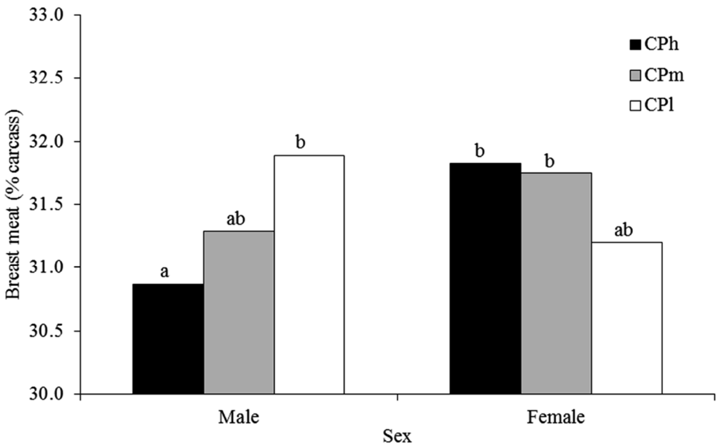

Figure 1. Effect of sex of the offspring and dietary protein level of the breeders on breast meat yield (% carcass) of the offspring. CPh = high dietary protein level; CPm = medium dietary protein level; CPl = low dietary protein level.
[a-b]Different letters within sex indicate significant differences among treatments ($P < 0.05$).

In conclusion, feeding pullets low dietary protein levels during the rearing period did not affect fertility, hatchability, embryonic mortality, and offspring performance; the only effect found was an increase in feed intake of their offspring.

CONCLUSION

The overall conclusion of the current study is that a higher growth pattern during the rearing period was effective in improving fertility, reducing early embryonic mortality, and improving offspring performance only in young broiler breeders, whereas decreasing the dietary protein level had less pronounced effects on these traits.

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Chapter 5

Effects of dietary protein levels during rearing and dietary energy levels during lay on body composition and reproduction in broiler breeder females

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ABSTRACT

A study with a 2 × 3 × 2 factorial arrangement was conducted to determine the effects of 2 dietary protein levels (high = CPh and low = CPl) during rearing, 3 dietary energy levels (3,000, MEh1; 2,800, MEs1; and 2,600, MEl1, kcal/kg AME_n, respectively) during the first phase of lay, and 2 dietary energy levels (2,800, MEs2; and 3,000, MEh2, kcal/kg AME_n, respectively) during the second phase of lay on body composition and reproduction in broiler breeders. No meaningful interactions for protein and energy treatments within the different phases of the experiment were found and, therefore, this paper focusses on the main effects. Pullets fed the CPl diet had a 12.8% higher feed intake, 14% lower breast muscle and 97% higher abdominal fat pad portion at 22 wk of age. The increased abdominal fat pad and decreased breast muscle of the CPl compared to the CPh birds increased hatchability during the first phase of lay, due to a decreased embryonic mortality between d 10 to 21 of incubation, and increased egg production during the second phase of lay. Feeding birds the MEh1 and MEl1 diets decreased egg production slightly compared to the MEs1 birds. Birds fed the MEh1 diet showed a higher mortality compared to the birds fed the MEs1 and MEl1 diets. Feeding birds the MEh2 diet did not affect egg production, increased hatchability of fertile eggs, decreased embryonic mortality between d 3 to 21 of incubation, and increased the number of first grade chicks. It was concluded that a low-protein diet during rearing changed body composition with positive effects on incubation traits during the first phase of lay and improved egg production during the second phase of lay in broiler breeders. A high- or low-energy diet compared to a standard diet during the first phase of lay slightly decreased total and settable egg numbers while a high-energy diet during the second phase of lay increased hatchability, decreased embryonic mortality and increased number of saleable chicks.

Key words: broiler breeder, diets, body composition, egg production, hatchability

INTRODUCTION

The last decades has shown an increased growth potential of broiler breeders due to selection on growth of the offspring (Havenstein et al., 2003a,b; Renema et al., 2007b). Besides the improvement in growth performance of broilers, body composition has changed as well during this period. Havenstein et al. (2003a) reported that a 2001 broiler strain (Ross 308) had a lower portion (% BW) of AF and carcass fat at 43 d (1.4 and 13.7%, respectively) than a 1957 strain at 85 d (2.0 and 17.9%), when both strains were fed a 2001 diet. Breast meat (% BW) was 20.0% and 12.2% for the 2001 and 1957 strain, respectively. This selection on increased feed efficiency and growth rate, and decreased body fat content has not only affected the offspring but also the parent stock (broiler breeders). It is suggested by some researchers (Sun and Coon, 2005; De Beer, 2009; Decuypere et al., 2010) that a certain proportion of body fat in broiler breeders at the onset of lay is necessary for maximum egg and chick production. It was shown that carcass fat at sexual maturity is between 11 and 15% of total BW (Joseph et al., 2000; Renema et al., 2001a,b; Sun et al., 2006; Renema et al., 2007a). Body composition can be adjusted by different feed allowances during rearing and lay (e.g. Fattori et al., 1993; Renema et al., 2001a,b; Chapter 2), but also by differences in nutrient composition of the diet (Chapter 2). The impact of feed management during rearing on reproductive performances can be attributed to a modified body composition at onset of lay but can also be achieved by changes in dietary composition during lay. Lopez and Leeson (1995a) showed that feeding a higher CP level (16 vs. 10%) to broiler breeders during the entire laying period increased infertility level from 4.6 to 9.4%. Moreover, Ekmay et al. (2013) showed that increasing levels of dietary lysine and isoleucine at peak production reduced fertility. The positive effect of decreasing lysine on fertility had been previously reported by Mejia et al. (2012a). Pearson and Herron (1982) reported, that low protein intake (21.3 vs. 27.0 g/d) improved hatchability as compared to high protein due to a decreased embryonic mortality. Similarly, Whitehead et al. (1985) reported a significant increase in saleable chicks per breeder when the breeder diet contained 13.7 compared to 16.8% CP. In contrast, no dietary effects (19 or 25 g CP and either 325, 385 or 450 ME/bird per d) on embryonic mortality were found by Spratt and Leeson (1987).

Research has shown that a high protein content of the diet during lay can decrease fertility and hatchability of fertile eggs in broiler breeders. However, little is known about effects of body composition on incubation traits and egg production. Therefore, the objective of the study was to determine the effects of different dietary protein levels during rearing and different dietary energy levels during lay on body composition and reproduction of the modern Ross 308 broiler breeders.

MATERIALS AND METHODS

The protocol for the experiment conformed to the standards for animal experiments and was approved by the Ethical Committee of Wageningen UR, the Netherlands. Animal care guidelines were used according to the Euro guide recommendations for animal use for experimental and other scientific purposes (Forbes et al., 2007).

Experimental design

The effects of different dietary protein levels during rearing and different dietary energy levels during lay on body composition and reproduction were studied using a 2 × 3 × 2 factorial completely randomized design with 2 CP levels (CPh = high protein; or CPl = low protein) during rearing between 2 and 22 wk of age; high, standard and low dietary energy levels during the first phase of lay between 22 and 45 wk of age (3,000, MEh1; 2,800, MEs1; and 2,600, MEl1, kcal/kg AME_n, respectively) with 14.4% CP, 0.53% dig Lys. and 0.50% dig. M+C; and standard and high dietary energy levels during the second phase of lay between 45 and 60 wk of age (2,800, MEs2; and 3,000, MEh2, kcal/kg AME_n, respectively) with 14.1% CP, 0.52% dig. Lys. and 0.49% dig. M+C.

Birds, housing, and management

A flock of 3,000 Ross 308 female broiler breeder day-old chicks (Aviagen-EPI, Roermond, The Netherlands) were allotted to 36 floor pens (4.5 × 2.5 m) in two identical climate controlled rooms. An extra pen was available with 120 pullets to replace dead chicks between d 1 and d 14. The experiment started at d 14 with 80 pullets per pen. Due to mortality, sex errors and culling (removing the smallest), the number of pullets was gradually reduced to 76 (6 wk of age), 73 (15 wk of age), and 70 (22 wk of age). Males were reared elsewhere and introduced into the pens when the breeders were 23 wk of age (8 per pen). The number of males per pen was gradually reduced to 7 and 5 at 25 and 27 wk of age, respectively. At 34 and 36 wk of age, 1 male per pen was replaced by a sexually mature spike male. At 47 wk of age 1 male per pen was removed leaving 4 males per pen. Small and inactive males were culled.

Floor pens contained an elevated floor (1.2 × 2.0 m) with plastic slats and wood shavings were used as litter on the remaining area. Pullets were fed manually till 15 wk of age, and from that age onward by automatic pan feeding (5 pans) containing a male exclusion system. One manual feeding pan per pen positioned at a minimum height of 50 cm to prevent female access to the feed, was provided for the males. Water was supplied by one bell drinker positioned above the litter floor till wk 11 and from that age onward above the slatted floor. Per pen, four laying nests (94 × 33 cm) were available to the breeders from 23 wk of age onward. During rearing and lay feed was provided at 0745 and 1030 h, respectively. For each specific dietary treatment, water was provided from 15 min prior to feed allowance and 2 h after all feed had been consumed. Water intake was restricted to prevent over-drinking. This meant that treatments with a longer eating time, due to a larger feed allowance, had water available for a longer period of time. Feed was provided *ad libitum* from 0 to 2 wk of age, and from wk 2 onward pullets were fed restricted amounts of mash feed daily. During rearing, pullets followed a four phase feeding system. The starter-1 diet was fed from 0 to 2, the starter-2 from 2 to 6, the grower from 6 to 15, and the pre-breeder from 15 to 22 wk of age. Within each phase during rearing, diets were formulated to be isocaloric. Digestible amino acid content was lowered by approximately 16% for the CPl diet, compared with the CPh diet. Diets during lay were formulated to be isonitrogenous. During the experiment, birds were maintained on the same target BW and feed allocation was adjusted to the predetermined body growth curve during rearing and a combination of the predetermined body growth curve and egg production (Aviagen-EPI, Roermond, The Netherlands). Males received a standard male diet (2,560 AME_n kcal/kg; 12.9% CP; 0.4% dig. Lys; 0.4% dig. M+C; 1.0% Ca; 0.3% aP). Ingredient composition and calculated nutrient contents of the female diets during rearing and lay are presented in Tables 1 and 2, respectively.

Room temperature was maintained at 33°C during the first 2 d, and from d 3 onward temperature was gradually reduced to 20°C at wk 5. The pullets were reared following a photoperiod of 24L:0D (20 lx) for the first 3 d which was gradually reduced to a photoperiod of 8L:16D (5 lx) at wk 3. Breeders were photo-stimulated with 11 h of light at 21 wk of age (40 lx), and day length was gradually increased by 1 h (later 0.5 h) per week to a photoperiod of 14:10D at wk 25. This was maintained until the end of the experiment at 60 wk of age, with lights on from 0400 to 1800 h. Pullets were IR beak trimmed at d 0 and vaccinated according to a standard protocol (Aviagen-EPI, Roermond, The Netherlands). Health status of the hens was monitored daily.

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

Item	Starter-1 (0-14 d)	Starter-2 (15-42 d)		Grower (43-105 d)		Pre-breeder (106-154 d)	
		CPh[1]	CPl	CPh	CPl	CPh	CPl
Ingredient							
Maize	440.0	440.0	440.0	399.5	399.5	400.0	400.0
Wheat	187.5	220.6	220.6	191.6	191.6	237.5	250.0
Soybean meal	233.3	109.2	46.3	24.4	-	107.5	38.9
Sunflower meal	-	50.0	50.0	50.0	-	-	-
Maize gluten meal	25.6	50.0	50.0	75.0	70.4	-	50.0
Rapeseed meal	35.0	35.0	35.0	35.0	12.5	35.0	35.0
Wheat middlings	20.0	21.4	90.0	100.0	180.0	150.0	150.0
Peas	-	-	-	23.7	23.7	-	-
Maize gluten meal	10.0	22.1	8.5	-	-	-	-
Maize starch	-	-	-	-	15.0	-	-
Alfalfa meal	-	-	-	60.1	70.0	5.0	9.4
Soya oil	5.0	6.4	13.3	4.4	----	2.0	3.4
Chalk	16.8	17.0	17.3	14.6	14.9	16.8	17.0
Monocalcium phosphate	9.9	10.1	10.1	4.4	4.7	9.5	9.4
Salt	1.5	0.7	0.6	0.2	0.1	1.3	0.8
Sodium carbonate	3.8	4.7	4.9	4.9	5.1	4.4	4.5
Premix rearing[2]	10.0	10.0	10.0	10.0	10.0	-	-
Premix lay[3]	-	-	-	-	-	30.0	30.0
L-Lysine	0.5	2.0	2.5	1.7	1.9	0.4	1.2
DL-Methionine	1.1	0.5	0.4	0.3	0.3	0.6	0.4
L-Threonine	-	0.3	0.5	0.2	0.3	-	-
Analyzed content[4]							
DM	867.5	876.8	874.0	875.7	874.4	879.1	877.6
Crude ash	51.1	48.9	48.1	49.4	46.3	56.4	55.1
CP	194.1	166.5	138.1	141.5	113.2	151.1	126.2
Crude fat	20.2	22.9	29.6	25.5	21.7	22.2	21.1
Starch	337.5	377.9	398.3	370.0	392.0	368.6	390.5
Reducing sugars[5]	36.0	34.1	28.1	32.8	30.0	38.1	31.4
Calculated content							
AME_n (kcal/kg)	2,795	2,800	2,800	2,600	2,600	2,700	2,700
Digestible lysine	8.60	7.18	6.04	5.36	4.50	5.59	4.71
Digestible M + C	6.70	5.70	4.80	4.50	3.80	5.04	4.38
Digestible threonine	6.01	5.17	4.36	4.00	3.30	4.18	3.41
Digestible tryptophan	1.95	1.50	1.23	1.20	1.00	1.47	1.16
Calcium	10.0	10.0	10.0	9.0	9.0	12.0	12.0
Total phosphorus	6.4	6.6	6.9	5.8	5.7	6.7	6.8
Available phosphorus	4.1	4.1	4.1	3.2	3.2	3.2	3.2
Linoleic acid	14.4	15.6	19.4	14.4	12.6	12.8	14.0
Physical characteristic							
Mean particle size (mm)	0.28	0.33	0.31	0.26	0.26	0.41	0.40

[1]Dietary protein level rearing. CPh = high dietary protein level rearing; CPl = low dietary protein level rearing.

[2]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 30 mg; vitamin K3, 1.5 mg; vitamin B1, 2.0 mg; vitamin B2, 7.5 mg; vitamin B6, 3.5 mg; vitamin B12, 0.02 mg; niacinamide, 35 mg; D-pantothenic acid, 10 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 12 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 85 mg (as MnO_2); zinc, 60 mg (as $ZnSO_4$); cobalt, 0.4 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 0.8 mg (as KI); selenium, 0.1 mg (as $Na_2SeO_3 \cdot 5H_2O$).

[3]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin K3, 5.0 mg; vitamin B1, 3.0 mg; vitamin B2, 12.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.03 mg; niacinamide, 55 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.4 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 10 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 120 mg (as MnO_2); zinc, 100 mg (as $ZnSO_4$); cobalt, 0.25 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 2.0 mg (as KI); selenium, 0.3 mg (as $Na_2SeO_3 \cdot 5H_2O$); choline chloride 50%, 2.0 g; Natuphos, 0.1 g; Rovabio Excel AP, 50 mg.

[4]Based on 2 analysis in duplicate per diet.

[5]Mono- and disaccharides as glucose units.

Table 2. Dietary ingredients, and analyzed and calculated nutrients of the lay diets (g/kg, as-fed basis).

	Breeder-1 (155-315 d)			Breeder-2 (316-420 d)	
Item	MEh1[1]	MEs1	MEl1	MEs2[2]	MEh2
Ingredient					
Maize	425.0	425.0	425.0	360.0	360.0
Wheat	140.0	140.0	140.0	183.0	160.7
Soybean meal	133.1	114.6	96.1	108.8	119.4
Maize gluten meal	40.0	30.0	20.0	25.0	50.0
Wheat middlings	10.0	96.8	183.5	105.0	10.0
Maize starch	100.0	52.5	5.0	50.0	137.4
Alfalfa meal	5.0	7.5	10.0	15.0	5.0
Soya oil	32.9	20.0	7.0	10.0	10.0
Palm oil	-	-	-	22.2	25.3
Chalk	30.0	30.0	30.0	30.0	30.0
Limestone	34.6	34.9	35.2	42.8	42.8
Monocalcium phosphate	11.5	10.5	9.6	9.9	11.2
Salt	1.5	1.3	1.1	1.2	1.4
Sodium carbonate	4.3	4.5	4.7	4.5	4.4
Premix lay[3]	30.0	30.0	30.0	30.0	30.0
Termin-8[4]	1.1	1.1	1.1	1.1	1.1
L-Lysine	0.4	0.6	0.9	0.7	0.7
DL-Methionine	0.6	0.7	0.8	0.8	0.6
Analyzed content[5]					
DM	875.3	866.4	865.3	874.7	872.5
Crude ash	99.6	99.3	97.9	102.1	105.8
CP	143.8	144.6	142.9	137.7	138.6
Crude fat	29.9	27.6	24.5	44.1	46.1
Starch	391.4	370.4	357.7	370.1	396.7
Reducing sugars[6]	25.8	28.4	31.2	28.7	24.9
Calculated content					
AME_n (kcal/kg)	3,000	2,800	2,600	2,800	3,000
Digestible lysine	5.30	5.30	5.30	5.20	5.20
Digestible M + C	5.00	5.00	5.00	4.90	4.90
Digestible threonine	4.26	4.09	3.91	3.92	4.13
Digestible tryptophan	1.25	1.28	1.30	1.27	1.19
Calcium	30.0	30.0	30.0	33.0	33.0
Total phosphorus	5.5	6.0	6.5	5.9	5.3
Available phosphorus	3.1	3.1	3.1	3.0	3.0
Linoleic acid	27.7	21.9	16.1	18.1	17.7
Physical characteristic					
Mean particle size (mm)	0.81	0.80	0.85	0.78	0.84

[1]Dietary energy level laying phase 1. MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay.
[2]Dietary energy level laying phase 2. MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.
[3]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin K3, 5.0 mg; vitamin B1, 3.0 mg; vitamin B2, 12.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.03 mg; niacinamide, 55 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.4 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 10 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 120 mg (as MnO_2); zinc, 100 mg (as $ZnSO_4$); cobalt, 0.25 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 2.0 mg (as KI); selenium, 0.3 mg (as $Na_2SeO_3 \cdot 5H_2O$); choline chloride 50%, 2.0 g; Natuphos, 0.1 g; Rovabio Excel AP, 50 mg.
[4]Mold inhibitor (Anitox Corp. Inc., Lawrenceville, GA).
[5]Based on 2 analysis in duplicate per diet.
[6]Mono- and disaccharides as glucose units.

Observations

Body weight. To monitor BW and BW gain, 10 birds per pen (as group) were weighed weekly from 0 to 35 wk of age and from 35 wk of age onward biweekly in the morning before feeding, and feeding levels were adjusted to similar growth rates throughout the experiment.

Feed, energy and protein intake. Cumulative feed, energy and CP intake were calculated for rearing, first phase, and second phase of lay.

Body composition. At 22, 35, and 59 wk of age, one randomly selected female bird per pen was killed and weighed. Breast muscle (BM) (total of pectoralis major and pectoralis minor) and abdominal fat pad (AF) were dissected from the carcass and weighed. The weights of BM and AF were expressed as a percentage of BW.

Egg production. All eggs per pen were collected and recorded daily. The total number of settable (above 50 g), small (under 50 g), double yolk, abnormal shell, and dirty eggs were calculated per pen, per week and per phase of lay.

Egg weight, ASM, and peak production. Egg weights of all hatching eggs (settable and small) were recorded on a daily basis. Weekly and average egg weight per phase were calculated. Age of sexual maturity (ASM) was defined as age at 50% production and was determined by a linear interpolation of the week (in d) where birds passed 50% rate of lay. Peak egg production was determined as a 3-wk rolling average.

Mortality. Mortality was recorded daily per pen to calculate total percentage of mortality per week, phase, and total laying period. Mortality was categorized as leg problems and other causes.

Fertility, hatchability, embryonic mortality and second grade chicks. Starting at 28 wk of age, at a 5-wk interval, 150 eggs per pen from a 3 to 4 d production period were transported and set, after a 5 d storage period, in an incubator at a commercial hatchery (Probroed & Sloot, Meppel, The Netherlands). Eggs were candled on d 10 of incubation and clear eggs and dead in shell were opened to determine infertility and the timing of embryonic mortality by visual appraisal (categorized in d 1 to 2 or d 3 to 9). At d 18, eggs were transferred to hatcher baskets and placed in a hatcher. At d 21 of incubation, unhatched eggs were calculated and scored as d 10 to 21 embryonic mortality. All chicks were graded as first or second grade chicks, where a first grade chicken was defined as dry, free of deformities, and bright eyes (Tona et al., 2004). Fertility was calculated as the percentage of fertile eggs of the set eggs. Hatchability of set and fertile eggs was calculated as the percentage of first grade chicks hatched of set and fertile eggs, respectively. Embryonic mortality was calculated as a percentage of fertile eggs. Second grade chicks were calculated as a percentage of total hatched chicks.

Statistical analysis

The REML variance component analysis procedure (Genstat 15 Committee, 2012) was used to test the effect of the nutritional factors on the determined traits, using the model:

$$Y_{ijk} = \mu + CP_i + ME1_j + ME2_k + CP_i \times ME1_j + CP_i \times ME2_k + ME1_j \times ME2_k + CP_i \times ME1_j \times ME2_k + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = overall mean; CP_i = fixed effect of protein level of the rearing diet i (i = 2; high and low); $ME1_j$ = fixed effect of energy level of the laying phase 1 diet j (j = 3; high, standard, and low); $ME2_k$ = fixed effect of energy level of the laying phase 2 diet k (k = 2; standard and high); e_{ijk} = the error term. The statistical model for incubation traits within the different phases included age.

P-values for CPi are presented for all parameters that were determined during rearing. P-values for CP_i, $ME1_j$ and $CP_i \times ME1_j$ are presented for all parameters that were determined during the first phase of lay. *P*-values for CP_i, $ME1_j$, $ME2_k$, $CP_i \times ME1_j$, $CP_i \times ME2_k$, $ME1_j \times ME2_k$ are presented for all parameters that were determined during the second phase of lay. The three way interactions between the treatments were never found to be significant and, therefore, not presented in the tables.

The statistical model for incubation traits per laying phase (first and second) included age. From the beginning until the end of the experiment, pen was considered as the experimental unit. Data are presented as means ± SEM. Unless otherwise noted, all statements of significance are based on testing at $P < 0.05$.

RESULTS

Body weight and body composition

Body weight at 22, 35 and 59 wk of age were not affected by the different treatments (Table 3).

Pullets receiving the CPl diet had 14% less BM and 97% more AF at 22 wk of age compared to the CPh pullets (Table 3). At 35 wk of age, differences in BM and AF had disappeared between the two dietary rearing treatments. Dietary energy levels during the first and second phase of lay did not affect measured body composition parameters during lay.

Table 3. BW, breast muscle (BM) and abdominal fatpad (AF) at 22, 35, and 59 wk of age as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age) of Ross 308 broiler breeders.

	22 wk of age			35 wk of age			59 wk of age		
Source[1]	BW (g)	BM (%)[2]	AF (%)[2]	BW (g)	BM (%)	AF (%)	BW (g)	BM (%)	AF (%)
Protein rearing (n=18)									
CPh	2,769	20.15[a]	0.68[b]	3,585	17.24	2.02	4,187	16.43	2.27
CPl	2,820	17.37[b]	1.34[a]	3,694	17.31	2.06	4,155	16.14	2.61
SEM	72	0.41	0.17	90	0.22	0.19	82	0.48	0.25
Energy lay 1 (n=12)									
MEh1	-	-	-	3,658	16.87	2.24	4,187	16.24	2.52
MEs1	-	-	-	3,699	17.24	2.12	4,227	16.53	2.02
MEl1	-	-	-	3,560	17.72	1.74	4,099	16.07	2.78
SEM	-	-	-	110	0.27	0.23	101	0.59	0.31
Energy lay 2 (n=18)									
MEs2	-	-	-	-	-	-	4,272	16.19	2.70
MEh2	-	-	-	-	-	-	4,070	16.37	2.18
SEM	-	-	-	-	-	-	82	0.48	0.25
P-value									
CP	0.620	<0.001	0.010	0.398	0.829	0.892	0.785	0.673	0.339
ME1	-	-	-	0.659	0.103	0.294	0.662	0.855	0.228
ME2	-	-	-	-	-	-	0.094	0.801	0.151
CP × ME1	-	-	-	0.583	0.470	0.208	0.747	0.600	0.211
CP × ME2	-	-	-	-	-	-	0.247	0.298	0.307
ME1 × ME2	-	-	-	-	-	-	0.723	0.871	0.808

[a-b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).

[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing. MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.

[2]Percentage of BW.

Feed, energy, and CP intake

In all phases, feed intake was adjusted to meet target BW. Cumulative feed, energy and CP intakes during the different phases of the experiment are shown in Table 4. To meet target BW at 22 wk of age, feed and energy intake between 2 and 22 wk of age for CPl birds was increased by 12.8%, while CP intake was decreased by 3.7%, compared with the CPh birds. Birds receiving the MEh1 diet showed a 7.5% decreased feed intake, 1.0% decreased energy intake and 6.9% decreased CP intake compared with the MEs1 birds. Birds receiving the MEl1 diet showed a 7.7% increased feed intake, 0.1% decreased energy intake and 6.8% increased CP intake compared with the MEs1 birds. Cumulative feed, energy, and CP intake were decreased by 8.5, 2.0, and 9.6% in MEh2 compared with the MEs2 birds, respectively (Table 4).

Table 4. Cumulative feed, energy, and CP intake as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age) of Ross 308 broiler breeders.[1]

	Rearing			First phase of lay			Second phase of lay		
Source[2]	Feed	Energy	CP	Feed	Energy	CP	Feed	Energy	CP
Protein rearing (n=18)									
CPh	10.31[b]	27515[b]	1,545[a]	24.64[b]	68700[b]	3,543[b]	15.22	44056	2,145
CPl	11.63[a]	31015[a]	1,488[b]	24.68[a]	68803[a]	3,548[a]	15.22	44058	2,145
SEM	0.01	7	1	0.01	10	1	0.01	29	1
Energy lay 1 (n=12)									
MEh1	-	-	-	22.79[c]	68300[c]	3,304[c]	15.23	44082	2,146
MEs1	-	-	-	24.65[b]	68998[a]	3,547[b]	15.22	44060	2,145
MEl1	-	-	-	26.54[a]	68957[b]	3,787[a]	15.21	44029	2,143
SEM	-	-	-	0.01	12	1	0.01	35	2
Energy lay 2 (n=18)									
MEs2	-	-	-	-	-	-	15.90[a]	44511[a]	2,253[a]
MEh2	-	-	-	-	-	-	14.55[b]	43603[b]	2,036[b]
SEM	-	-	-	-	-	-	0.01	29	1
P-value									
CP	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.977	0.968	0.978
ME1	-	-	-	<0.001	<0.001	<0.001	0.570	0.563	0.571
ME2	-	-	-	-	-	-	<0.001	<0.001	<0.001
CP × ME1	-	-	-	0.350	0.455	0.365	0.781	0.784	0.780
CP × ME2	-	-	-	-	-	-	0.790	0.782	0.791
ME1 × ME2	-	-	-	-	-	-	0.759	0.765	0.758

[a-c]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]Feed = cumulative feed intake (kg/bird); Energy = cumulative AME_n intake (kcal/bird); CP = cumulative CP intake (g/bird).
[2]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing. MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.

Egg production

Despite a higher hen-day egg production for the CPh birds at 28, 30, and 31 wk of age (Figure 1) no effects were found on total and settable eggs (Table 5) or peak egg production (Table 6). Feeding birds the CPl diet during rearing resulted in 0.3 more small and 0.4 more double yolk eggs during the first phase of lay (Table 5), and 3.0 more total and 3.6 more settable eggs during the second phase of lay (Table 7), compared to the birds fed the CPh diet.

Birds fed the MEs1 diet produced slightly more total ($P = 0.063$) and settable ($P = 0.086$) eggs during the first phase of lay, compared to the birds fed the MEh1 and MEl1 diet, irrespective the dietary protein level during rearing (Table 5). A linear decrease in dietary energy level during the first phase of lay resulted in a linear decrease in double-yolk eggs during this period. No carryover effects of treatments during the first phase of lay on the second phase were found.

Despite a small effect on double-yolk eggs, dietary energy level during the second phase of lay did not affect egg production (Table 7).

Table 5. Egg production traits and defective egg production (n/hen) during the first phase of the laying period (22 to 45 wk of age) as affected by dietary protein level (CP) during rearing (2 to 22 wk of age) and dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age) of Ross 308 broiler breeders.

Source[1]	Total eggs	Settable eggs[2]	Small eggs[3]	Double yolk eggs	Abnormal shell eggs[4]	Dirty eggs
Protein rearing (n=18)						
CPh	113.8	107.7	1.5[b]	1.5[b]	0.6	2.5
CPl	113.9	107.4	1.8[a]	1.9[a]	0.4	2.4
SEM	0.5	0.7	0.1	0.1	0.1	0.3
Energy lay 1 (n=12)						
MEh1	113.8	107.4	1.8	2.1[a]	0.5	2.0
MEs1	115.0	109.1	1.6	1.6[b]	0.5	2.2
MEl1	112.7	106.2	1.5	1.3[c]	0.5	3.1
SEM	0.7	0.9	0.1	0.1	0.1	0.3
P-value						
CP	0.911	0.754	0.021	<0.001	0.060	0.707
ME1	0.063	0.086	0.201	<0.001	0.975	0.070
CP × ME1	0.553	0.403	0.350	0.039	0.576	0.381

[a-c]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay.
[2]Settable eggs = normal egg ≥ 50 g.
[3]Small eggs = normal egg < 50 g.
[4]Abnormal shell eggs = cracked, soft shell and shell less eggs.

Table 6. Egg weight, ASM, peak egg production, and age at peak egg production as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age) of Ross 308 broiler breeders.

Source[1]	Egg weight phase 1 (g)[2]	Egg weight phase 2 (g)	Egg weight total lay (g)	ASM (d)[3]	Peak egg prod. (%)[4]	Age peak egg prod. (d)
Protein rearing (n=18)						
CPh	60.2	69.0	63.7	181.3	90.3	217.7
CPl	60.2	68.8	63.7	180.8	89.2	221.7
SEM	0.1	0.1	0.1	0.3	0.4	2.4
Energy lay 1 (n=12)						
MEh1	60.3	69.2[a]	63.9	180.8[b]	89.8	225.2[a]
MEs1	60.3	68.9[ab]	63.8	180.4[b]	89.6	219.9[b]
MEl1	60.1	68.6[b]	63.5	182.0[a]	89.9	214.1[c]
SEM	0.1	0.1	0.1	0.4	0.5	2.9
Energy lay 2 (n=18)						
MEs2	-	69.1[a]	63.8	-	-	-
MEh2	-	68.7[b]	63.7	-	-	-
SEM	-	0.1	0.1	-	-	-
P-value						
CP	0.677	0.349	0.837	0.243	0.094	0.253
ME1	0.408	0.012	0.099	0.019	0.912	0.037
ME2	-	0.024	0.404	-	-	-
CP × ME1	0.700	0.467	0.504	0.102	0.377	0.006
CP × ME2	-	0.680	0.599	-	-	-
ME1 × ME2	-	0.368	0.113	-	-	-

[a-c]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.
[2]Egg weight is determined for all hatching eggs (small and settable).
[3]ASM = age of sexual maturity, defined as age at 50% production.
[4]Determined as a 3-wk rolling average.

Table 7. Egg production traits and defective egg production (n/hen) during the second phase of lay (45 to 60 wk of age) as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age) of Ross 308 broiler breeders.

Source[1]	Total eggs	Settable eggs[2]	Small eggs[3]	Double yolk eggs	Abnormal shell eggs[4]	Dirty eggs
Protein rearing (n=18)						
CPh	63.6[b]	61.5[b]	0.07	0.13	0.6	1.3
CPl	66.6[a]	65.1[a]	0.08	0.09	0.3	1.0
SEM	0.7	0.9	0.03	0.03	0.2	0.2
Energy lay 1 (n=12)						
MEh1	65.9	64.2	0.11	0.11	0.3	1.2
MEs1	65.1	63.5	0.06	0.13	0.3	1.0
MEl1	64.3	62.2	0.07	0.09	0.7	1.1
SEM	0.8	1.1	0.04	0.03	0.3	0.3
Energy lay 2 (n=18)						
MEs2	65.5	63.4	0.10	0.16[a]	0.6	1.2
MEh2	64.7	63.3	0.06	0.06[b]	0.3	1.0
SEM	0.7	0.9	0.03	0.03	0.2	0.2
P-value						
CP	0.005	0.008	0.785	0.291	0.315	0.351
ME1	0.396	0.436	0.632	0.783	0.464	0.929
ME2	0.449	0.926	0.406	0.022	0.263	0.572
CP × ME1	0.875	0.917	0.284	0.745	0.343	0.133
CP × ME2	0.027	0.082	0.333	0.829	0.346	0.224
ME1 × ME2	0.443	0.647	0.375	0.278	0.264	0.730

[a-b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.
[2]Settable eggs = normal egg ≥ 50 g.
[3]Small eggs = normal egg < 50 g.
[4]Abnormal shell eggs = cracked, soft shell and shell less eggs.

Egg weight, ASM, and peak production

No effects of rearing diets were found on egg weight, ASM, and peak production (Table 6). Dietary energy level during the first phase of lay did not affect egg weight during that period. However, a linear decrease in dietary energy level during the first phase of lay resulted in a linear decrease in egg weight during the second phase of lay. An interaction effect on age at peak egg production between dietary protein level during rearing and dietary energy level during the first phase of lay was found. Birds fed the low compared to the high protein diet during rearing showed a delayed age (229.8 vs. 210.0 d) at peak egg production when they received the standard energy diet during the first phase of lay. This effect was not present when birds were fed the high or low energy diet during the first phase of lay.

Birds fed the MEl1 diet showed a delayed ASM of 1.2 and 1.6 d, while age at peak production was advanced by 11.1 and 5.8 d compared to the MEh1 and MEs1 diets, respectively.

Feeding birds the MEh2 diet decreased egg weight during the second phase of lay.

Mortality

Cumulative mortality (2.1 vs. 3.1% for CPh and CPl, respectively) during rearing was not affected by dietary protein level in that period. However, a trend was found for an increased carryover effect ($P = 0.079$) on mortality for the CPl compared to the CPh birds during the entire laying period (Table 8).

Birds fed the MEh1 diet showed an increased mortality during the first phase of lay, as well as the entire laying period, compared to the birds fed the MEs1 and MEl1 diets. The latter difference was caused by an increased mortality due to leg problems. Birds fed the high compared to the standard energy diet during the second phase of lay showed a higher mortality (11.7 vs. 6.0 and 4.8%, respectively) when they received the high energy diet during the first phase of lay, whereas this effect was not present when birds were fed the standard or the low energy diet.

Dietary energy level during the second phase of lay did not affect mortality.

Table 8. Mortality and cause of mortality as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age) of Ross 308 broiler breeders.

Source[1]	Mortality phase 1 (%)	Mortality phase 2 (%)	Mortality lay (%)	Leg problems mortality (%)	Other mortality (%)
Protein rearing (n=18)					
CPh	5.2	1.2	6.3	4.7	1.7
CPl	6.0	2.1	8.1	5.0	3.0
SEM	0.7	0.4	0.7	0.5	0.5
Energy lay 1 (n=12)					
MEh1	7.5^{a}	1.9	9.4^{a}	6.3^{a}	3.1
MEs1	4.9^{b}	1.7	6.5^{b}	4.3^{b}	2.3
MEl1	4.3^{b}	1.4	5.7^{b}	4.0^{b}	1.7
SEM	0.8	0.5	0.8	0.7	0.6
Energy lay 2 (n=18)					
MEs2	-	2.1	7.0	4.4	2.6
MEh2	-	1.2	7.5	5.4	2.1
SEM	-	0.4	0.7	0.5	0.5
P-value					
CP	0.424	0.116	0.079	0.605	0.065
ME1	0.027	0.802	0.011	0.043	0.262
ME2	-	0.116	0.622	0.186	0.433
CP × ME1	0.412	0.424	0.233	0.142	0.846
CP × ME2	-	0.422	0.622	0.605	0.910
ME1 × ME2	-	1.000	0.021	0.114	0.125

$^{a-b}$Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.

Fertility, hatchability, embryonic mortality and second grade chicks

Fertility during the first phase of lay was not affected by the CP diets during rearing (Table 9). However, hatchability of set eggs (89.0 vs. 87.7%) and fertile eggs (92.2 vs. 91.1%) from CPl birds were increased compared to CPh birds due to a decreased embryonic mortality between d 10 to 21 of incubation. The portion of second grade chicks during the first phase of lay was not affected by the dietary protein level during rearing (Table 9).

Birds fed the MEh2 diet showed an increased hatchability of fertile eggs, a decreased embryonic mortality between d 3 to 9 and d 10 to 21 of incubation, and a lower portion of second grade chicks compared to birds fed the MEs2 diet (Table 10). No effects on fertility and hatchability of set eggs were found for the treatments during the second phase of lay.

Table 9. Fertility, hatchability, embryonic mortality, and second grade chicks during the first phase of lay (22 to 45 wk of age) as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), and dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age) of Ross 308 broiler breeders.

Source[1]	Fertility (%)	Hatch. of set eggs (%)	Hatch. of fertile eggs (%)	Embryonic mortality of fertile eggs (%)				2nd grade chicks (%)[2]
				1 to 2 d	3 to 9 d	10 to 21 d	1 to 21 d	
Protein rearing (n=18)								
CPh	96.5	87.7[b]	91.1[b]	1.1	2.2	5.1[a]	8.4[a]	0.5
CPl	96.7	89.0[a]	92.2[a]	1.1	2.0	4.2[b]	7.4[b]	0.7
SEM	0.3	0.5	0.3	0.1	0.2	0.2	0.3	0.1
Energy lay 1 (n=12)								
MEh1	96.9	88.7	91.7	1.1	2.0	4.7	7.8	0.6
MEs1	96.6	88.4	91.6	1.1	2.2	4.4	7.8	0.8
MEl1	96.3	88.0	91.6	1.1	2.0	5.0	8.0	0.5
SEM	0.4	0.6	0.4	0.1	0.2	0.3	0.4	0.1
P-value								
CP	0.537	0.050	0.017	0.815	0.571	0.013	0.039	0.374
ME1	0.560	0.616	0.930	0.938	0.832	0.380	0.757	0.339
CP × ME1	0.120	0.102	0.535	0.078	0.314	0.092	0.259	0.607

[a-b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay.
[2]Expressed as a percentage of total number of hatched chicks.

Table 10. Fertility, hatchability, embryonic mortality, and second grade chicks during the second phase of lay (45 to 60 wk of age) as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age) of Ross 308 broiler breeders.

Source[1]	Fertility (%)	Hatch. of set eggs (%)	Hatch. of fertile eggs (%)	Embryonic mortality of fertile eggs (%) 1 to 2 d	3 to 9 d	10 to 21 d	1 to 21 d	2nd grade chicks (%)[2]
Protein rearing (n=18)								
CPh	89.2	79.5	89.3	1.9	2.0	5.2	9.0	1.7
CPl	88.6	78.9	89.2	1.5	2.3	5.2	9.0	2.3
SEM	1.1	1.2	0.5	0.2	0.2	0.3	0.4	0.2
Energy lay 1 (n=12)								
MEh1	87.3	77.9	89.4	1.7	1.9	5.4	9.0	1.9
MEs1	90.6	80.5	89.1	1.7	2.3	5.1	9.2	2.2
MEl1	88.8	79.3	89.3	1.6	2.3	5.1	8.9	2.0
SEM	1.4	1.5	0.6	0.2	0.2	0.4	0.5	0.2
Energy lay 2 n=18)								
MEs2	88.7	78.5	88.5[b]	1.6	2.4[a]	5.7[a]	9.7[a]	2.3[a]
MEh2	89.1	80.0	90.0[a]	1.7	1.9[b]	4.7[b]	8.4[b]	1.7[b]
SEM	1.2	1.2	0.5	0.2	0.2	0.3	0.4	0.2
P-value								
CP	0.733	0.708	0.807	0.157	0.165	0.994	0.948	0.173
ME1	0.276	0.447	0.918	0.846	0.265	0.826	0.898	0.878
ME2	0.813	0.376	0.030	0.597	0.048	0.033	0.020	0.040
CP × ME1	0.671	0.924	0.382	0.448	0.844	0.180	0.151	0.653
CP × ME2	0.886	0.886	0.510	0.988	0.042	0.208	0.061	0.022
ME1 × ME2	0.409	0.780	0.218	0.645	0.034	0.226	0.317	0.689

[a-b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.
[2]Expressed as a percentage of total number of hatched chicks.

DISCUSSION

No meaningful interactions for protein and energy treatments within the different phases of the experiment were found and, therefore, this paper focusses on the main effects.

Effects of dietary protein level during rearing

The 12.8% higher feed intake needed to achieve target BW, when pullets were fed the low-CP diet, in the current study was in line with the 10.0% higher feed intake of a previous study in our laboratory (Chapter 2) and those of Lilburn and Myers-Miller (1990), Miles et al. (1997), Hudson et al. (2000) and Hocking et al. (2002). Since the diets were isocaloric, this increase of feed intake was driven by a need for protein to reach BW. Effects of a low-CP diet during rearing on body composition (14% decreased BM and 97% increased AF) at the end of rearing are in agreement with earlier findings (Spratt and Leeson, 1987; Miles et al., 1997; Hudson et al., 2000; Mba et al., 2010; Chapter 2). The differences in body composition could be explained by the higher daily energy and lower CP intake by the CPl birds during rearing (Table 4). The differences in body composition at the end of rearing in the current study disappeared during lay, as shown in the earlier study presented in Chapter 2.

Despite some small effects on number of small and double yolk eggs, no carryover effects of dietary protein level during rearing were found on the number of total or settable eggs during the first phase of lay. However, a significant carryover effect of dietary protein level during rearing on the number of total and settable egg production during the second phase of lay was observed (Figure 1). Pullets fed the CPl diet produced between 45 and 60 wk of age 3.0 more total and 3.6 more settable eggs than pullets fed the CPh diet. This effect was not been observed in our previous study (Chapter 2) due to the fact that that experiment was terminated at 40 wk of age. The better persistency of lay of birds fed low-protein diet during lay might be explained by the higher proportion of AF and lower proportion of BM at the end of rearing. Breeders with a higher body fat content are probably more able to mobilize energy reserves in periods of a negative energy balance (Renema et al., 2013) which probably prevent them for molting. The lower muscle content of the body may decrease the daily energy requirement for maintenance and increase the amount of energy that would be available for egg production (Ekmay et al., 2013).

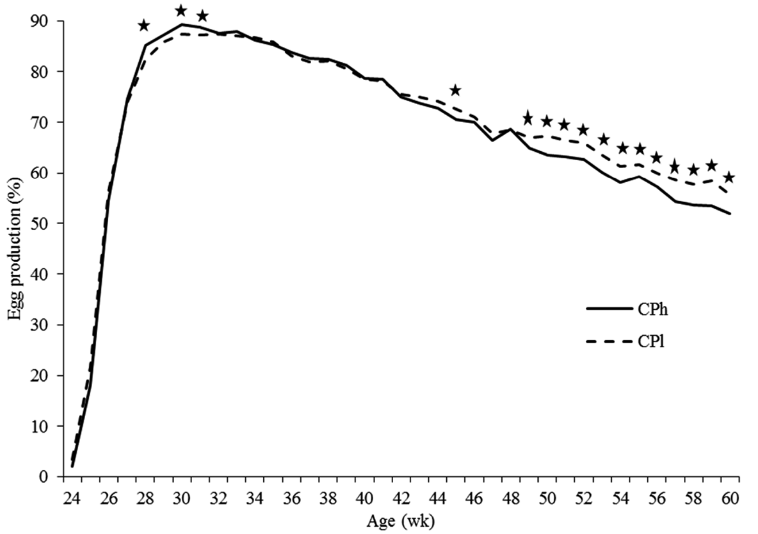

Figure 1. Broiler breeder hen-day total egg production curves as affected by dietary protein level during rearing (CPh = high crude protein; CPl = low crude protein).
*Stars indicate significant differences ($P < 0.05$) between treatments.

Contrary to the current study, Miles et al. (1997) did not find any effects of a low protein diet during rearing on total egg production. This may be caused by the different breeds, or moreover the different properties due to 15 years of advances in selection and breeding resulting in differences in body composition (Renema et al., 2013). On the other hand, when pullets were fed very low protein diets (approximately 10%) during rearing, total egg production was negatively affected (Hudson et al., 2000; Hocking et al., 2002). In line with our previous trial (Chapter 2), no effects of dietary protein level during rearing were found on egg weight.

The increased AF and decreased BM of the pullets fed the CPl diet decreased embryonic mortality of the offspring between d 10 and 21 of incubation of 0.9% (4.2 vs. 5.1%) during the first phase of lay. This resulted in a 1.3 and 1.1% increased hatchability of set and fertile eggs, respectively. Hocking et al. (2002) and the previous experiment (Chapter 2) did not find any effects on incubation traits when pullets received different dietary protein levels during rearing. The explanation of the positive effects of changed body composition (higher AF and lower BM portion) of the CPl birds on lower embryonic mortality in the current study is unclear. However, it could be hypothesized that egg composition was influenced by body composition at end of rearing.

Fertility of hatching eggs in the current study was not affected by different dietary protein levels during rearing. This is contrary to the findings of Walsh and Brake (1997, 1999) who found that a low CP intake during rearing could reduce fertility during lay. These studies, however, were conducted with broiler breeders two decades ago and may therefore not comparable to the breeders in the current study. Current broiler breeders maybe showed smaller effects on fertility due to differences in body compositions at start of lay.

Effects of dietary energy level during the first phase of lay

In the current study, a linear decreased dietary energy level during the first phase of lay resulted in a linear increased feed and CP intake, while energy intake was almost equal for reaching the same target BW. These results are in agreement with Sun and Coon (2005), Enting et al. (2007) and Pishnamazi et al. (2011).

Despite some numerical trends, dietary energy level did not affect body composition at 35 wk of age. Contrary to our results, Sun and Coon (2005) and Pishnamazi et al. (2011) found an increased fat content of the body when birds were fed a higher energy diet during the initial or entire laying period, respectively. However, both authors also did not find an effect on protein content of the body. The explanation for the absence of a difference in abdominal fat pad in the current study as a result of a change in dietary energy level could be maybe due to the too low number of birds per pen allocated for dissection, thereby allowing an increased standard error of mean within the treatments.

Birds fed the MEs1 diet produced a slightly higher number of total and settable eggs during the first phase of lay compared to the birds fed the MEh1 and MEl1 diets. In contrast to these findings, Pishnamazi et al. (2011) found no effect on number of eggs when birds were fed two different dietary energy levels (2,900 vs. 2,800 kcal/kg ME) to 33 wk of age. Also Joseph et al. (2000) did not find an effect of a similar daily energy and 10% lower CP intake on total egg production. However, the latter observed a decreased number of settable eggs due to a decreased egg weight.

In the current study, no effect of different dietary energy levels during the first phase of lay on egg weight during that phase was observed. However, a carryover effect on increased egg weight (+0.6 g) during the second phase of lay was found when birds were fed the MEh1 diet during the first phase of lay (low daily CP/AA intake) compared to the MEl1 birds (high daily CP/AA intake). A comparable effect was observed by Sun and Coon (2005) when birds were fed a 5.5% higher dietary energy level. They found no effect on egg weight between 24 to 30 wk of age, however, egg weight between 30 and 65 wk of age was increased when birds received an isonitrogenous diet with a higher energy level during lay. No effect on egg weight between 23 and 48 wk of age was observed by Joseph et al. (2002) when birds received an 8% decreased daily CP intake. However, in an earlier experiment they observed a decreased effect on egg weight during initial lay (24 to 29 wk of age) when birds receive an approximately 10% lower daily CP intake (Joseph et al., 2000). In contrast to the current study, Spratt and Leeson (1987) found an higher egg weight (between 34 and 38 wk of age) when breeders were fed a high compared to a low energy diet between 19 and 40 wk of age. The difference in the latter study can be explained by the substantial difference (38%) in energy content between the diets.

Birds fed the MEl1 diet showed a small delayed sexual maturity of 1.4 d compared to those fed the MEh1 and MEs1 diets. Contrary to our findings, Pishnamazi et al. (2011) found that providing a low energy diet (2,800 vs. 2,900 kcal/kg ME) advanced sexual maturity with 2 d, while Sun and Coon (2005) did not find an effect on sexual maturity when different dietary energy levels were provided. Thereafter the increase in rate of lay in the current study differed between the treatment groups. An opposite effect of 11.1 and 5.8 d on age at peak production was found for the MEl1 birds compared to the MEh1 and MEs1 birds, respectively. This may be due to the fact that birds fed the MEl1 diet received a 7.3 or 14.6% higher daily CP intake compared to the MEh1 and MEs1 diet, respectively. A high daily CP intake during initial lay could be a stimulus for development of the reproductive tract, resulting in an earlier peak egg production (Lilburn and Myers-Miller, 1990). Moreover, Pishnamazi et al. (2011) stated that a lower protein intake (19.7 vs. 20.4 g/d) during initial lay may hamper ovary development and, as a consequence, egg production.

Feeding birds the MEh1 diet increased mortality during the first phase of lay and entire laying period compared to the birds fed the MEs1 and MEl1 diet. Contrary to our results, Sun and Coon (2005) did not find an effect on mortality when Cobb 500 birds were fed a high or low energy diet (2,955 vs. 2,800 AME_n kcal/kg) during the entire laying period. The difference in total mortality in the current experiment was caused by a higher mortality due to leg problems and more specifically ruptures of the gastrocnemius tendon. It can be speculated that differences in behavior due to differences in feed intake played a role in this cause of mortality. Birds fed the high energy diet, compared to the other treatments, were much more aggressive around feeding resulting in (hyper)activity like running and jumping, inducing a higher risk of damaging the tendons (data not shown).

No effects on incubation traits were found when birds were fed different dietary energy levels during the first phase of lay. This was in agreement with Enting et al. (2007), who fed Cobb breeders three dietary energy levels (2,200 vs. 2,500 vs. 2,800 kcal/kg AME) during the entire laying period.

Effects of dietary energy level during the second phase of lay

Subjecting birds to a high energy diet during the second phase of lay resulted in a lower feed and CP intake compared to a standard energy diet. This was in line with the results of the first phase of lay. Contrary to our expectations, the birds fed the high energy diet showed a 2% lower daily energy intake compared to the standard energy diet. A sound explanation for this phenomenon was not found. It may be suggested that the difference in daily energy intake was too small to cause a difference in AF at the end of lay. The absence of a relative lower BM weight due to the 10% lower daily CP intake of the MEh2 birds may be due to broiler breeders who at this age partitioned CP to sustain their BM above maintaining egg weight in favor of their offspring. In contrast to the current study, Sun and Coon (2005) found an increased egg weight when birds received a high energy diet. The difference between the current study and the study of Sun and Coon (2005) is that in the latter study, not only energy intake was increased, but also CP intake.

Egg production and mortality were not affected by dietary energy level which is in agreement with Sun and Coon (2005) who fed breeders a high or low energy diet (2,970 vs. 2,816 kcal/kg ME) during the entire laying period. Egg weight of the birds fed the MEh2 diet was 0.4 g lower than for birds fed the MEs2 diet, possibly due to the lower daily CP/AA (effect on albumen and yolk) and lower daily linoleic intake acid intake (effect on yolk only). However, we did not examine egg composition.

A high energy diet (and as a consequence a 10% lower daily CP/AA intake) resulted in no effects on fertility but improved hatchability of fertile eggs, which in turn was caused by decreased embryonic mortality between d 3 to 21 of incubation. This is in agreement with the studies of Pearson and Herron (1982) and Whitehead et al. (1985). Lopez and Leeson (1995a) observed an increased fertility and hatchability of set eggs and lower embryonic mortality when birds received a lower daily CP intake during the entire laying period.

The improved incubation traits in the current study probably also positively affected the quality of the embryos resulting in a lower number of second grade chicks. This was previously underlined by Pearson and Herron (1982) who found that the portion of dead and malpositioned embryos could be reduced if daily protein intake was decreased from 27.0 to 21.3 g protein per animal.

CONCLUSION

A low-protein diet during rearing fed to broiler breeder pullets changes body composition with positive effects on incubation traits during the first phase and an improved egg production during the second phase of lay. Changing the dietary energy level compared to the standard (2,800 Kcal/kg) during the first phase of lay resulted in slightly negative effects on production while a high-energy diet (3,000 Kcal/kg) during the second phase of lay showed positive effects on incubation traits.

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Chapter 6

Effects of dietary protein levels during rearing and dietary energy levels during lay on behavior and feather cover in broiler breeder females

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ABSTRACT

An experiment was conducted to determine the effects of different dietary protein levels during rearing and different dietary energy levels during lay in broiler breeder on behavior and feather cover. A 2 × 3 × 2 factorial arrangement of treatments was used. A total of 2,880 Ross 308 14-d-old broiler breeder pullets were fed between wk 2 to 22 a high (CPh) or low (CPl) CP diet. Between wk 22 to 45 breeders were fed either a high, standard, or low energy diet (3,000, MEh1; 2,800, MEs1; and 2,600, MEl1, kcal/kg AME_n, respectively). Between wk 45 to 60 breeders were fed either a standard or high energy diet (2,800, MEs2; and 3,000, MEh2, kcal/kg AME_n, respectively). During rearing CPl pullets received 12.1% more feed, resulting in a 137% increased eating time and 47% decreased eating rate. This increased feeding, sitting and comfort behaviors while walking, foraging and stereotypic object pecking behaviors were reduced. Feather cover was poorer when pullets were fed a low protein diet during rearing but this effect was not present during lay. A 7.6% lower feed intake for the MEh1 birds resulted in a 21% decreased eating time and 19% increased eating rate compared to the MEs1 birds. Birds on the MEh1 diet spent less time feeding and more time sitting, expressing comfort behavior, and stereotypic object pecking compared to the MEs1 birds. Birds fed the MEh1 diet showed a poorer feather cover during the entire lay compared to the MEs1 birds. A 7.7% higher feed intake for the MEl1 birds resulted in a 31% increased eating time and 18% decreased eating rate compared to the MEs1 birds. MEl1 birds spent more time feeding and less time foraging, comfort and stereotypic object pecking compared to the MEs1 birds. No effect on feather cover was found for the MEl1 compared to the MEs1 birds. The MEh2 birds received 8.8% less feed resulted in a 16% decreased eating time and 9% increased eating rate. The lower feed intake resulted in less time spent feeding and standing, and more time spent foraging and expressing comfort behavior. Feather cover was not affected by dietary energy level during the second phase of lay. In conclusion, feeding broiler breeders a higher amount of feed due to a higher energy to protein ratio resulted in an increased eating time and less stereotypic object pecking what is an indication of reduced hunger and frustration. This was much more expressed during rearing than lay. However, a low daily protein intake during rearing and first phase of lay can lead to a poorer feather cover.

Key words: broiler breeder, diets, rearing, laying, behavior

INTRODUCTION

Due to the directed selection for faster growth and feed efficiency of broilers during the last decades, also broiler breeders show these traits (Renema et al., 2007b). Modern broiler strains show a 2.5 times higher daily growth while FCR is less than half compared to strains in use 60 years ago. The detrimental impact of a high genetic growth potential of breeder pullets has been observed by several researchers who found significant negative effects on production and mortality when breeders provided feed *ad libitum* (see Renema and Robinson, 2004 for a review). To prevent these problems, broiler breeders are feed restricted between 67 to 75% of *ad libitum* feed intake during the rearing period (Savory et al., 1996; De Jong et al., 2002) and up to 50% during the laying period (Bruggeman et al., 1999). It is suggested that feed restriction level for modern-day breeders is more severe due to the still increasing growth potential of the offspring (Chapter 3). Feed restriction compared to *ad libitum* feed intake can lead to a 7 times lower mortality, improved egg quality, 1.5 times higher peak production and 20-25% higher egg production (Heck et al., 2004). On the other hand, numerous studies have shown that feed restricted broiler breeders show behavioral disorders (stereotypic object pecking, over-drinking, and pacing) that are indicative of frustration, boredom and hunger (De Jong and Jones, 2006; D'eath et al., 2009). Stereotypic object pecking normally starts after feeding, and is mostly performed at the litter, drinkers, feeders and the walls of the pen or to flock mates (Kostal et al., 1992; Savory and Maros, 1993; Savory and Kostal, 1996; De Jong et al., 2002; Hocking et al., 2002). To prevent over-drinking, also water intake is often restricted in practice (Van Krimpen and De Jong, 2014). Pacing is mainly observed before feed is provided to the birds (Savory and Maros, 1993). Improving behavior and, thereby, welfare in broiler breeders is not a simple task because feed restriction is regular practiced (Decuypere et al., 2010). During the past two decades, researchers have investigated alternative management strategies (feeding and diet composition) to improve welfare by reducing the negative effects of feed restriction in combination with an adequate growth pattern and reproduction results. Scatter feeding and feeding twice a day during rearing resulted in an increased eating time but no positive effects on physiological indicators of stress and hunger (De Jong et al., 2005a). Scatter feeding in combination with high levels of dietary insoluble fiber may improve welfare of broiler breeders due to the absent of stereotypic pecking and increased dust bathing and comfort behavior (Nielsen et al., 2011). Dilution of the feed increased the time spent eating, which is noted as a promising method for improving breeder welfare (De Jong and Van Krimpen, 2011). In some studies, dietary dilution (by adding fiber) reduced stereotypic object pecking (De Jong et al., 2005b; Hocking et al., 2004), although these effects were not observed in other studies (Hocking, 2006; Jones et al., 2004). Recently, a partly diluted diet (isocaloric and low CP/AA) was applied to breeder pullets which resulted in an increased eating time, decreased eating rate, increased resting and decreased stereotypic pecking behavior (Chapter 3).

Besides feed restriction, a poor feather cover also has negative effects on welfare of the broiler breeders (Van Emous and De Jong, 2013). A good feather cover is important to protect broiler breeders from skin damage caused by objects or rough mating behavior (De Jong et al., 2009) and for thermoregulation of the birds as shown in layers (Peguri and Coon, 1993). The quality of feather cover of broiler breeders has decreased over the last decade due to hitherto, unknown reasons (Van Emous and De Jong, 2013). Based on the results of Twining et al. (1976), who found that feeding broilers over 16% CP improved feather growth and molting, it can be suggested that diet composition could play a role in feather development. Moreover, in an previous experiment it was found that feeding low protein diets during rearing to breeder pullets negatively affected feather cover during the initial rearing period (Chapter 3).

Most studies, which are conducted to improve behavior of breeders, focused on effects of different feeding strategies during the rearing period. The current study was conducted to investigate the effects of different dietary protein levels during the rearing period as well as the effects of different dietary energy levels during the laying period on behavior and feather cover in broiler breeder females.

MATERIALS AND METHODS

The protocol for the experiment conformed to the standards for animal experiments and was approved by the Ethical Committee of Wageningen UR, the Netherlands.

Experimental design

The study consisted of a 2 × 3 × 2 factorial completely randomized design with 2 CP levels (CPh = high protein; or CPl = low protein) during rearing between 2 and 22 wk of age; high, standard and low dietary energy levels during the first phase of lay between 22 and 45 wk of age (3,000, MEh1; 2,800, MEs1; and 2,600, MEl1, kcal/kg AME_n, respectively) with 14.4% CP, 0.53% dig Lys and 0.50% dig. M+C; and standard and high dietary energy levels during the second phase of lay between 45 and 60 wk of age (2,800, MEs2; and 3,000, MEh2, kcal/kg AME_n, respectively) with 14.1% CP, 0.52% dig. Lys and 0.49% dig. M+C.

Birds, housing, and management

A total of 3,000 Ross 308 female broiler breeder one-day-old chicks (Aviagen-EPI, Roermond, The Netherlands) were allotted to 36 floor pens (4.5 × 2.5 m) in two identical climate rooms. The experiment started at d 14 with 80 pullets per pen (2,880 in total). From the first day, an extra pen was available for 120 extra pullets to replace dead chicks until 14 days. Based on unforeseen culling (small or sick), mortality and sex errors, the number of pullets gradually reduced to 76 (wk 6), 73 (wk 15), and 70 (wk 22). Males were reared elsewhere and introduced into the pens when the hens were 23 wk of age (8 per pen; male/female ratio of 11.4%). Male/female ratio was gradually reduced to 10.0% (7 males/pen) and 7.1% (5 males/pen) at 25 and 27 wk of age, respectively. At 34 and 36 wk of age, one originally male per pen was replaced by a sexually mature spike male. At 47 wk of age one (small and / or inactive) male per pen was culled leaving 4 males per pen.

The pullets were housed in pens with wood shavings and an elevated floor (1.2 × 2.0 m) with plastic slats. The sidewalls of the pens were constructed from wire mash so that pullets could see birds in other pens. Pullets were fed by manual feeding pans till 15 wk of age and from that age onward an automatic pan feeding system (5 pans) with a male exclusion system was used. Males were provide feed in one manual feeding pan per pen which was located at a minimum height of 50 cm to prevent female access to the feed. Water was provided by one bell drinker per pen located above the litter floor till 11 wk of age and from that age onward above the slatted floor. Per pen, four laying nests (94 × 33 cm) were available to the hens from 23 wk of age onward. During the rearing and laying period, feed was provided at 0745 and 1030 h, respectively. Water was provided, for each specific dietary treatment, from 15 min before provision of the feed to 2 h after the feeding pans were emptied. This meant that treatments with a longer eating time, due to a larger amount of feed, had access for a longer time to water. Feed was provided *ad libitum* during the first two wk of the rearing period and from wk 3 onward pullets were fed restricted amounts of feed every day. Birds were fed amounts of feed to maintain the breeder recommended target BW during the entire experiment (Aviagen-EPI, Roermond, The Netherlands).

During the rearing period pullets followed a four phase feeding system (diets in mash form). The starter-1 diet was fed from wk 0 to 2, the starter-2 diet from wk 2 to 6, the grower diet from wk 6 to 15, and the pre-breeder diet from wk 15 to 22. Within each phase, diets were formulated to be isocaloric (AME_n basis) and digestible amino acids were lowered by 16% for the CPl diet as compared to the CPh diet. Diets during lay were formulated to be isonitrogenous. Treatments comprised of 18, 6 and 3 replicates per treatment in the rearing period, first and second phases of the laying period, respectively. Males received a standard male diet with lower levels for energy, crude protein, amino acids, calcium, and phosphorus. Ingredient composition and calculated nutrient contents of the diets during the rearing and laying period are presented in Tables 1 and 2, respectively.

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

	Starter-1 (0-14 d)	Starter-2 (15-42 d)		Grower (43-105 d)		Pre-breeder (106-154 d)	
Item		CPh[1]	CPl	CPh	CPl	CPh	CPl
Ingredient							
Maize	440.0	440.0	440.0	399.5	399.5	400.0	400.0
Wheat	187.5	220.6	220.6	191.6	191.6	237.5	250.0
Soybean meal	233.3	109.2	46.3	24.4	-	107.5	38.9
Sunflower meal	-	50.0	50.0	50.0	-	-	-
Maize gluten meal	25.6	50.0	50.0	75.0	70.4	-	50.0
Rapeseed meal	35.0	35.0	35.0	35.0	12.5	35.0	35.0
Wheat middlings	20.0	21.4	90.0	100.0	180.0	150.0	150.0
Peas	-	-	-	23.7	23.7	-	-
Maize gluten meal	10.0	22.1	8.5	-	-	-	-
Maize starch	-	-	-	-	15.0	-	-
Alfalfa meal	-	-	-	60.1	70.0	5.0	9.4
Soya oil	5.0	6.4	13.3	4.4	-	2.0	3.4
Chalk	16.8	17.0	17.3	14.6	14.9	16.8	17.0
Monocalcium phosphate	9.9	10.1	10.1	4.4	4.7	9.5	9.4
Salt	1.5	0.7	0.6	0.2	0.1	1.3	0.8
Sodium carbonate	3.8	4.7	4.9	4.9	5.1	4.4	4.5
Premix rearing[2]	10.0	10.0	10.0	10.0	10.0	-	-
Premix lay[3]	-	-	-	-	-	30.0	30.0
L-Lysine	0.5	2.0	2.5	1.7	1.9	0.4	1.2
DL-Methionine	1.1	0.5	0.4	0.3	0.3	0.6	0.4
L-Threonine	-	0.3	0.5	0.2	0.3	-	-
Analyzed content[4]							
DM	867.5	876.8	874.0	875.7	874.4	879.1	877.6
Crude ash	51.1	48.9	48.1	49.4	46.3	56.4	55.1
CP	194.1	166.5	138.1	141.5	113.2	151.1	126.2
Crude fat	20.2	22.9	29.6	25.5	21.7	22.2	21.1
Starch	337.5	377.9	398.3	370.0	392.0	368.6	390.5
Reducing sugars[5]	36.0	34.1	28.1	32.8	30.0	38.1	31.4
Calculated content							
AME_n (kcal/kg)	2,795	2,800	2,800	2,600	2,600	2,700	2,700
Digestible lysine	8.60	7.18	6.04	5.36	4.50	5.59	4.71
Digestible M + C	6.70	5.70	4.80	4.50	3.80	5.04	4.38
Digestible threonine	6.01	5.17	4.36	4.00	3.30	4.18	3.41
Digestible tryptophan	1.95	1.50	1.23	1.20	1.00	1.47	1.16
Calcium	10.0	10.0	10.0	9.0	9.0	12.0	12.0
Total phosphorus	6.4	6.6	6.9	5.8	5.7	6.7	6.8
Available phosphorus	4.1	4.1	4.1	3.2	3.2	3.2	3.2
Linoleic acid	14.4	15.6	19.4	14.4	12.6	12.8	14.0
Physical characteristic							
Mean particle size (mm)	0.28	0.33	0.31	0.26	0.26	0.41	0.40

[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing.
[2]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 30 mg; vitamin K3, 1.5 mg; vitamin B1, 2.0 mg; vitamin B2, 7.5 mg; vitamin B6, 3.5 mg; vitamin B12, 0.02 mg; niacinamide, 35 mg; D-pantothenic acid, 10 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; iron, 80 mg (as $FeSO_4{\cdot}7H_2O$); copper, 12 mg (as $CuSO_4{\cdot}5H_2O$); manganese, 85 mg (as MnO_2); zinc, 60 mg (as $ZnSO_4$); cobalt, 0.4 mg (as $CoSO_4{\cdot}7H_2O$); iodine, 0.8 mg (as KI); selenium, 0.1 mg (as $Na_2SeO_3{\cdot}5H_2O$).
[3]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin K3, 5.0 mg; vitamin B1, 3.0 mg; vitamin B2, 12.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.03 mg; niacinamide, 55 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.4 mg; iron, 80 mg (as $FeSO_4{\cdot}7H_2O$); copper, 10 mg (as $CuSO_4{\cdot}5H_2O$); manganese, 120 mg (as MnO_2); zinc, 100 mg (as $ZnSO_4$); cobalt, 0.25 mg (as $CoSO_4{\cdot}7H_2O$); iodine, 2.0 mg (as KI); selenium, 0.3 mg (as $Na_2SeO_3{\cdot}5H_2O$); choline chloride 50%, 2.0 g; Natuphos, 0.1 g; Rovabio Excel AP, 50 mg.
[4]Based on 2 analysis in duplicate per diet.
[5]Mono- and disaccharides as glucose units.

Table 2. Dietary ingredients, and analyzed and calculated nutrients of the laying diets (g/kg, as-fed basis).

Item	Breeder-1 (155-315 d)			Breeder-2 (316-420 d)	
	MEh1[1]	MEs1	MEl1	MEs2[2]	MEh2
Ingredient					
Maize	425.0	425.0	425.0	360.0	360.0
Wheat	140.0	140.0	140.0	183.0	160.7
Soybean meal	133.1	114.6	96.1	108.8	119.4
Maize gluten meal	40.0	30.0	20.0	25.0	50.0
Wheat middlings	10.0	96.8	183.5	105.0	10.0
Maize starch	100.0	52.5	5.0	50.0	137.4
Alfalfa meal	5.0	7.5	10.0	15.0	5.0
Soya oil	32.9	20.0	7.0	10.0	10.0
Palm oil	-	-	-	22.2	25.3
Chalk	30.0	30.0	30.0	30.0	30.0
Limestone	34.6	34.9	35.2	42.8	42.8
Monocalcium phosphate	11.5	10.5	9.6	9.9	11.2
Salt	1.5	1.3	1.1	1.2	1.4
Sodium carbonate	4.3	4.5	4.7	4.5	4.4
Premix lay[3]	30.0	30.0	30.0	30.0	30.0
Termin-8[4]	1.1	1.1	1.1	1.1	1.1
L-Lysine	0.4	0.6	0.9	0.7	0.7
DL-Methionine	0.6	0.7	0.8	0.8	0.6
Analyzed content[5]					
DM	875.3	866.4	865.3	874.7	872.5
Crude ash	99.6	99.3	97.9	102.1	105.8
CP	143.8	144.6	142.9	137.7	138.6
Crude fat	29.9	27.6	24.5	44.1	46.1
Starch	391.4	370.4	357.7	370.1	396.7
Reducing sugars[6]	25.8	28.4	31.2	28.7	24.9
Calculated content					
AME_n (kcal/kg)	3,000	2,800	2,600	2,800	3,000
Digestible lysine	5.30	5.30	5.30	5.20	5.20
Digestible M + C	5.00	5.00	5.00	4.90	4.90
Digestible threonine	4.26	4.09	3.91	3.92	4.13
Digestible tryptophan	1.25	1.28	1.30	1.27	1.19
Calcium	30.0	30.0	30.0	33.0	33.0
Total phosphorus	5.5	6.0	6.5	5.9	5.3
Available phosphorus	3.1	3.1	3.1	3.0	3.0
Linoleic acid	27.7	21.9	16.1	18.1	17.7
Physical characteristic					
Mean particle size (mm)	0.81	0.80	0.85	0.78	0.84

[1]MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay.
[2]MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.
[3]Provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 100 IU; vitamin K3, 5.0 mg; vitamin B1, 3.0 mg; vitamin B2, 12.0 mg; vitamin B6, 5.0 mg; vitamin B12, 0.03 mg; niacinamide, 55 mg; D-pantothenic acid, 15 mg; folic acid, 2.0 mg; biotin, 0.4 mg; iron, 80 mg (as $FeSO_4 \cdot 7H_2O$); copper, 10 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 120 mg (as MnO_2); zinc, 100 mg (as $ZnSO_4$); cobalt, 0.25 mg (as $CoSO_4 \cdot 7H_2O$); iodine, 2.0 mg (as KI); selenium, 0.3 mg (as $Na_2SeO_3 \cdot 5H_2O$); choline chloride 50%, 2.0 g; Natuphos, 0.1 g; Rovabio Excel AP, 50 mg.
[4]Mold inhibitor (Anitox Corp. Inc., Lawrenceville, GA).
[5]Based on 2 analysis in duplicate per diet.
[6]Mono- and disaccharides as glucose units.

Room temperature was maintained during the first 2 d at 33°C and from d 3 onward temperature was gradually reduced to 20°C at 5 wk of age. The pullets were reared following a photoperiod of 24L:0D (20 lx) for the first 3 d which was gradually reduced to a photoperiod of 8L:16D (5 lx) at 3 wk of age. Hens were photo-stimulated with 11 h of light at wk 21 (40 lx), and day length was gradually increased by 1 h (later 0.5 h) per wk to a photoperiod of 14:10D at wk 25. This was maintained until the end of the experiment at 60 wk of age, with lights on from 0400 to 1800 h. Pullets were IR beak trimmed at d 0, vaccinated according to a standard vaccination program (Aviagen-EPI, Roermond, The Netherlands), and health status of the hens was monitored daily.

Observations

Eating time and eating rate. Eating time (min/d) was defined as the time between provision of the feed and the moment that the feed pans were empty. Eating time was determined by visual inspection at 11, 17, 27, 38, 46 and 54 wk of age. Eating rate (g feed/min) was calculated as feed intake (g/bird per d) divided by the eating time (Van Krimpen et al., 2009).

Behavioral observations. Behavior of the birds was observed by using the scan sampling technique at 11, 17, 27, 38, 46 and 54 wk of age. Behavior was scored by counting the birds in the pen performing different behaviors according to an ethogram (Table 3). Behavior observations during the rearing period started 30 min after feed was provided (0745 h), while during the laying period observations started 1.5 h before feed was provided (1030 h). During the rearing period (0815, 0900, 0945, 1030, 1115, 1200, 1330 and 1415 h) and laying period (0900, 0945, 1030, 1115, 1200, 1245, 1330 and 1415 h) behavior was scored 8 times at two consecutive days (1 day in phase 2). Feeding and drinking was only recorded when feed and water was available. During the feeding and drinking period, stereotypic object pecking was defined as pecking at the side walls of the pens. When the feed pans and the bell drinker were empty, pecking at these objects were, besides pecking to walls, also scored as stereotypic object pecking.

Table 3. Ethogram showing the behavioral measurements, according to De Jong et al. (2005a).

Behavior	Definition
Feeding	Pecking at feed at the feeding pans
Drinking	Pecking at water at the bell drinker
Standing	Standing without performing other behavior
Sitting	Sitting without performing other behavior
Walking	Walking or running without performing other behavior
Foraging	Pecking, scratching the litter
Comfort	All comfort behavior like, preening, auto pecking, nibbling, stroking, wing flapping, stretching, and dust bathing
Object pecking	Stereotypic pecking at parts of the pen, wall, empty feeding pans, or empty bell drinker
Bird pecking	All pecking at other birds
Laying nest	Oviposition (only during the laying period)

Feather cover. Feather cover score was recorded during the rearing period in a 5-wk interval between 5 and 20 wk of age. During the laying period, feather cover score was recorded at 30, 40, 50, and 60 wk of age. Feather cover was scored from 5 individual birds per pen according to the method described by Bilcik and Keeling (1999). Scores, varying from 0 (intact feathers) to 5 (completely denuded area), were given for each of seven body parts (neck, breast, belly, back, wings, tail, and legs). The average of these seven scores was calculated and also used for statistical analysis.

Statistical analysis

The experiment was set up as a 2 × 3 × 2 factorial design with two 2 CP levels during rearing (2 to 22 wk of age), 3 dietary energy levels during the first phase of lay (22 to 45 wk of age), and 2 energy levels during the second phase of lay (45 to 60 wk of age). From the beginning until the end of the experiment, pen was treated as the experimental unit. The data were analyzed by the REML variance component analysis procedure (Genstat 15, Committee, 2012). Dietary protein level during the rearing, dietary energy level during the first phase of lay, dietary energy level during the second phase of lay and their interactions were used as factors in the statistical model of the data. The statistical model for behavioral traits and feather cover within the different phases included age. Parameters were tested for normal distribution before analysis. After inspection of diagnostic plots of residuals, it was decided to analyze the behavioral variables with a logistic regression model (data expressed as percentages). Data are presented as means ± SEM. None of the three way interactions between the treatments reached statistical significance at any time and, therefore, are not presented in the tables. All statements of significance are based on testing at $P < 0.05$.

RESULTS

Feed intake, eating time, and eating rate

To meet target BW at 22 wk of age, feed intake between 2 and 22 wk of age for CPl birds was increased by 12.8%, compared to the CPh birds, as reported in Chapter 5. Average feed intake at 11 and 17 wk of age was 12.1% higher for the CPl birds compared to the CPh birds resulting in a 2.4 times longer eating time and 47% decreased eating rate (Table 4). Average feed intake at 27 and 36 wk of age was 7.6% lower and 7.7% higher for the MEh1 and MEl1 birds compared to the MEs1 birds, respectively. This resulted in a 21% decreased and 31% increased eating time and 19% increased and 18% decreased eating rate for the MEh1 and MEl1 birds compared to the MEs1 birds, respectively. Birds fed the MEh2 diet received an average 8.8% lower feed intake at 46 and 54 wk of age compared to the MEs2 birds and as a consequence had a 16% decreased eating time and a 9% increased eating rate.

Table 4. Feed intake (g/bird per d), eating time (min/d), and eating rate (g feed/min) of Ross 308 broiler breeders during the different phases.[1]

	Rearing			First phase of lay			Second phase of lay		
Source[2]	Feed intake	Eating time	Eating rate	Feed intake	Eating time	Eating rate	Feed intake	Eating time	Eating rate
Protein rearing									
CPh	80.2[b]	70.7[b]	1.18[a]	157.6	127.2	1.29	145.2	109.9	1.33
CPl	89.9[a]	167.4[a]	0.62[b]	157.7	125.1	1.32	145.1	108.5	1.35
SEM	0.0	0.6	0.01	0.0	1.6	0.02	0.1	0.9	0.01
Energy lay 1									
MEh1	-	-	-	145.6[c]	96.2[c]	1.55[a]	145.2	108.9	1.34
MEs1	-	-	-	157.6[b]	122.4[b]	1.30[b]	145.2	108.2	1.35
MEl1	-	-	-	169.7[a]	159.8[a]	1.07[c]	145.1	110.5	1.32
SEM	-	-	-	0.1	1.9	0.02	0.1	1.1	0.01
Energy lay 2									
MEs2	-	-	-	-	-	-	151.8[a]	119.0[a]	1.28[b]
MEh2	-	-	-	-	-	-	138.5[b]	99.4[b]	1.40[a]
SEM	-	-	-	-	-	-	0.1	0.9	0.01
P-value									
CP	<0.001	<0.001	<0.001	0.396	0.342	0.344	0.673	0.254	0.392
ME1	-	-	-	<0.001	<0.001	<0.001	0.735	0.318	0.346
ME2	-	-	-	-	-	-	<0.001	<0.001	<0.001
CP × ME1	-	-	-	0.143	0.943	0.857	0.351	0.114	0.102
CP × ME2	-	-	-	-	-	-	0.655	0.012	0.017
ME1 × ME2	-	-	-	-	-	-	0.755	0.404	0.449

[a-c]Means within a column and source without a common superscript differ significantly ($P < 0.05$).
[1]Each value represents the mean of 2 observations periods during the rearing period (11 and 17 wk of age), first phase of the laying period (27 and 38 wk of age), and second phase of the laying period (46 and 54 wk of age).
[2]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.

Behavioral observations

Behavior traits per treatment during the rearing period are summarized in Table 5. During the rearing period CPl birds spent 110% more time feeding, 171% more time sitting, 35% more time comfort, and 7% less time standing, 11% less time walking, 24% less time foraging, 70% less time stereotypic object pecking, and 75% less time bird pecking compared to the CPh birds.

Table 5. Behavioral traits (% of time) during the rearing period of Ross 308 broiler breeders fed diets with different crude protein levels during rearing.[1]

Source[2]	Feed[3]	Drink	Stand	Sit	Walk	Forag	Comf	Obj	Bird
Protein rearing									
CPh	10.6[b]	5.1	29.9[a]	5.2[b]	7.0[a]	14.8[a]	5.5[b]	21.5[a]	0.4[a]
CPl	22.3[a]	4.8	27.7[b]	14.1[a]	6.2[b]	11.2[b]	7.4[a]	6.4[b]	0.1[b]
SEM	0.3	0.2	0.4	0.4	0.2	0.4	0.1	0.5	0.0
P-value									
CP	<0.001	0.322	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a-b]Means within a column without a common superscript differ significantly ($P < 0.05$).
[1]Each value represents the average of 2 observation periods (11 and 17 wk of age).
[2]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing.
[3]Feed = pecking at feed at the feeding pans; drink = pecking at water at the bell drinker; stand = standing; sit = sitting; walk = walking; forag = foraging; comf = comfort; obj = stereotypic object pecking; bird = pecking at other birds.

The time spent on the different behavioral traits during the rearing period during the day is shown in Figure 1. Feeding behavior decreased in an exponential manner for the birds fed the CPl and CPh diets with the decrease being more rapid for birds fed the CPh diet. The development of the time spent on drinking, standing, and walking during the day was similar for both treatments. Sitting was somewhat higher at the first observation time and increased substantially for the CPl birds whereas it increased slightly for the CPh birds and remained lower during the day. Birds fed the CPl diet showed less foraging at the first observation time whereas it slightly increased during the day. CPh birds showed more foraging during the first part of the day whereas it was more or less constant during the rest of the day. Comfort behavior was rapidly increase for the CPl and remains on a higher level during the day compared to the CPh birds. For birds fed the CPl diet, stereotypic object pecking slightly increased during the day to a maximum of approximately 10% whereas it substantially and more pronounced increased (to almost 30%) for birds fed the CPh diet. Pecking at other bids was almost zero during the day for the CPl birds whereas CPh birds showed an increase frequency during the day.

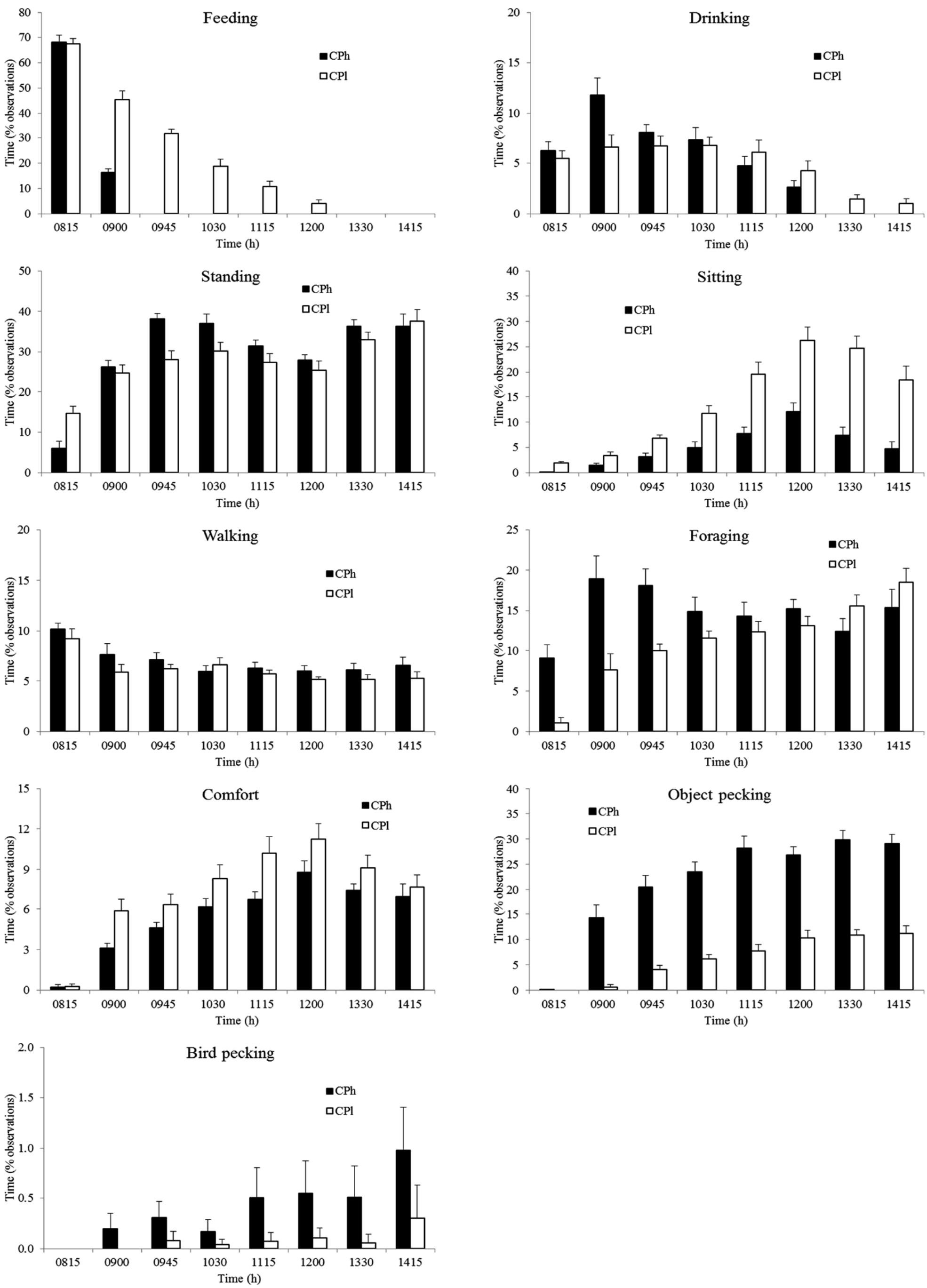

Figure 1. Time (% observations) (mean ± SEM) spent on different behaviors during the rearing period (average of 11 and 17 wk of age) of bird fed diets with different crude protein levels (CPh = high protein; CPl = low protein) in the eight observation periods between 0815 and 1415 h.

No carryover effect of the dietary treatments during the rearing period was observed on behavior during the first phase of the laying period (Table 6). However, during the second phase of the laying period, CPl birds spent 4% less time on feeding and 35% more on sitting than the CPh birds (Table 7).

Birds fed the MEh1 diet showed 17% less feeding, and, as a consequence 19% more sitting, 16% more comfort, and 34% more stereotypic object pecking compared to the birds fed the MEs1 diet (Table 6). Birds fed the MEl1 diet showed 22% more feeding, and, as a consequence 17% less foraging, 11% less comfort, and 31% less stereotypic object pecking compared to the birds fed the MEs1 diet.

Table 6. Behavioral traits (% of time) during the first phase of the laying period of Ross 308 broiler breeders fed diets with different crude protein levels during rearing and energy levels during the first phase of lay.[1]

Source[2]	Feed[3]	Drink	Stand	Sit	Walk	Forag	Comf	Obj	Bird	Nest
Protein rearing										
CPh	26.2	5.9	25.1	7.8	7.5	12.4	8.1	3.1	0.1	3.9
CPl	25.7	6.1	24.7	8.0	7.3	13.2	8.2	2.8	0.0	3.9
SEM	0.5	0.2	0.5	0.3	0.1	0.4	0.2	0.2	0.0	0.1
Energy lay 1										
MEh1	21.2[c]	5.7	24.8	9.2[a]	7.4	14.4[a]	9.3[a]	3.9[a]	0.1	4.0
MEs1	25.5[b]	5.9	25.7	7.7[b]	7.4	13.1[a]	8.0[b]	2.9[b]	0.1	3.6
MEl1	31.1[a]	6.3	24.3	6.9[b]	7.5	10.9[b]	7.1[c]	2.0[c]	0.0	4.0
SEM	0.6	0.3	0.6	0.4	0.2	0.5	0.3	0.2	0.0	0.1
P-value										
CP	0.513	0.407	0.521	0.633	0.354	0.161	0.733	0.265	0.058	0.768
ME1	<0.001	0.354	0.266	<0.001	0.925	<0.001	<0.001	<0.001	0.139	0.063
CP × ME1	0.911	0.620	0.862	0.226	0.167	0.754	0.107	0.780	0.257	0.510

[a-c]Means within a column and treatment without a common superscript differ significantly ($P < 0.05$).
[1]Each value represents the average of 2 observation periods (27 and 38 wk of age).
[2]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay.
[3]Feed = pecking at feed at the feeding pans; drink = pecking at water at the bell drinker; stand = standing; sit = sitting; walk = walking; forag = foraging; comf = comfort; obj = stereotypic object pecking; bird = pecking at other birds; nest = sitting in laying nest.

Feeding birds the MEh2 diet resulted in 11% less feeding, 9% less standing, 43% more foraging, and 7% more comfort behavior compared to birds fed the MEs2 diet (Table 7).

Table 7. Behavioral traits (% of time) during the second phase of the laying period of Ross 308 broiler breeders fed diets with different crude protein levels during rearing and energy levels during the first and second phase of lay.[1]

Source[2]	Feed[3]	Drink	Stand	Sit	Walk	Forag	Comf	Obj	Bird	Nest
Protein rearing										
CPh	25.5[a]	5.4	31.2	3.1[b]	8.1	12.5	7.2	2.7	0.1	4.3
CPl	24.4[b]	5.9	30.4	4.2[a]	8.0	13.5	7.1	2.6	0.1	3.8
SEM	0.4	0.2	0.6	0.3	0.2	0.5	0.2	0.1	0.0	0.2
Energy lay 1										
MEh1	25.0	5.2	30.9	3.3	8.0	12.9	7.5	2.8	0.1	4.3
MEs1	24.3	5.7	30.7	3.7	8.1	13.8	7.1	2.5	0.1	4.0
MEl1	25.5	6.0	31.0	3.9	7.9	12.2	6.9	2.7	0.1	3.9
SEM	0.5	0.3	0.7	0.4	0.3	0.6	0.2	0.1	0.0	0.2
Energy lay 2										
MEs2	26.4[a]	5.8	32.2[a]	3.5	7.9	10.7[b]	6.9[b]	2.5[b]	0.1	4.0
MEh2	23.5[b]	5.4	29.4[b]	3.8	8.2	15.2[a]	7.4[a]	2.8[a]	0.1	4.0
SEM	0.4	0.2	0.6	0.3	0.2	0.5	0.2	0.1	0.0	0.2
P-value										
CP	0.046	0.132	0.360	0.022	0.798	0.193	0.777	0.959	0.860	0.088
ME1	0.202	0.165	0.957	0.567	0.862	0.225	0.202	0.529	0.589	0.514
ME2	<0.001	0.301	0.003	0.426	0.345	<0.001	0.047	0.049	0.126	0.984
CP × ME1	0.628	0.782	0.196	0.821	0.565	0.542	0.061	0.056	0.890	0.540
CP × ME2	0.467	0.674	0.098	0.229	0.958	0.710	0.199	0.310	0.211	0.713
ME1 × ME2	0.341	0.873	0.127	0.369	0.923	0.476	0.018	0.131	0.697	0.579

[a-b]Means within a column and treatment without a common superscript differ significantly ($P < 0.05$).
[1]Each value represents the mean of 2 observation periods (46 and 54 wk of age).
[2]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.
[3]Feed = pecking at feed at the feeding pans; drink = pecking at water at the bell drinker; stand = standing; sit = sitting; walk = walking; forag = foraging; comf = comfort; obj = stereotypic object pecking; bird = pecking at other birds; nest = sitting in laying nest.

Feather cover

In Tables 8 and 9, the average feather cover score of the different body parts are presented as well as scores for the individual parts of the body (if effects were significant). All individual parts of the body, and, as a consequence, average feather cover score during the rearing period of the CPl birds were higher than those of the CPh birds (Table 8). Feather cover score of the wings for the CPl birds was still elevated during the first phase of the laying period, however, this did not result in a higher average feather cover score (Table 9).

Table 8. Feather cover score during the rearing period of Ross 308 broiler breeders fed diets with different crude protein levels during rearing.[1]

Source[2]	Neck	Breast	Belly	Back	Wings	Tail	Legs	Average[3]
Protein rearing								
CPh	1.18[b]	1.50[b]	2.05[b]	0.07[b]	0.93[b]	0.67[b]	2.06[b]	1.21[b]
CPl	1.55[a]	2.03[a]	2.33[a]	0.13[a]	1.51[a]	1.00[a]	2.53[a]	1.58[a]
SEM	0.04	0.03	0.04	0.01	0.03	0.03	0.04	0.02
P-value								
CP	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a-b]Means within a column without a common superscript differ significantly ($P < 0.05$).
[1]Feather cover score ranges from 0 (intact feathers) to 5 (completely denuded area). Each value represents the mean of 4 replicates during the rearing period (at 5, 10, 15, and 20 wk of age).
[2]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing.
[3]Average represents the means of the seven parts of the body.

An interaction effect between dietary protein level and age was found on average feather cover score during the entire rearing period (Figure 2). Average feather cover score of the pullets decreased for both treatments with age and birds fed the CPl diet showed a higher feather cover score during the entire rearing period compared to the CPh birds.

Birds fed the MEh1 diet showed an increased score for the back, legs and average feather cover during the first phase of the laying period compared to the birds fed the MEs1 and MEl1 (Table 9). This effect remains during the second phase of the laying period.

Feather cover score was not affected by dietary treatments during the second phase of the laying period (Table 9).

Table 9. Feather cover score during the first and second phase of the laying period of Ross 308 broiler breeders fed diets with different crude protein levels during rearing and energy levels during the first and second phase of lay.[1]

	First phase of lay				Second phase of lay				
Source[2]	Back	Wings	Legs	Average[3]	Back	Wings	Tail	Legs	Average[3]
Protein rearing									
CPh	1.51	1.51[b]	1.68	1.53	4.48	3.36	3.99[a]	4.53	3.74
CPl	1.37	1.82[a]	1.76	1.61	4.34	3.19	3.78[b]	4.44	3.64
SEM	0.08	0.06	0.06	0.04	0.08	0.07	0.07	0.06	0.06
Energy lay1									
MEh1	1.65[a]	1.78	1.90[a]	1.70[a]	4.64[a]	3.57[a]	4.16[a]	4.69[a]	3.91[a]
MEs1	1.37[b]	1.63	1.68[b]	1.52[b]	4.43[ab]	3.20[b]	3.88[b]	4.54[a]	3.66[b]
MEl1	1.29[b]	1.59	1.58[b]	1.49[b]	4.18[b]	3.06[b]	3.63[c]	4.23[b]	3.50[b]
SEM	0.10	0.07	0.07	0.05	0.09	0.08	0.08	0.08	0.08
Energy lay2									
MEs2	-	-	-	-	4.37	3.27	3.91	4.46	3.66
MEh2	-	-	-	-	4.46	3.28	3.87	4.51	3.72
SEM	-	-	-	-	0.08	0.07	0.07	0.06	0.06
P-value									
CP	0.245	<0.001	0.333	0.206	0.201	0.108	0.028	0.357	0.264
ME1	0.044	0.160	0.014	0.020	0.006	<0.001	<0.001	<0.001	0.003
ME2	-	-	-	-	0.380	0.864	0.679	0.578	0.552
CP × ME1	0.715	0.452	0.987	0.446	0.812	0.507	0.513	0.869	0.583
CP × ME2	-	-	-	-	0.116	0.462	0.120	0.246	0.612
ME1 × ME2	-	-	-	-	0.488	0.767	0.486	0.286	0.656

[a-c]Means within a column and treatment without a common superscript differ significantly ($P < 0.05$).
[1]Feather cover score ranges from 0 (intact feathers) to 5 (completely denuded area). Each value represents the mean of 2 replicates during the first phase of the laying period (at 30 and 40 wk of age) and 2 replicates during the second phase of the laying period (at 50 and 60 wk of age).
[2]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.
[3]Average represents the means of the seven parts of the body.

DISCUSSION

The aim of this study was to evaluate the effects of different dietary protein levels during the rearing period and different dietary energy levels during the first and second phase of the laying period on behavior and feather cover of Ross 308 broiler breeder females.

Effect of dietary protein level during rearing

Providing a low compared to a high protein diet to pullets, resulted in a 12.8% increase in feed and energy intake during the entire rearing period. This is in line with a previous study of Hocking et al. (2002) and the experiment described in Chapter 2. In these studies, they found that providing low protein diets resulted in an 11.1 and 10.0% higher feed intake, respectively. The higher feed intake for the birds fed the low protein diet resulted in a 137% prolonged eating time and a 47% lower eating rate. The effect of a higher feed intake on prolonged eating time and lower eating rate was in agreement with previously reported studies of De Jong et al. (2005b) and the experiment reported in Chapter 3. The remarkable prolonged eating time could not be explained by the relatively small increase in feed intake of approximately 13% only. A reduced passage rate due to a higher daily crude fiber intake (+12%) in the low protein diet may be another additional effect.

As expected, behavioral traits were affected by the prolonged eating time and lower eating rate of the birds fed the CPl diet. These birds spent more time on feeding, sitting and comfort, and less time on standing, walking, foraging, stereotypic object pecking and bird pecking. These results are in close agreement with those of Hocking et al. (2001) and the first experiment (Chapter 3) who found that birds fed low protein diets spent more time resting and less time on stereotypic object pecking.

It can be concluded that birds fed the CPl diet were more tranquil (more sitting and comfort behavior) and less eager to the feed provided (Figure 1). This is maybe an indication that these birds experienced less hunger and frustration than the birds fed the high protein diet. The positive relationship between a higher amount of feed consumed and the time budget for resting was previously found by Hocking (1996) and De Jong et al. (2003). Birds fed the low protein diet spent more time on comfort behavior during all observation periods.

During the observation times, it was observed that when feeding pans were empty, birds directly switched from feeding to foraging and stereotypic object pecking (Figure 1). The relation between feeding and stereotypic object pecking has been noted by Mason et al. (2006) who postulated that for birds that spent more time eating, a smaller time budget remains for exhibiting stereotypic object pecking. Moreover, Hocking et al. (2004) suggested that a higher feed intake results in more feeding and less pecking behavior which might improve the welfare of the birds.

The reduced activity (walking and foraging) and moreover the 3.4 times lower stereotypic pecking behavior of the birds fed the CPl diet in the current study are important indicators of reduced hunger and frustration of the eating motivation (Savory and Maros, 1993; Hocking et al., 1996; Savory et al., 1996; Savory and Kostal, 1996). Moreover, these findings are potential indicators of an improved welfare.

Pullets fed the CPl diet had a poor feather cover during the entire rearing period (Figure 2). The effect of daily protein intake on feather growth has been shown by Twining et al. (1976). Feeding broilers a high protein diet results in an increased number of feathers on the litter indicating a fast feather development and molting. It could be suggested that the levels of the sulfur-containing amino acids methionine and cystine (essential for feather growth) in the current study were marginal in the CPl diet. Moran (1984) explained that marginal dietary levels of sulfur-containing amino acids resulted in reduced and abnormal feathering. When using a low protein diet in pullet rearing practice, it is therefore suggested to, in particularly increase methionine and cystine levels of the starter-2 diet to prevent disturbed feather growth and abnormal feather shape.

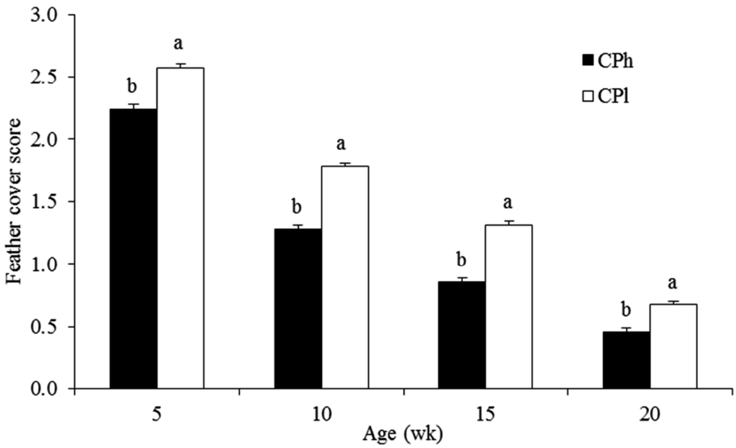

Figure 2. Feather cover score (means and ± SEM) during rearing for pullets reared on a high (CPh) or low (CPl) crude protein diet. Feather cover was scored from 0 (intact feathers) to 5 (complete denuded area) for each of 7 different body parts (neck, breast, belly, back, wings, tail, and legs) and averaged to a total feather cover score per bird (Bilcik and Keeling, 1999).
[a,b]Different letters within age indicate significance differences among treatments ($P < 0.05$).

Effect of dietary energy level during the first phase of lay

Feed intake on the different dietary energy levels was adjusted to obtain similar weekly BW gains. Feeding birds a 7.1% higher dietary energy level compared to a standard diet resulted in a 7.5% lower feed intake. Conversely, feeding birds a 7.1% lower dietary energy level compared to the standard diet resulted in a 7.7% higher feed intake (Chapter 5). These finding are in close agreement with Sun and Coon (2005), Enting et al. (2007) and Pishnamazi et al. (2011). The result from the current study confirms that body weight development of broiler breeders is primarily affected by daily energy intake.

A higher feed intake of the birds on the low energy diet increased eating time whereas eating rate was decreased. Conversely, a lower feed intake of the birds on the high energy diet decreased eating time whereas eating rate was increased. It is, therefore, suggested that not diet composition as such but the amount of feed consumed is the primary factor for regulating eating time and eating rate.

The different feed intake and eating time resulted, as expected, in differences in time spent on different behaviors. During the first phase of the laying period differences in behavior time budget, except feeding, was less pronounced than during the rearing period. Feeding birds the low compared to the high energy diet resulted in 1.5 times more feed related behaviors. As such the time spent on other behaviors was therefore also affected. When birds spent more time on a certain behavior, less time is available to exhibit other behaviors as postulated by Mason et al. (2006). This is probably the explanation for the increased time spent on sitting, foraging and comfort behavior of the birds fed the high energy diet.

Remarkable was that birds fed the low energy diet, with a feed intake of almost 170 g/bird per d showed, on average, 2.0% stereotypic object pecking. This could be explained by the relative short eating time of approximately 160 min per d (Table 4) leaving enough time to express other behaviors as suggested by Hocking et al. (2004) and Mason et al. (2006). More reasonable, this phenomenon might be explained by the fact that this acquired behavior was programmed during the rearing period. Another interesting issue was that stereotypic object pecking during the rearing period was, on average, for all birds 14% while this was 3% during the first phase of the laying period. The reason for this reduction is due to the difference in feed restriction level between the two phases of life of breeders. In the current study, feed intake during the rearing and laying period at the time observations moments were made was, on average, 85 and 158 g/bird per d, respectively. It has been previously shown that during the rearing period feed intake of broiler breeders is restricted to 25-33% of *ad libitum* intake (Savory et al., 1996; De Jong et al., 2002) while this is 50-90% during the laying period (Bruggeman et al., 1999). This means that during the rearing period the eagerness to feed and thereby the eating rate of the birds will be at a higher level resulting in a shorter eating time and more hunger and frustration which resulted in more stereotypic object pecking.

Providing the high energy diet during the first phase of the laying period resulted in a poor feather cover score during the entire laying period compared to the standard and low energy diet. The reason for this difference is the approximately 10% lower daily crude protein or more reasonable the 11% lower daily methionine and cysteine intake. It was shown by Stilborn et al. (1997) that feathers contain high concentrations of protein and amino acids especially sulfur-containing amino acids. Moreover, dietary concentrations of these nutrients may affect feather quality and development (Leeson and Walsh, 2004). This finding implies that also a low daily intake of methionine and cysteine during the laying period can negatively affect feather cover which also can remain during the second phase of the laying period.

The average feather cover score of all birds increased rapidly from 0.8 to 2.3 (+188%) between 27 and 38 wk of age (data not shown) while in Chapter 3 a less pronounced effect from 1.1 to 1.7 (+55%) was observed between 30 and 40 wk of age. The discrepancies between the current and the previous experiment may be due to the absence of males and the more feeding space in the previous study.

Effect of dietary energy level during the second phase of lay

Birds fed the high, compared to the standard, energy diet during the second phase of the laying period showed quite the same decreased effect on feed intake as was found during the first phase of the laying period (high vs. standard dietary energy phase 1). These birds also showed more or less the same effects on eating time, eating rate, and different behavior. Thus, feeding birds a high energy diet during the first and second phase of the laying period resulted in less feeding and more foraging, comfort, and stereotypic object pecking.

Contrary to expectations, stereotypic object pecking was on the same level in the second compared to the first phase of the laying period. It was expected that stereotypic object pecking would diminish or disappear when birds become older. On the other hand, feed intake decreased, on average, from 158 to 145 g/bird per d for the first and second phase, respectively, resulting in a decreased average eating time from 130 to 109 min leaving more time for expression of other behaviors. It is therefore suggested that stereotypic object pecking behavior was programmed during the earlier life.

It was observed, that while birds age, different dietary energy levels, and thereby different daily protein intake, did not affect feather cover. This is in contrast with the findings during the first phase of the laying period when birds were fed a high energy diet and as a consequence a low daily protein intake, resulted in a poorer feather cover. It can be speculated that differences between treatments seems to be masked due to the fact that broiler breeder birds, especially at the back, tail, and legs, at older age were almost bold.

CONCLUSION

Providing a low protein diet during rearing improved breeder pullet welfare during the rearing period, as showed by increased sitting and comfort behavior. Moreover these birds showed less stereotypic object pecking, which is a major indicator of reduced hunger and frustration. Despite the positive effects on behavior, feather cover was negatively affected by a low protein diet which can result in negative effects on welfare due to the fact that birds are less protected. A high energy diet fed to birds during the first and second phase of the laying period resulted in a decreased feed intake and eating time and increased eating rate. More or less similar effects during the first and second phase of the laying period were found on behavior. In general, a higher energy diet resulted in less feeding and more foraging, comfort associated behaviors, and stereotypic object pecking indicating that breeder welfare was impaired. On the other hand, a low energy diet during the first phase of the laying period showed opposite effects indicating that breeder welfare was slightly improved.

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Chapter 7

General discussion

INTRODUCTION

Poultry meat is one of the most important protein sources in human diet and production is worldwide growing. Global poultry meat production in 2000 was 69 million tons and this increased to over 97 million tons in 2010 (Windhorst, 2011). This equates to an annual production of approximately 70 billion broilers originating from approximately 600 million broiler breeders. So a relatively small number of broiler breeders has a major impact on the poultry meat chain and optimizing management of breeders will have benefits for the total chain. Broiler breeders need to produce first class and healthy chicks (Zuidhof et al., 2007). Due to the continuing increase in the genetic potential of the offspring (e.g., Havenstein et al., 2003a,b; Renema et al., 2007b) this is becoming increasingly challenging.

In the past, obesity, mainly in the second phase of the laying period, was a major problem in broiler breeder flocks and resulted often in a decreased reproduction rate during the laying period (Bornstein et al., 1984; Leclercq et al., 1985; Cahanar et al., 1986; Robinson et al., 1993). Overweight hens have sperm storage problems (due to the fat deposition in the sperm storage glands) and physical problems during the cloacal contact during natural mating (Mc Daniel et al., 1981). The body composition of breeders, however, has changed dramatically during the last five to six decades (Havenstein et al., 2003a; De Beer, 2009). In modern broiler breeders, obesity is not an issue anymore, due to the selection of strains with increased breast muscle and decreased fat pad deposition characteristics (Havenstein et al., 2003a). The selection for increased feed efficiency, growth rate and body fat content has not only affected the offspring but also the parent stock. This was recently confirmed by Eitan et al. (2014) who compared a 1980 to a 2000 breeder strain. The 2000 strain contained 42% more breast meat (21.2 vs. 14.9% of BW) and 50% smaller abdominal fat pad (2.7 vs. 5.4% of BW) compared to the 1980 strain.

Feeding high yield breeders high levels of amino acids (e.g. lysine) will lead to more muscle production and this extra muscle requires more energy to maintain (De Beer, 2009). Therefore, during the last decade several researchers have reported that broiler breeders need a certain proportion of body fat at the onset of lay for subsequent reproductive performance (Sun and Coon, 2005; De Beer, 2009; Mba et al., 2010). Because tissue growth is directly affected by dietary nutrient composition, a nutritional approach to this topic was highly relevant. Therefore, the overall practical objective of the present study was to develop new feeding strategies during the rearing and laying period for broiler breeders in order to alter body composition with positive effects on reproduction, offspring and welfare, for a more sustainable approach of broiler breeder production.

IMPACT OF FEEDING STRATEGIES DURING REARING ON BODY COMPOSITION

Changes in feed allowance or a change in diet composition (energy and/or protein levels) have been used as generally applied dietary interventions. Several authors have evaluated the use of a change in feed allowances on body composition during rearing (Fattori et al., 1993; Renema et al., 2001a; Robinson et al., 2007) or laying (Bornstein et al., 1984; Bowmaker and Gous, 1989; Renema et al., 2001b). Other studies evaluated the effects of a change in diet composition on body composition during rearing (Miles et al., 1997; Hudson et al., 2000; Mba et al., 2010) or laying (Pearson and Herron, 1981; Spratt and Leeson, 1987). The combination of different feed allocations and different dietary protein levels in a single trial during the rearing period and its effects on body composition has received limited attention with the exception of a trial by Hocking et al. (2002). Such an experiment has been the focus of Chapter 2, however, no interactions of the different feeding strategies on body composition were found. Moreover, differences in dietary protein levels during the rearing period were more effective than modifying the growth pattern by different feed allocations in changing body composition. This was probably due to the rather small differences (8%) in BW between treatments at the end of the rearing period as described in Chapter 2. For example, Renema et al. (2001a) did not find an effect of an 11% higher BW, while a 21% higher BW increased abdominal fat content at the end of the rearing period. On the other hand, feeding broiler breeders to a 20% higher BW (2,640 vs. 2,200 g) at the end of rearing is relatively beyond practical conditions. From the results of the first experiment (Chapters 2, 3 and 4), it was decided to use dietary protein level as treatment during the rearing period in the second experiment (Chapters 5 and 6) instead of different growth patterns.

A, on average, 16% lower dietary CP during the rearing period in the studies reported in Chapters 2 and 5 resulted in a decreased breast meat and increased abdominal fat pad content at 10 wk of age (recorded only in Chapter 2) and at the end of rearing (20 and 22 wk of age as reported in Chapters 2 and 5, respectively). This was in close agreement with Mba et al. (2010) who found the same effects of a 12.5% reduction in crude protein content of the diet (14 vs. 16%) on body composition at 12 and 23 wk of age. In fact, not the dietary crude protein or amino acid content influenced body composition, but the differences in daily or total intake of the macro nutrients. On average, in both experiments of this thesis, the 16% lower protein diets (low vs. high protein diet) resulted in a 11% higher total energy, 5% lower total crude protein, and 7% lower total amino acid intake during the rearing period. Results of the experiment described in

Chapter 2, for example, showed a clear relationship between total energy intake and abdominal fat pad (*P* < 0.001; Figure 1).

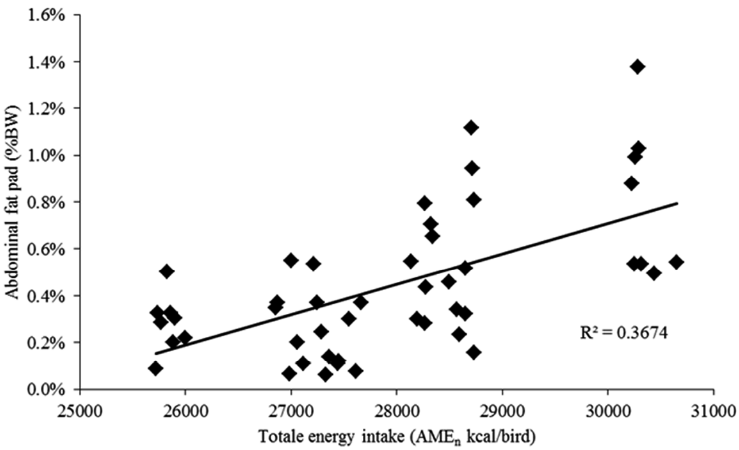

Figure 1. Relationship between total energy intake (AME_n kcal/bird) during the rearing period (2-22 wk of age) and abdominal fat pad (% BW), based on the results in Chapter 2. Each point in the graph is an individual pen.

Average breast muscle (18.8 vs. 17.2%) and abdominal fat pad (1.0 vs. 0.4%) content at the end of the rearing period of all birds was relative higher in the study reported in Chapter 5 than the study reported in Chapter 2. The differences between the experiments in breast muscle content might be explained by the differences in the higher total dietary protein and moreover total digestible lysine intake (+4.5%) in the second compared to the first experiment. Particularly dietary lysine is known as the major essential amino acid for breast muscle deposition in broilers and thus also for broiler breeders (Leeson and Summers, 2005).

The abdominal fat pad content roughly doubled in the study reported in Chapter 5 compared to the study reported in Chapter 2 could be explained by two different factors. Firstly, body composition at the end of the rearing period was determined at 20 (Chapter 2) and 22 (Chapter 5) wk of age. In this pullet to breeder transition period, body composition or moreover fat content of the body changes dramatically (Figure 2). Secondly, the differences could be explained by the, on average, 4.5% higher cumulative energy intake in the study reported in Chapter 5.

At 15 wk of age, no effects of dietary protein level on abdominal fat pad (% BW) were found while this was present at wk 10 (Chapter 2). This phenomenon was also reported by Mba et al. (2010) who observed a difference in abdominal fat pad affected by differences in dietary protein level at wk 12 while this disappeared at 19 wk of age. It seems that abdominal fat pad and fat contents of the body follows a specific pattern during rearing with ageing. This pattern in body composition was previously reported by Bennet and Leeson (1990) who found a decreased total fat content between 2 and 14 wk of age but an increased fat content between 14 and 24 wk of age. Combining the data presented here and those of other authors yields a quadratic relationship between age and abdominal fat pad content (% BW) during the rearing and pullet to breeder transition period ($P < 0.001$; Figure 2).

The decreased abdominal fat pad weight around 12 wk of age is caused by the severe feed restriction levels (67 to 75%) between 7 and 16 wk of age, as described by De Jong and Guéméne (2011). It is likely that due to the severe feed restriction program during the midterm phase of rearing, pullets are required to use body (fat) reserves to meet energy requirements. This explains that the fat content of the body decreased during the severe feed restriction period while it increased again when energy intake increases substantially after 15 wk of age.

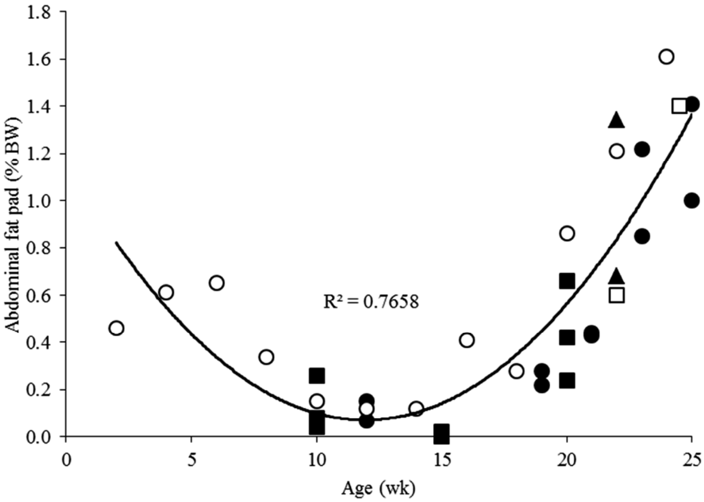

Figure 2. Relationship between age (wk) and abdominal fat pad content (% BW) during the rearing and pullet to breeder transition period (□ Sun et al., 2006; ○ Robinson et al., 2007; ● Mba et al., 2010; ■ Chapter 2; ▲Chapter 5).

IMPACT OF BODY COMPOSITION AT END OF REARING ON BREEDER TRAITS

Breeder performance

In Chapter 5, it was concluded that the higher abdominal fat pad weight and lower breast muscle content of the body at the end of the rearing period positively affected the number of settable eggs during the second phase of lay. When the data of Chapter 5 were used to analyze the relationship between breast muscle and abdominal fat pad content at 22 wk of age on total number of settable eggs, this conclusion has to be refined (Figure 3). These graphs show that the number of settable eggs during the second phase of lay was affected by abdominal fat pad content ($P < 0.001$; panel D) and not by breast muscle content ($P = 0.154$; panel C). It also shows that either breast muscle ($P = 0.529$; panel A) or abdominal fat pad content ($P = 0.115$; panel B) at the end of the rearing period did not affect the number of settable eggs during the first phase of lay.

The results of the experiment described in Chapter 5 confirm the hypothesis that, pullets with an altered body composition at the end of rearing can be more persistent, resulting in an improved egg production during the second phase (45 to 60 wk of age) of lay. Particularly abdominal fat pad is the cause of this difference while breast muscle content has no effect on persistency of lay. Breeders with a higher body fat content are probably more able to mobilize energy reserves during periods of a negative energy balance (Renema et al., 2013) which may prevent them start molting. On the other hand, breeders with a low body fat content lack energy to meet their energy requirements if dietary energy intake is limited and, thereby, may lost BW over time that finally initiate molting as part of a natural process. It is observed that natural molting starts with a voluntary reduction in food intake resulting in an approximately 20% BW loss (Mrosovsky and Sherry, 1980).

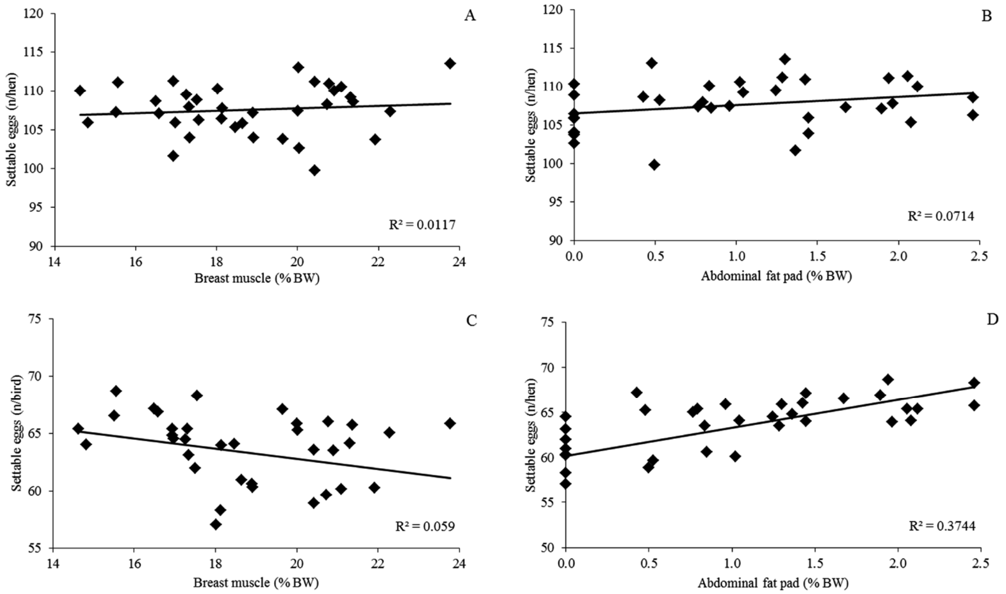

Figure 3. Relationship between breast muscle (panel A and C) or abdominal fat pad content (panel B and D) at 22 wk of age on settable eggs (n/hen) during the first phase of lay (panel A and B) or second phase of lay (panel C and D), based on the results in Chapter 5. Each point in the graphs is an individual pen.

In contrast to expectations, the differences in abdominal fat pad at the end of the rearing period, due to the different dietary protein levels, was not found at 35 wk of age. A possible explanation could be that only abdominal fat and not total body fat content was measured. At the end of the rearing period, the total body fat content of broiler breeders is around 10% (Miles et al., 1997; Renema et al., 2001a) whereas abdominal fat pad is a small portion (about 15%) of total body fat content (Joseph et al., 2000; Renema et al., 2001a,b; Renema et al., 2007a). Research with broilers showed that 85% of total body fat is stored in adipose tissue (subcutaneous, intramuscular, abdominal fat, etc.) as energy reserve (Evans, 1977). It is hypothesized, that in the pullets fed the low protein diets in the present work contained a higher proportion of easier mobilizing abdominal fat (Ricard et al., 1983; Becker et al., 1984) so that they metabolized more abdominal than subcutaneous and intramuscular fat compared to the birds fed the high protein diets during the initial laying period. This may have resulted in a diminished effect on abdominal fat pad after peak production for both treatments but probably more pronounced for the birds fed the high protein diet. It can also be speculated that while ageing, body fat reserves, and thereby BW, of individual birds can decrease below a certain critical level so that these birds spontaneously start molting. Unfortunately, during the experiments, the number of molted birds was not recorded to confirm this hypothesis.

No effect of differences in body composition at the end of the rearing period on breeder performance was found during the first phase of the laying period (Chapters 2 and 5). This was probably caused by the fact that even modern lean breeders have enough body fat reserve to overcome this severe period of negative energy balance due to the increase in BW and high egg production.

It can be concluded that feeding a low protein diet during rearing, results in more abdominal fat pad reserves, which seems to have a positive effect on persistency of lay.

Mortality

Hocking et al. (2002) indicated that diets with a low CP (10%) content between 15 and 18 wk of age could result in a doubling of mortality during lay. In line with these results, mortality of pullets fed the low protein diet during rearing in Chapter 5 showed a tendency to an increased mortality over the entire laying period (6.3 vs. 8.1%; $P = 0.079$). It is hypothesized that feeding low protein levels during the rearing period may negatively affect the immune system due to the indispensable need for certain amino acids (arginine, glutamine, and cysteine) for the development of the immune system (Kidd, 2004).

Incubation traits

A higher BW of pullets at the end of the rearing period improved incubations traits of 29 wk old breeders while these effects disappeared while ageing (Chapter 4). It is postulated that there was a synergistic effect between BW and fat content on incubation traits. The combination of an 8% higher BW and a 44% higher abdominal fat pad content of the HGP breeders at the end of the rearing period compared to the SGP breeders probably caused the positive effect on early incubation.

The largely improved fertility at 29 wk of age disappeared at 33 and 37 wk of age, which was probably caused by smaller differences in BW and body fat content between breeders with these growth patterns when they aged. At 33 and 37 wk of age, the difference in BW between the two treatments in the current study was halved compared to the difference at 29 wk of age. Moreover, the difference in abdominal fat pad content had totally disappeared at 40 wk of age (Chapter 2).

In Chapter 5, it was postulated that differences in late embryonic mortality during the first phase of lay between birds fed different dietary protein levels during the rearing period was caused by differences in body composition at the onset of lay. Data of Chapter 5 were used to analyze the relationship between breast muscle and abdominal fat pad content of the body at 22 wk of age on embryonic mortality during the first of the laying period (Figure 4). No effect on embryonic mortality was found during the second phase of lay. These graphs show that particularly abdominal fat pad content (P = 0.003; panel B) and not breast muscle content (P = 0.269; panel A) at the end of the rearing period seems to be an important factor to affect embryonic mortality during the first phase of lay. Thus a higher abdominal fat pad content at the end of rearing resulted in a decreased embryonic mortality. A sound explanation for this phenomenon may be that egg composition might be affected by the differences in abdominal fat pad content of the body.

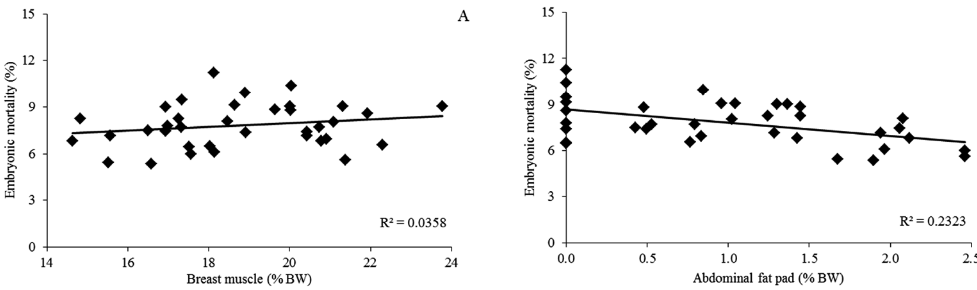

Figure 4. Relationship between breast muscle content (panel A) or abdominal fat pad content (panel B) at 22 wk of age on embryonic mortality (%) during the first phase of lay, based on the results in Chapter 5. Each point in the graphs is an individual pen.

Offspring performance

From the results of Chapter 4, it was concluded that the combination of a higher target BW and increased abdominal fat pad content of the body of pullets at the end of the rearing period was slightly positive for the offspring BW gain. On the other hand, a changed body composition of pullets at the end of the rearing period by decreasing dietary protein levels showed no benefits on BW gain (Chapter 4). No comparable studies in the literature are available with regards to the effect of a different target BW at the end of the rearing period or different dietary protein levels fed to breeder pullets on offspring performance.

In Chapter 4 an interaction effect between sex of the offspring and dietary protein level of the breeder pullets on offspring breast meat gain was observed. Male broilers from breeders fed the low protein diet had higher breast meat gain than male broilers from breeders fed the high protein diet, while breast meat gain of female broilers was not affected by dietary protein levels. It was suggested that this effect was caused by differences in protein deposition between the sexes. Feeding breeders a low protein diet during the rearing period may cause a gene-expression for maximum protein efficiency which is transferred to the offspring. This suggesting was underlined by the results of Rao et al. (2009) who found that offspring of Langshan breeders fed 10 vs. 15% CP diets had higher breast meat content at d 28. This finding and the results on breast meat gain (% of BW; males and females) of Moraes et al. (2011) who fed breeder pullets different energy and protein levels all show that feeding different levels of macronutrients to pullets, resulting in differences in body composition, can alter offspring processing yields.

Although, the underlying mechanisms behind these effects are still unknown, they may be related to metabolic changes by altering gene expression as suggested by (Rao et al., 2009; Choi and Friso, 2010). For example, as shown by Heijmans et al. (2008) and Rao et al. (2009), strong epigenetic effects on the offspring were found when humans or breeders, respectively, where exposed to suboptimal nutrition. It is therefore recommended that these effects need to be further studied in order to understand the physiological processes. This also will aid the feed industry in optimizing pullet and breeder diets for maximum performance of pullets, breeders and broilers, potentially differentiation to sexes.

IMPACT OF FEEDING STRATEGIES DURING REARING ON REARING TRAITS

Feed intake, eating time, and eating rate

Either a higher feed allowance or a decreased dietary protein level during the rearing period, resulted in an increased feed intake, prolonged eating time and decreased eating rate (Table 1). A prolonged eating time and decreased eating rate caused by a higher feed intake was previously observed in broiler breeders (De Jong et al., 2005b) and layers (Van Krimpen et al., 2008). The effect of a higher feed intake resulting in a prolonged eating time was expected. But unexpected was the additional increased eating time for the low compared to the high protein diets. While feed intake was 10 (Chapter 3) to 12% (Chapter 6) higher for the low compared to the high protein diet eating time was remarkably prolonged (163 to 237%, respectively). This large difference in eating time between the low and high CP diets may be due to the combination of the following factors:

1. The higher feed intake for birds fed the low CP diets resulted in an earlier physical gut/crop fill resulting in a slower eating rate.
2. The low protein diets contained more crude fiber resulting in a higher degree of satiety, increasing eating time as previously reported by Van Krimpen et al. (2007) with layers.
3. It was observed, during behavioral observations, that birds fed the low protein diets were less eager and less aggressive to the feed and showed earlier resting after feed distribution (Chapter 6).

The differences in eating time and eating rate were more pronounced for the dietary than the BW treatments (Chapter 3). This supports the suggestion of the previous paragraph that the contrast in BW (200 g) between the high and the low growth pattern probably was not sufficiently large to provide significant differences.

The effect on eating time and eating rate of the low compared to the high protein diet during rearing was more pronounced in Chapter 6 than in Chapter 3 (Table 1). This was in the first place due to the differences in feed intake between both treatments at the moments of observation in Chapter 3 (10%) and Chapter 6 (12%). In the second place, pen size probably played a role in the differences between the two experiments. Pen size was in the first experiment 1.35 m^2 (Chapter 3) and 11.25 m^2 (Chapter 6) in the second, provoking different feeding behavior and walking distances between feeding and water systems. The observations in the current study on the relationship between feed intake, eating time and eating rate are in agreement with De Jong et al. (2005b). Breeders in that experiment, however, were fed different diluted diets (Table 1). In conclusion, eating time and eating rate are directly affected by the amount of feed provided to pullets.

Table 1. Effect of dietary treatment on feed intake (g/bird per d), eating time (min/d) and eating rate (g feed/min) during rearing.

Exp.	Treat-ment	Age (wk)[1]	Feed intake (g/b/d)	Feed intake (%)	Eating time (min/d)	Eating time (%)	Eating rate (g/min)	Eating rate (%)	Reference
BW	2,200	7, 12, 17	69.2	100	111	100	0.77	100	Chapter 3
(g)	2,400		74.9	108	131	118	0.71	92	
CP[2]	15.3	7, 12, 17	68.7	100	94	100	0.83	100	Chapter 3
(%)	14.2		72.0	105	115	122	0.76	91	
	13.2		75.5	110	153	163	0.62	74	
CP[2]	15.1	11, 17	80.2	100	71	100	1.14	100	Chapter 6
(%)	13.0		89.9	112	167	237	0.54	47	
AME_n[3]	11.7	6, 10, 13,	61.0	100	37	100	1.63	100	De Jong et al.,
(MJ/kg)	10.3[4]	16, 20	69.4	114	54	144	1.29	79	2005b
	9.0		76.6	126	78	209	0.98	60	

[1]Age at which observations were taken.
[2]Weighted average of different phases.
[3]Recalculated to AME_n.
[4]Average from two treatments. In the original papers diets, were diluted with different ingredients.

Behavior and physiological parameters

The results from the studies reported in Chapters 3 and 6 shows that the largest increase in feed intake (10-12%) was realized when birds were fed the low protein diets (Table 1). Such increased feed intakes and prolonged eating times positively affected welfare-related behavior during the rearing period. Feeding birds a low protein diet resulted in both experiments in more time spent on feeding and sitting and resultantly less stereotypic object pecking and bird pecking (Chapters 3 and 6). Particularly stereotypic object pecking is mentioned as an important indicator of hunger and frustration in broiler breeders (Savory et al., 1996; Savory and Kostal, 1996; De Jong et al., 2005b). Data of Chapter 3 (7, 12, and 17 wk of age) was used to analyze the linear relationship between feeding and stereotypic object pecking during the rearing period (Figure 5). It clearly shows that when pullets spent more time on feeding, they spent less time on stereotypic object pecking ($P < 0.001$) at different ages during the rearing period. This graph also shows that this effect is independent of age, however, the absolute level of stereotypic object pecking increased while ageing which is directly correlated with increased feed restriction.

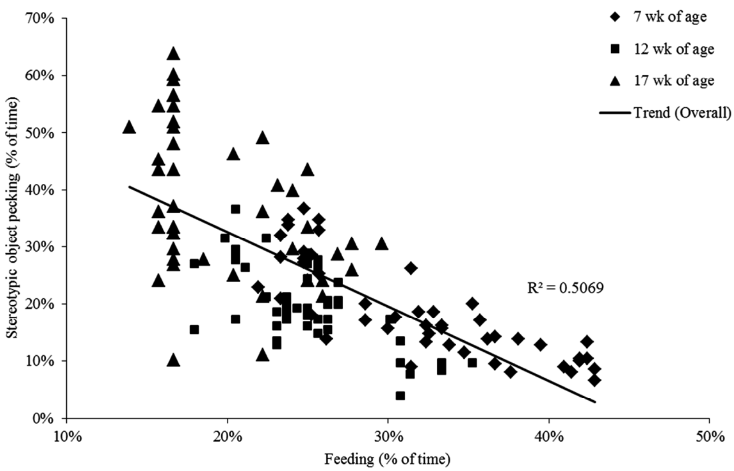

Figure 5. Relationship between feeding (% of time) and stereotypic object pecking (% of time), based on the results of 7, 12, and 17 wk of age in Chapter 3. Each point in the graph is an individual pen.

Previously, Mason et al. (2006) postulated that birds which spent more time feeding will spent less time on stereotypic object pecking. This phenomenon of exchange of behavior could be explained by the 'law of communicating vessels' and this principle is clearly shown in Figure 6. This graph shows the development of feeding and stereotypic object pecking during the day during rearing (Chapter 6). Pullets fed the low compared to the high protein diet showed a prolonged feeding behavior which reduced and delayed stereotypic object pecking. This exchange in behavior was also observed during the rearing period in Chapter 3. It can be concluded that feeding pullets a low protein diet can improve welfare due to the decreased time spent on stereotypic object pecking.

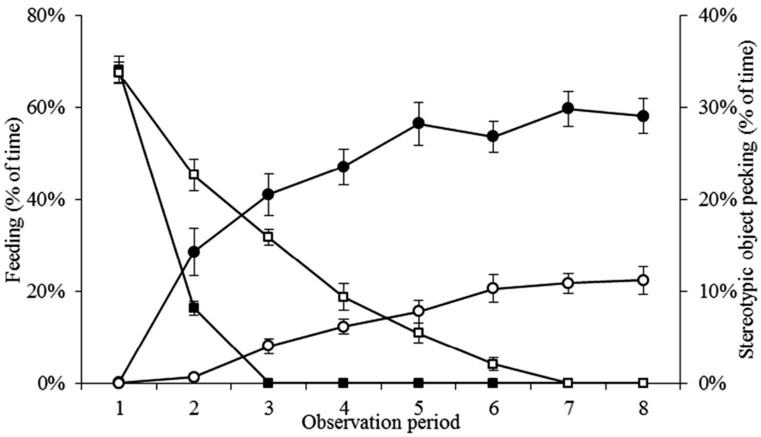

Figure 6. Development of feeding and stereotypic object pecking (% of time) during the day based on the results in Chapter 6 during the rearing period (■ feeding on the high protein diet; □ feeding on the low protein diet; ● stereotypic object pecking on the high protein diet; ○ stereotypic object pecking on the low protein diet).

It could be expected that birds fed the low protein diet also showed decreased plasma corticosterone concentrations. These profiles were measured in the study reported in Chapter 3. The different feeding strategies, however, did not resulted in changes in plasma corticosterone concentrations. It is suggested that modern broiler breeders are less responsive with respect to changes in plasma corticosterone concentrations to differences in feed allowance than breeders decades ago. On average, plasma corticosterone concentrations in the current study were about five times higher than in the previous studies reported by De Jong et al. (2003, 2005b). High plasma corticosterone concentrations were also observed by Nielsen et al. (2011) with breeder pullets at wk 15. This increased plasma corticosterone concentration may be a result of the increased growth potential of broilers and breeders (Havenstein et al., 2003a,b; Renema et al., 2007b).

Feather cover

Feathers are high in protein and amino acids (Stilborn et al., 1997), especially the sulfur-containing amino acids methionine and cysteine which are needed for the synthesis of feather keratin (Wheeler and Latshaw, 1981). Feather cover development is not well described in broiler breeder nutrition research. No effects were found when birds were fed following a higher growth pattern during the rearing period (Chapter 3). A low protein diet during the rearing period, however, had a negative effect on feather cover quality (Chapters 3 and 6). In Chapter 3, feather cover was inferior on the low protein diet at 6 and 11 wk of age while this difference disappeared from 16 wk of age onward. In Chapter 6, feather coverage was inferior on the low protein diet during the entire rearing period. It is, therefore, suggested that the protein and amino acid levels of the diets in the studies here were critical or deficient, in particular those amino acids needed for feather growth and development. The effect of daily protein intake on feather growth in broilers was previously reported by Twining et al. (1976), Aktara et al. (1996), Melo et al. (1999) and Urdaneta-Rincon and Leeson (2004). This suggestion was underlined by the malformed cover feathers on the wings in the current study what might be an indication of amino acids deficiency. Moran (1984) already showed that marginal dietary deficiencies of sulfur containing amino acids resulted in abnormal feathering. Data of Chapter 3 were used to analyze the linear relationships between the total crude protein intake at different phases during the rearing period on feather cover score (Figure 7). These graphs show that the effect of a low total CP intake on feather cover score was much more pronounced between 2 and 6 wk of age ($P < 0.001$; panel A) than between 6 and 15 wk of age ($P = 0.182$; panel B). It is, therefore, important to conclude that total CP (and AA) is a critical factor in development of feathers cover during rearing till approximately 6 wk of age.

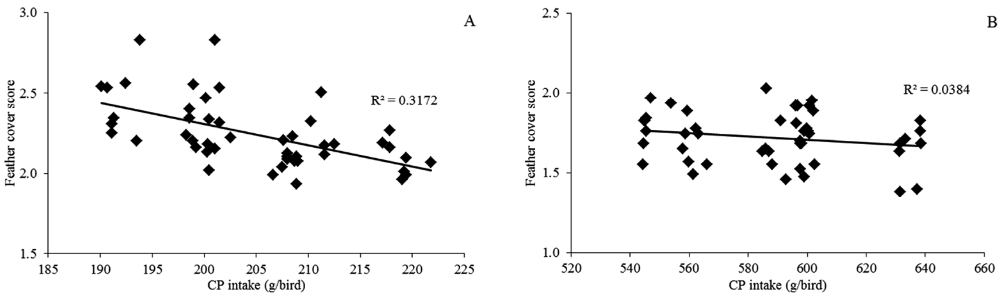

Figure 7. Relationship between the total crude protein (CP) intake (g/bird) between 2 to 6 wk of age (starter 2 diet; panel A) or between 6 to 15 wk of age (grower diet; panel B) and feather cover scores at 6 wk of age (panel A) or at 16 wk of age (panel B), based on the results in Chapter 3. Each point in the graphs is an individual pen.

IMPACT OF FEEDING STRATEGIES DURING LAY ON BREEDER TRAITS

Body composition

Different dietary energy levels during the laying period did not significantly affect body composition at 35 and 59 wk of age (Chapter 5). The reason for the lack of an effect could be explained by a similar daily energy intake among the different treatments to achieve the same target BW. This may explain the absence of an effect on abdominal fat pad content. The absence of a difference in breast muscle content was much more surprising because in the different feeding strategies, daily protein intake differed (e.g., high energy diet is low daily protein intake). It could be hypothesized that breeders during the laying period prioritize offspring above their own.

Breeder performance

The different feeding strategies during the laying period in Chapter 5 did not affect breeder performance in the second phase, however, the high and low energy diet during the first phase resulted in a slightly lower number of eggs. It is not really clear what caused the difference in egg production during this laying phase. It was observed that a high energy diet resulted in a decreased feed intake and decreased eating time (Chapter 6). The most aggressive breeders ate a larger amount of feed resulting in decreased flock uniformity (Renema et al., 2013). A less uniform flock will reach peak production somewhat later caused by the larger variation in sexual development of individual birds (Laughlin, 2009). However uniformity during initial lay was not recorded during the current study, the more than 5 d delayed peak egg production (Chapter 5) for the birds fed the high energy diet is an indication of a decreased uniformity.

It could also be hypothesized that a low energy diet (and thus high daily protein intake) resulted in more breast muscle deposition. This might increase the daily energy requirement for maintenance and decreases, therefore, the amount of energy that remains for egg production (Ekmay et al., 2013).

In general, egg weight is affected by daily dietary protein and amino acids intake (Lopez and Leeson, 1995a; Fisher, 1998; Joseph et al., 2000). More specific, a higher egg weight is caused by a higher daily intake of sulfur amino acids (effect on albumen and yolk) and/or higher daily intake of linoleic acid (effect on yolk). This was confirmed in the current study in the second phase of the laying period when daily amino acids and linoleic acid were increased (Chapter 5). In the first phase of lay, the higher daily intake of amino acids was compensated by the lower daily linoleic acid intake potentially resulting in similar egg weights during that phase.

Total mortality during the entire laying period was increased when breeders were fed the high (9.4%) compared to the standard (6.5%) and low (5.7%) energy diets during the first phase of lay (Chapter 5). The majority (on average, 67% of the different treatments) of the total mortality was due to ruptures of the gastrocnemius tendons. The weekly and cumulative mortality due to ruptures of the tendons and other causes, and total mortality are presented in Figure 8. These graphs show that the ruptures of the tendons started at the onset of lay (around 25 wk of age) and ceased at around 40 wk of age. It also shows that mortality due to ruptures of the tendons was approximately 1.5 times higher in the breeders fed the high (6.3%; panel A) compared to the standard and low energy diet (4.2%; averaged in panel B).

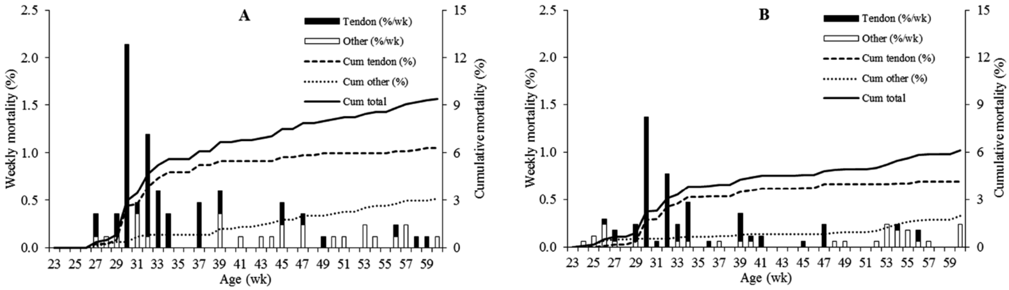

Figure 8. Weekly and cumulative mortality due to ruptures of the tendons and other causes, and total mortality during the laying period in breeders fed the high energy diet (panel A) or the standard and low energy diet (averaged in panel B), based on the results in Chapter 5.

The precise reason for the ruptures of the tendons is unknown. However, in the literature several possible factors are mentioned:

1. Genetic selection for fast growth and increased body weight (Duff and Randall, 1986).
2. Tenosynovitis caused by reovirus or staphylococcus infections could be a predisposing factor (Crespo and Shivaprasad, 2008).
3. Feed restriction could be a predisposing factor (Riddell, 1983).
4. Aggressiveness of (spiked) males resulted in chasing the females and jumping from the slatted floor to the ground or over equipment (Crespo and Shivaprasad, 2011).
5. An increased height between the slatted floor and the ground may cause damage to the gastrocnemius tendons (Crespo and Shivaprasad, 2011).
6. Season effect (Sokale et al., 2013).

Since all external factors were equal among treatments in the experiment reported here, these factors cannot explain the increased ruptures of the tendons. A more plausible cause is the difference in behavior of the birds due to the differences in the amount of feed consumed between the dietary treatments (Chapter 6). The next potential factors are more likely to account for inducing ruptures of the tendons:

7. A lower daily feed intake resulting in a lower daily macro- and micronutrients intake. No literature is available, however, regarding the effect of a lower nutrient intake on tendon development during rearing.

8. Lowering the daily amount of feed decreased time spent on feeding behavior (Chapter 6). It was observed that these birds were aggressive at feeding time resulting in (hyper)activity like running and jumping, inducing a higher risk of damaging the tendons.
9. Providing less feed leads to more competition at feeding time and a possible decrease in flock uniformity (unfortunately not recorded) during initial lay. More aggressive birds will develop higher BW due to the higher feed intake with possible effects on the tendon.

A combination of factor 8 and 9 seems to be the most reasonable explanation of the differences in mortality caused by ruptures of the tendons reported here.

Incubation traits

Feeding a low dietary energy level, resulting in a higher daily protein intake, during the first as well the second phase of the laying period did not affect fertility (Chapter 5), which is also found by other authors (Whitehead et al., 1985; Mejia et al., 2012b). In other studies, feeding broiler breeders a high daily protein level during the laying period resulted in decreased fertility (Lopez and Leeson, 1995a; Ekmay et al., 2013). It is not clear what caused the differences between the cited studies but probably causative factors are: housing system, diet, and breed. E.g., birds were individually housed and artificially inseminated (Mejia et al., 2012b; Ekmay et al., 2013) or group housed with natural mating (Whitehead et al., 1985; Lopez and Leeson, 1995a; Chapter 5). Besides the different ways of housing birds, also different dietary treatments were used for changing dietary protein or amino acids levels. For example, in the study of Lopez and Leeson (1995a) birds received different dietary crude protein levels while essential amino acids levels were equal. Some researchers used semi-purified diets (Mejia et al., 2012a,b; Ekmay et al., 2013) and changed specific amino acids levels while other researchers used more practical diets with reduced levels of crude protein or amino acids (Whitehead et al., 1985; Chapter 5). In the studies of Pearson and Herron (1982) and Whitehead et al. (1985), older Ross strains were used, while here Ross 308 birds were used. In the studies of Mejia et al. (2012a,b) and Ekmay et al. (2013) Cobb 500 breeders were used while Lopez and Leeson (1995a) used Hubbard breeders.

The results from Chapter 5 showed conclusively that feeding birds a high energy diet (less daily protein intake) during the second phase of lay improved hatchability of fertilized eggs. These results are in agreement with Pearson and Herron (1982), Whitehead et al. (1985) and Lopez and Leeson (1995a). The differences in hatchability of the fertile eggs were caused by differences in embryonic mortality. A higher or lower embryonic mortality leads to a lower or higher hatchability of fertile eggs, respectively. This observation supports the earlier work of

Pearson and Herron (1982) who found that lowering daily protein intake (27.0 vs. 21.3 g/bird) resulted in a decreased mortality and malformation of embryos. The decreased embryonic mortality in birds fed the high energy diet (low daily protein intake) in the current study might be explained by the lower egg weight (68.7 vs. 69.1 g; Chapter 5). Larger eggs have a higher eggshell conductance (EC) due to an increased pore density or pore size (Shafey, 2002). A higher EC increased vital gas exchange and water loss which causes, respectively, an increased early and late embryonic mortality (Peebles et al., 1987).

The effect of an improved hatchability and decreased embryonic mortality, while feeding the high energy diet (low daily protein intake), was underlined by the decreased proportion of second grade chicks (Chapter 5). The relationship between low embryonic mortality and less second grade chicks was previously found in studies of Reijrink et al. (2010) and Molenaar et al. (2011).

Offspring performance

Relatively few papers are available on the effects of specific protein or amino acids intake of broiler breeders on offspring performance and processing yields (Wilson and Harms, 1984; Lopez and Leeson, 1995b; Mejia et al., 2013). In general, little or no effect of a change in maternal daily protein intake on growth and processing yields of the offspring has been reported. This is in agreement with the results of the two broiler trials obtained of hatching eggs from 28 and 53 wk of age during the second experiment (Table 2). Breeders fed similar amounts of daily energy, but 7% more or less protein during the first phase of the laying period showed no difference in performance and processing yields of offspring from 28 wk old breeders. Moreover, feeding breeders a 9% lower daily protein intake during the second phase of the laying period did not, except a decreased mortality, affect broiler performance and processing yields of offspring from 53 wk old breeders as well. The lack of an effect of daily protein intake on offspring performance and processing yields can be explained by the research of Ekmay et al. (2011). They found that 60 to 70% of egg albumen lysine is derived from skeletal muscle reserves and the remainder from dietary resources. They suggested that skeletal muscles probably functioned as a transient protein pool from which lysine can be mobilized.

Table 2. Performance of broilers (at d 34) obtained of hatching eggs from 28 or 53 wk of age broiler breeders as affected by dietary protein level (CP) during rearing (2 to 22 wk of age), dietary energy level (ME1) during the first phase of lay (22 to 45 wk of age), and dietary energy level (ME2) during the second phase of lay (45 to 60 wk of age).

	28 wk of age					53 wk of age				
Source[1]	BW gain (g/b/d)	FI (g/b/d)	FCR (g/g)	Mort. (%)	Breast meat (%)	BW gain (g/b/d)	FI (g/b/d)	FCR (g/g)	Mort. (%)	Breast meat (%)
Protein rearing										
CPh	62.3	92.9	1.493	1.3	32.0	65.6	93.8	1.428	1.5	31.9
CPl	62.5	94.0	1.504	2.3	32.1	66.4	94.9	1.430	1.1	32.1
SEM	0.5	0.8	0.005	0.6	0.2	0.4	0.7	0.004	0.4	0.2
Energy lay 1										
MEh1	63.0	94.8	1.505	2.8	32.2	66.2	94.5	1.428	0.6	32.4
MEs1	62.7	94.0	1.499	1.3	32.2	65.7	93.9	1.431	1.9	31.9
MEl1	61.5	91.7	1.491	1.3	31.7	66.1	94.6	1.429	1.3	31.8
SEM	0.6	1.0	0.006	0.7	0.2	0.5	0.8	0.004	0.5	0.3
Energy lay 2										
MEs2	-	-	-	-	-	65.6	94.0	1.431	1.9^{a}	32.1
MEh2	-	-	-	-	-	66.4	94.7	1.427	0.6^{b}	32.0
SEM	-	-	-	-	-	0.4	0.7	0.004	0.4	0.2
P-value										
CP	0.721	0.535	0.094	0.191	0.794	0.221	0.236	0.720	0.515	0.295
ME1	0.190	0.089	0.209	0.183	0.225	0.732	0.849	0.913	0.268	0.201
ME2	-	-	-	-	-	0.245	0.450	0.451	0.048	0.787
CP × ME1	0.945	0.879	0.798	0.183	0.957	0.795	0.630	0.470	0.630	0.945
CP × ME2	-	-	-	-	-	0.883	0.856	0.838	0.191	0.593
ME1 × ME2	-	-	-	-	-	0.501	0.899	0.188	0.277	0.499

[a-b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]CPh = high dietary protein level rearing; CPl = low dietary protein level rearing; MEh1 = high dietary energy level first phase of lay; MEs1 = standard dietary energy level first phase of lay; MEl1 = low dietary energy level first phase of lay; MEs2 = standard dietary energy level second phase of lay; MEh2 = high dietary energy level second phase of lay.

Feed intake, eating time and eating rate

An effect of an increased feed intake (+8 to +17%) due to a 7 to 14% lower energy density on eating time (+20 to +66%) and eating rate (+69 to 92%) was observed during first and second phase of lay (Table 3). This effect was previously found by De Jong et al. (2005b), however, their data were more pronounced.

Table 3. Effect of dietary treatment on feed intake (g/bird per d), eating time (min/d) and eating rate (g feed/min) during lay.

Exp.	Treat-ment	Age (wk)[1]	Feed intake (g/b/d)	Feed intake (%)	Eating time (min/d)	Eating time (%)	Eating rate (g/min)	Eating rate (%)	Reference
AME_n	12.5	27, 38	145.6	100	96	100	1.55	100	Chapter 6
(MJ/kg)	11.7		157.6	108	122	127	1.30	84	
	10.9		169.7	117	160	166	1.07	69	
AME_n[2]	11.5	26, 30,	157.0	100	184	100	0.85	100	De Jong et al.,
(MJ/kg)	10.3[3]	40	177.2	113	311	169	0.57	67	2005b
	9.0		200.7	128	464	253	0.43	51	
AME_n	12.5	46, 54	138.5	100	99	100	1.40	100	Chapter 6
(MJ/kg)	11.7		151.8	110	119	120	1.28	92	
AME_n[2]	11.5	60	148.0	100	128	100	1.16	100	De Jong et al.,
(MJ/kg)	10.3[3]		168.0	114	241	188	0.70	60	2005b
	9.0		188.0	127	265	207	0.71	61	

[1]Age at which observations were taken.
[2]Recalculated to AME_n.
[3]Average from two treatments. In the original papers diets, were diluted with different ingredients.

Table 3 also shows that today's breeders compared to a 10 years old strain, react less to an increased feed intake. Feeding breeders a 10.3 compared to a 11.5 MJ/kg diet (De Jong et al., 2005b) during the first phase of lay resulted in a 13% higher feed intake, a 69% prolonged eating time and a 33% slower eating rate. Feeding breeders in the current study an 11.3 (average of 11.7 and 10.9 MJ/kg) compared to a 12.5 MJ/kg diet resulted in a 13% higher feed intake, a 47% prolonged eating time and a 23% slower eating rate. This could be explained by the increased growth potential of broiler breeders during the last 10 years and, thereby, the increased feed restriction level (Renema et al., 2007b).

Behavior

Data of the experiment reported in Chapter 6 show a linear relationship ($P < 0.001$) between feeding time and stereotypic object pecking during the first phase of the laying period while this effect disappeared during the second phase ($P = 0.585$) (Figure 9). The relationship between feeding and stereotypic object pecking during the first phase of lay is comparable with the results during the rearing period (Figure 5), however, differences are less pronounced during lay. This can be explained by differences in feed restriction between rearing and lay. It has previously been shown that during the rearing period feed intake of broiler breeders is restricted to 25-33% of *ad libitum* intake (Savory et al., 1996; De Jong et al., 2002) while this is 50-90% during the laying period (Bruggeman et al., 1999).

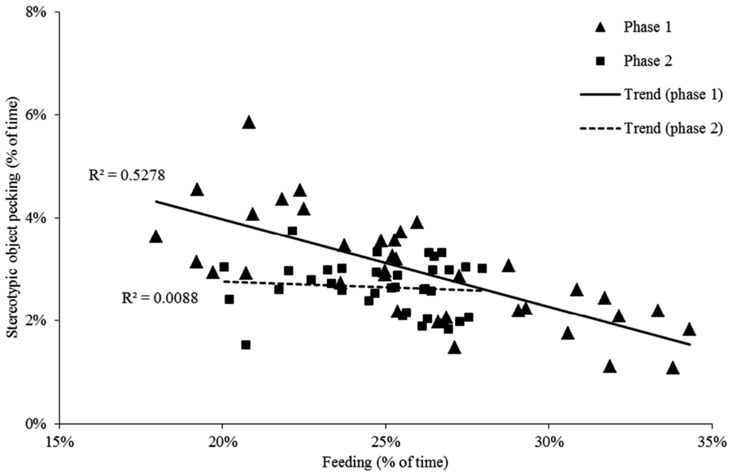

Figure 9. Relationship between time spent on feeding (% of time) and stereotypic object pecking (% of time) during the laying period, based on the results in Chapter 6. Each point in the graph is an individual pen.

Breeders showed around 2.5% stereotypic object pecking behavior during the entire laying period. This was unexpected due to the, on average, approximately 80% increase in amount of feed between the rearing and laying period. This absent of an effect while ageing could be explained by two different reasons; (1) feeding time during the laying period was only 2.5 h per day leaving enough time to express other behaviors as suggested by Hocking et al. (2004) and Mason et al. (2006); (2) it is suggested that stereotypic object pecking behavior was programmed during earlier life.

Feather cover

A low daily protein intake during the first phase of the laying period resulted in a poor feather cover during the entire laying period. This phenomenon (low CP intake and poor feather cover) was observed during the rearing period as well. This effect was more pronounced in the first than in the second phase of lay, potentially because feather cover during the second phase was already almost completely damaged, thereby, masking treatment differences.

Feather cover score of all birds during the first phase of lay increased slowly in Chapter 3 from 1.1 to 1.7 (+55%) while in Chapter 6 this decreased more rapidly from 0.8 to 2.3 (+188%). This discrepancy between studies could be explained by the absence of males and the more feeding space in the study reported in Chapter 3.

A NEW FEEDING STRATEGY

Practical implications

Based on the research reported here, several recommendations regarding feed formulations for pullets and breeders can be made:

- Increase the energy-protein ratio in diets for broiler breeder pullets to improve reproduction and welfare. Because of the severe feed restriction and a mandatory daily feeding program during rearing in the nearby future, it is advised to decrease the crude protein and amino acid levels with 10 to 15%. In order to follow a similar daily growth pattern, feed allowance should be increase by approximately 7 to 11%. To prevent a possible negative effect on feather cover during initial rearing, two options are available for rearing diets:
  1. Do not decrease the CP/AA levels in the starter-1 diet (0 to 2 wk), decrease the CP/AA levels in the starter-2 diet (2 to 6 wk) with at a maximum of approximately 5%, but decrease the CP/AA levels in the grower and pre-breeder feeds by approximately 15%, or

2. Decrease CP/AA levels in the starter-2 diet by 15% and decrease the amino acids methionine and cysteine by a maximum of 5% or keep them at the same level as advised by the breeder company. This means an alternation in amino acids profile of the starter-2 diet.

- Do not alter the energy to protein ratio of the diet in the first phase of the laying period for breeders.
- Increase the energy to protein ratio to about 21 kcal/g CP for breeders during the second phase of the laying period to improve incubation traits. This can be achieved by three different methods:
  1. Increased energy level (3,000 vs. 2,800 AME_n kcal/kg) and a standard crude protein level (142 CP g/kg), or
  2. Increased energy level (2,900 vs. 2,800 AME_n kcal/kg) and a 3% decreased crude protein level (137 vs. 142 CP g/kg), or
  3. Standard energy (2,800 AME_n kcal/kg) and a 6.5% decreased crude protein level (133 vs. 142 CP g/kg).

Economic implications

The profitability of the broiler breeder business is mainly dependent of the feed costs and the number of fertilized settable eggs and/or the number of day-old chicks (Laughlin, 2009). In order to estimate the economic impact of the different feeding strategies, the results of Chapter 5 are used in this paragraph. The Economical Poultry-computer model of Wageningen UR Livestock Research was used to calculate these effects. Standard costs and prices from KWIN-V (2013) were used and calculations were based on a standard broiler breeder flock of 21,000 females and 2,000 males. Feed prices of the first quarter of 2013 were used. Only significant differences and near-significant trends ($0.05 < P < 0.10$) on breeder performance between treatments were used in the evaluation. Non-significant numerical differences were averaged for the different treatments to prevent under- or overestimating of the effects. The economic analysis, expressed per 100, 22 wk old breeders is shown in Table 4.

Feeding pullets a low compared to a high CP diet resulted in a 6% higher gross margin (€944 vs. €891). This difference was caused by the increased hatchability of settable eggs during the first phase of lay and increased number of eggs during the second phase of lay period. The 1.3 kg (11.9 vs. 10.6 kg; +12%) higher feed intake during rearing was partly compensated by the 5% lower feed price resulting in 7% higher feed costs (€279 vs. €261). Extrapolated to a standard flock of 21.000 females the annual increase in gross margin could be more than €11,000.

Table 4. Economic analysis per phase, expressed per 100, 22 wk old breeders.[1]

Phase	Rearing		Phase 1			Phase 2	
Treatment	High CP	Low CP	High energy	Stand. energy	Low energy	Stand. energy	High energy
Technical and economical parameters							
Settable eggs phase 1 (n/hen)	102.8	102.8	101.6	104.8	102.1	102.8	102.8
Settable eggs phase 2 (n/hen)	56.9	59.6	58.3	58.3	58.3	58.3	58.3
Egg price (€/100 eggs)	17.70	17.70	17.70	17.70	17.70	17.70	17.70
Non settable eggs (n/hen)	7.8	7.8	7.8	7.2	8.2	7.8	7.8
Hatchability phase 1 (%)	87.7	89.0	88.4	88.4	88.4	88.4	88.4
Hatchability phase 2 (%)	79.2	79.2	79.2	79.2	79.2	79.2	79.2
Mortality (%)	6.3	8.1	9.4	6.5	5.7	7.2	7.2
Feed intake rearing (kg/hen)	10.6	11.9	10.6	10.6	10.6	10.6	10.6
Feed intake phase 1 (kg/hen)	23.8	23.8	21.7	23.8	25.6	23.8	25.6
Feed intake phase 2 (kg/hen)	14.0	14.0	14.0	14.0	14.0	14.7	13.3
Feed price rearing (€/100 kg)	24.70	23.45	24.70	24.70	24.70	24.70	24.70
Feed price phase 1 (€/100 kg)	24.90	24.90	26.25	24.90	23.55	24.90	24.90
Feed price phase 2 (€/100 kg)	23.85	23.85	23.85	23.85	23.85	23.85	24.40
Yields (€)							
Settable eggs phase 1	1,978	2,005	1,968	2,030	1,978	1,992	1,992
Settable eggs phase 2	998	1,045	1,022	1,022	1,022	1,022	1,022
Non settable eggs	4	4	4	4	4	4	4
Slaughtering	154	151	149	153	155	152	152
Costs (€)							
22 wk old breeder	1,035	1,035	1,035	1,035	1,035	1,035	1,035
Feed rearing period	261	279	261	261	261	261	261
Feed laying period	925	925	904	927	937	942	916
Spike males	21	21	21	21	21	21	21
Gross margin (€)	891	944	922	966	905	911	937

[1]Rearing (2 to 22 wk of age), first phase of lay (22 to 45 wk of age), and second phase of lay (45 to 60 wk of age).

It can be concluded from Table 4 that providing a high or low energy diet during the first phase of the laying period resulted in an, on average, 5% lower gross margin (€914 vs. €966). This difference was mainly caused by the decreased egg production for the birds fed the high and low energy diet during the first phase of the laying period.

Feeding birds the high energy diet during the second phase of the laying period resulted in a 3% higher gross margin (€ 937 vs. € 911) which was due to the lower feeding costs. For a standard flock of 21.000 females the annual increase in gross margin could be up to €5,500.

It should be stated that this evaluation is only based on one experiment, standard costs and prices of KWIN-V (2013), and feed prices of the first quarter of 2013. It is, therefore, only an indication on how different diets may affect economical results and how the new recommendations for feeding broiler breeders can affect profitability.

OVERALL CONCLUSIONS

Based on the results of the studies reported here, the following conclusions can be drawn:

- Feeding a low protein diet during rearing results in a larger abdominal fat pad and less breast muscle content at the end of rearing.
- A higher abdominal fat pad content in the body of breeder pullets increased hatchability, due to a decreased embryonic mortality, in the first phase and number of eggs during the second phase of lay.
- Providing more feed during rearing (a higher growth pattern) increased fertility and hatchability of settable eggs, and increased BW gain of offspring of young breeders.
- Feather cover development was negatively influenced by a low daily protein intake during rearing and during first phase of lay.
- A high-energy diet during the first phase of lay increased mortality due to ruptures of the tendons.
- A high-energy diet (lower daily protein intake) in the second phase of lay increased hatchability of fertile eggs due to a decreased embryonic mortality.
- Male broilers of breeders fed a low-protein diet during rearing have higher breast meat yield.
- A 10% increase in daily feed intake, due to feeding low protein diets during rearing, resulted in a twofold eating time.
- Time budgets in behavior showed that eating time was inversely related to time spent on stereotypic object pecking during rearing and during first phase of lay.

SUGGESTIONS FOR FUTURE RESEARCH

Different growth patterns during the laying period

In this study, the impact of different energy to protein ratios and feed intake levels on body composition, breeder performance, incubation traits, offspring performance, feather cover, and behavior were studied. From the current study it was concluded that the differences in BW at the end of the rearing period probably were not large enough to cause permanent differences in body composition during the laying period. Differences in BW targets were diminished during the initial laying period caused by a relative higher feed restriction for the high BW groups, inhibiting production. Therefore, future experiments should target a larger difference in BW (e.g.

300 g) at the end of rearing in combination with either maintaining (or not) these differences in BW during the initial laying period.

Feather cover

Inferior feather cover of broiler breeders has negative effects on welfare of the birds (Van Emous and De Jong, 2013). In our studies, a low daily protein intake during the rearing and first phase of the laying period resulted in an inferior feather cover which is an indication that specific amino acids levels were deficient for feather development. A new experiment might, therefore, focus on improving feather cover during the initial rearing period while maintaining the benefits on behavior and reproduction. This can be achieved by changing dietary amino acid profiles of the initial rearing diets.

Strain and stocking density

It will be interesting to study the effects of different feeding strategies during rearing and laying on reproduction, behavior, and offspring with different breeds and stocking density. For instance two different commercial genotypes (i.e. Ross and Cobb) and two stocking densities (i.e.: high (EU) 7.5 versus low (US) 5.0 breeders/m^2) can be used to test the genotype × feeding strategy interaction and the stocking density × feeding strategy interaction, in order to avoid the potential generalization of feeding strategies effects across genotypes and stocking densities.

Epigenetic

Most of the effects of nutrition on offspring performance, metabolism and immune response are unknown or, moreover, the underlying mechanisms are unclear. Therefore, future research should focus on the physiological effects of nutrition in broiler breeders during the rearing and laying period on offspring growth performance, endocrine status and lipid metabolism in the offspring. Of specific interest is the effect of gene expressions in the parents on the offspring performance.

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Summary

The basis of the modern poultry meat industry dates back to the late 1950s. Poultry meat is one of the most important protein sources within the global human diet and increased during the last decades with almost 50%. The impressive growth of the poultry meat industry is supported by improvements in health, nutrition and environmental management. Modern broiler strains have a 2.5 times higher daily growth while FCR is less than half compared to strains in use 60 years ago. The selection on production traits has not only affected the offspring but also the parents (broiler breeders). When broiler breeder pullets were fed *ad libitum* during the rearing period, they increased BW by about 2.5 times compared to restricted breeders (5 vs. 2 kg at 20 wk of age) which results in a significantly decreased reproductive performance. Therefore, feed intake of broiler breeders during rearing is severely restricted. However, feed restriction in broiler breeders contributes to behavioral disorders such as stereotypic object pecking which is indicative of frustration, boredom and hunger. The main goal in broiler breeder production is to provide fertilized eggs to produce a maximum number of healthy and robust day-old broilers chicks. These previous issues lead to a situation that the management of broiler breeders is becoming more and more challenging.

Based on literature and field observations, it was concluded that body composition of broiler breeders at the end of the rearing period has changed during the last five decades (i.e. more breast meat and less body fat). It was hypothesized that this change in body composition may negatively affect breeder performance, incubation traits and offspring performance. Because tissue growth is directly affected by dietary nutrient composition and feed intake, studies were designed to develop new feeding strategies during the rearing and laying period for broiler breeders in order to alter body composition of these birds with positive effects on reproduction, offspring and welfare, for a more sustainable approach of broiler breeder production.

In Chapter 2, the combined effects of growth pattern (GP) and dietary CP level during rearing (2 to 22 wk of age) on body composition and performance were investigated in broiler breeder females from 0 to 40 wk of age. One-day-old pullets (n = 768) were randomly allotted to 48 pens according to 2 growth patterns (standard = SGP and high = HGP) and fed 1 of 3 dietary CP levels (high = CPh, medium = CPm, and low = CPl). From 19 to 22 wk of age, feeding level was gradually adjusted to obtain a similar target BW for all birds, and then until 40 wk of age, all birds received similar amounts of a standard breeder diet. During the rearing period, the HGP pullets were fed a higher feed intake level (6.5%) than SGP pullets. To meet BW targets at 22 wk of age, feed intake from d 14 onward had to be increased for the CPm (4.6%) and CPl (10.0%) treatments. Breast muscle percentages of HGP and SGP pullets were similar at any age, although abdominal fat pad at 20 wk was 0.18% higher for HGP pullets. Pullets fed the CPl diet

had a lower breast muscle percentage compared with pullets fed the CPm and CPh diets (0.46 and 0.85% at wk 10, 0.81 and 1.45% at wk 20, respectively). Abdominal fat pad in CPl pullets were 0.18 and 0.22% (wk 10), and 0.24 and 0.42% (wk 20) higher compared with CPm and CPh pullets, respectively. At 40 wk of age, no effects on breast muscle and abdominal fat pad were found among all treatments. Egg production, sexual maturation, and egg weight were not affected by GP and CP levels during rearing. *A low CP diet during rearing decreases breast muscle and increases abdominal fat pad, whereas a high GP only increases abdominal fat pad, at the end of the rearing period. Decreasing dietary CP level seems to be more effective in increasing abdominal fat pad than increasing GP.*

In Chapter 3, the effects of the different rearing feeding strategies on feed intake, eating time, eating rate, behavior, plasma corticosterone concentration, and feather cover in broiler breeder females during the rearing and laying period were studied. These parameters were investigated in the experiment as described in Chapter 2. During rearing, HGP birds were fed, on average, 6.5% more feed than SGP birds. In HGP birds, eating time (min/d) during the rearing period increased by 17%, whereas eating rate (g feed/min) decreased by 8%, compared to SGP birds. This prolonged feeding behavior of HGP birds, but stereotypic object pecking and animal pecking was not reduced. Feather cover was not affected by growth pattern during the rearing and laying period. Only at 16 wk of age a lower plasma corticosterone concentration was found for the HGP birds. HGP birds showed more feeding and sitting behavior, but less foraging behavior during the rearing period, while during the laying period only more walking behavior was observed. In order to maintain target weights, feed intake levels of CPm and CPl during rearing were set 4.6 and 10.0% higher than CPh, whereas eating time was increased by 22 and 63% and eating rate was decreased by 9 and 26%, respectively. A prolonged eating time during rearing for CPm and CPl birds resulted in more time spent on feeding and resting and less stereotypic object pecking and animal pecking compared to CPh birds during rearing. In contrast to the rearing period, feed intake and eating time were not affected by CP level during rearing at 22 wk of age, whereas eating rate was increased by 8 and 16% for CPm and CPl birds, respectively, compared to CPh birds. At 27 wk of age the effect of CP level during rearing on eating rate had disappeared. Plasma corticosterone concentrations were not affected by dietary protein level during the rearing and laying period. Feather cover was inferior by lowering the dietary protein level, in particularly during the first 11 wk of rearing. No effect of GP was found on feather cover. *Dietary protein level positively affects some behavioral traits during the rearing period, whereas these traits are only slightly affected by growth patterns. However, the physiological parameter (plasma corticosterone concentration) is not affected.*

The effects of the different feeding strategies during the rearing period on fertility, hatchability, embryonic mortality, offspring performance, and characteristics of chicks and eggs are discussed in Chapter 4. Fresh egg composition and organ development in hatchlings were determined. Offspring of the different groups were reared till an age of 34 d and feed intake, BW gain, mortality, and carcass composition were determined. In 29 wk old HGP compared to SGP breeders, fertility and hatchability of set eggs were increased; embryonic mortality between d 1 to 9 was decreased whereas hatchability of fertile eggs was not affected. Breeders fed the CPm diet showed a decreased hatchability of fertile eggs caused by an increased embryonic mortality between d 18 and 21 compared to breeders fed the CPh and CPl diets. Offspring of 29-wk old HGP breeders tended ($P = 0.059$) to have a higher BW at d 34 than offspring of SGP breeders, which was achieved by a tendency for a higher BW gain ($P = 0.057$). Offspring of breeders fed the CPm and CPl diet showed a higher feed intake between d 18 to 27 and during the total growth period, compared to offspring of CPh breeders. Male broilers of CPl breeders had higher breast meat yield than male broilers of CPh breeders, while breast meat yield of female broilers was not affected by dietary protein levels. *A higher growth pattern during the rearing period increases fertility, decreases embryonic mortality, and improves offspring performance in young breeders, whereas decreased dietary protein level has a less pronounced effect on these traits.*

Based on the results reported in Chapters 2 to 4, a study was conducted where different dietary protein levels were chosen as treatment during the rearing period while during the laying period different dietary energy levels were applied. Chapter 5 describes the effects of different dietary protein levels during rearing period and different dietary energy levels during lay period on body composition, breeder performance and incubations traits. A study with a 2 × 3 × 2 factorial arrangement was conducted to determine the effects of 2 dietary protein levels (high = CPh and low = CPl) during rearing, 3 dietary energy levels (3,000, MEh1; 2,800, MEs1; and 2,600, MEl1, kcal/kg AME_n, respectively) during the first phase of lay, and 2 dietary energy levels (2,800, MEs2; and 3,000, MEh2, kcal/kg AME_n, respectively) during the second phase of lay on body composition and reproduction in broiler breeders. Pullets fed the CPl diet had a 12.8% higher feed intake, 14% lower breast muscle and 97% higher abdominal fat pad portion at 22 wk of age. The increased abdominal fat pad and decreased breast muscle of the CPl compared to the CPh birds increased hatchability during the first phase of lay, due to a decreased embryonic mortality between d 10 to 21 of incubation, and increased egg production during the second phase of lay. Feeding birds the MEh1 and MEl1 diets decreased egg production slightly compared to the MEs1 birds. Birds fed the MEh1 diet showed a higher mortality compared to the birds fed the MEs1 and MEl1 diets. Feeding birds the MEh2 diet did not affect egg production, increased hatchability of fertile eggs, decreased embryonic mortality between d 3 to 21 of incubation, and increased the number of first grade chicks. *A low-protein diet during rearing changes body composition with positive effects on incubation traits during the first phase of lay and improves egg production during the second phase of lay in broiler breeders. A high- or low-energy diet compared to a standard diet during the first phase of lay decreases total and settable egg numbers slightly while a high-energy diet during the second phase of lay increases hatchability, decreases embryonic mortality and increases the number of saleable chicks.*

In Chapter 6, the effects of different rearing and laying feeding strategies, as described in Chapter 5, on behavior and feather cover during rearing and lay were studied. During rearing, CPl pullets received 12.1% more feed, resulting in a 137% increased eating time and 47% decreased eating rate. This increased feeding, sitting and comfort behaviors while walking, foraging and stereotypic object pecking behaviors were reduced. Feather cover was poorer when pullets were fed a low protein diet during rearing but this effect was not present during lay. A 7.6% lower feed intake for the MEh1 birds resulted in a 21% decreased eating time and 19% increased eating rate compared to the MEs1 birds. Birds on the MEh1 diet spent less time feeding and more time sitting, expressing comfort behavior, and stereotypic object pecking compared to the MEs1 birds. Birds fed the MEh1 diet showed a poorer feather cover during the entire lay compared to the MEs1 birds. A 7.7% higher feed intake for the MEl1 birds resulted in a 31% increased eating time and 18% decreased eating rate compared to the MEs1 birds. MEl1 birds spent more time feeding and less time foraging, comfort and stereotypic object pecking compared to the MEs1 birds. No effect on feather cover was found for the MEl1 compared to the MEs1 birds. The MEh2 birds received 8.8% less feed resulted in a 16.0% decreased eating time and 9.0% increased eating rate. The lower feed intake resulted in less time spent feeding and standing and more time spent foraging and expressing comfort behavior. Feather cover was not affected by dietary energy level during the second phase of lay. *Feeding broiler breeders a higher amount of feed due to a higher energy to protein ratio resulted in an increased eating time and less stereotypic object pecking what is an indication of reduced hunger and frustration. This was much more expressed during rearing than lay. However, a low daily protein intake during rearing and first phase of lay can lead to a poorer feather cover.*

The General Discussion (Chapter 7) provides an evaluation and integration of the results reported in Chapters 2 to 6. The results obtained are compared with data from other experiments and explanations are provided. Practical and economic implications for new feeding strategies aimed at improving broiler breeder reproduction and welfare during the rearing and laying period are also evaluated in this section. Feeding broiler breeder pullets a low protein diet during the

rearing period resulted in a 6% higher gross margin. Changing the energy to protein ratio of the diets during the first phase of lay resulted in an, on average, 5% lower gross margin. An increased energy to protein ratio of the diet during the second phase, however, resulted in a 3% higher gross margin. *An increased energy to protein ratio in broiler breeder pullet diets by 15 to 20% is a successful strategy to improve reproduction traits and behavior. It is not recommended to alter the energy to protein ratio during the first phase of lay, however, an increased energy to protein ratio of the diet during the second phase of lay by approximately 6% will improve incubation traits.*

Samenvatting

De basis voor de huidige productie van pluimveevlees gaat terug naar de jaren 50. Pluimveevlees is een van de meest belangrijke bronnen van eiwit in de wereldwijde humane voedselconsumptie en is gedurende de laatste decennia met bijna 50% gegroeid. De indrukwekkende groei van de productie van pluimveevlees is mogelijk gemaakt door verbeteringen op het gebied van gezondheid, voeding en huisvesting. Moderne vleeskuikenrassen hebben een 2,5 keer zo hoge daggroei terwijl de voederconversie meer dan gehalveerd is ten opzichte van rassen die zestig jaar geleden werden gebruikt. De selectie op productie parameters heeft niet alleen effect gehad op vleeskuikens, maar ook op de ouders van deze kuikens (vleeskuikenouderdieren). Wanneer vleeskuikenouderdieren tijdens de opfokperiode onbeperkt voer krijgen verstrekt, worden de dieren ongeveer 2,5 keer zo zwaar als beperkt gevoerde dieren (5 t.o.v. 2 kg op 20 weken leeftijd) wat resulteert in een significante afname van de reproductie. Om dit te voorkomen worden ouderdieren tijdens de opfokperiode sterk beperkt in de voeropname. Voerbeperking bij opfok ouderdieren kan gedragsafwijkingen als stereotiep object pikken veroorzaken wat indicatief is voor frustratie, verveling en honger. Het belangrijkste doel van het houden van vleeskuikenouderdieren is het produceren van bevruchte broedeieren, die na uitkomst een maximaal aantal gezonde en robuuste eendagskuikens opleveren. De hierboven genoemde zaken geven aan dat het managen van vleeskuikenouderdieren een steeds grotere uitdaging wordt.

Vanuit de literatuur en praktijkobservaties werd geconcludeerd dat de lichaamssamenstelling van vleeskuikenouderdieren aan het einde van de opfokperiode gedurende de laatste vijf decennia is veranderd; het aandeel borstvlees is toegenomen en het aandeel lichaamsvet is afgenomen. De veronderstelling was dat deze verandering in lichaamssamenstelling een negatief effect zou hebben op de technische resultaten van de ouderdieren, de broedresultaten en de resultaten van de nakomelingen. De groei van lichaamsweefsel kan direct beïnvloed worden door de samenstelling van de verstrekte voeders en de voergift. Daarom zijn in het kader van dit project experimenten opgezet om nieuwe voerstrategieën voor vleeskuikenouderdieren tijdens de opfok- en legperiode te ontwikkelen. Het doel hiervan was om de lichaamssamenstelling van deze dieren zodanig te veranderen, dat dit positieve effecten heeft op reproductie, nakomelingen en welzijn, met als uiteindelijk doel om tot een meer duurzame benadering van de vleeskuikenouderdieren sector te komen.

In hoofdstuk 2 zijn de gecombineerde effecten van groeicurve (GP) en eiwitniveau van het voer (CP) gedurende de opfokperiode (2 tot 22 weken leeftijd) op lichaamssamenstelling en technische resultaten van vrouwelijke vleeskuikenouderdieren tussen 0 en 40 weken leeftijd onderzocht. Vleeskuikenouderdieren henkuikens (768 stuks) werden willekeurig verloot over 48 grondhokken met 2 groeicurves (standaard = SGP en hoog = HGP) en 3 voeders met verschillende eiwitniveaus (hoog = CPh, gemiddeld = CPm en laag = CPl). Tussen 19 en 22 weken leeftijd werd het voerniveau geleidelijk aangepast om een vergelijkbaar lichaamsgewicht voor alle dieren te bereiken. Vanaf 22 weken leeftijd, tot het einde van het experiment (40 weken leeftijd), kregen alle dieren dezelfde hoeveelheid van hetzelfde standaard foktoomvoer. Gedurende de opfokperiode kregen de HGP dieren een 6,5% hogere voergift dan de SGP dieren. Om hetzelfde lichaamsgewicht op 22 weken leeftijd te bereiken was de voergift vanaf 14 dagen leeftijd respectievelijk 4,6 en 10,0% hoger voor de CPm en CPl behandelingen. Het percentage borstvlees bij de HGP en SGP dieren was op alle leeftijden gelijk, terwijl het percentage buikvet op 20 weken leeftijd 0,18% hoger was bij de HGP dieren. De dieren die het CPl voer kregen, hadden in vergelijking met de dieren die het CPm en CPh voer kregen een lager percentage borstvlees (respectievelijk 0,46 en 0,85% op 10 weken leeftijd en 0,81 en 1,45% op 20 weken leeftijd). Het percentage buikvet bij de CPl dieren was respectievelijk 0,18 en 0,22% (10 weken leeftijd) en 0,24 en 0,42% (20 weken leeftijd) hoger in vergelijking met de CPm en CPh dieren. Op 40 weken leeftijd was er geen effect meer van de behandelingen op borstvlees- en buikvetpercentage. Het aantal eieren, leeftijd geslachtsrijpheid en eigewicht werden niet beïnvloed door GP en CP gedurende de opfokperiode. *Een laag eiwitvoer tijdens de opfokperiode verlaagt het aandeel borstvlees en verhoogt het aandeel buikvet, terwijl een hogere groeicurve alleen het aandeel buikvet aan het einde van de opfokperiode verhoogt. Het verlagen van het eiwitniveau van het opfokvoer lijkt, effectiever te zijn in het verhogen van het aandeel buikvet, dan het verhogen van de groeicurve tijdens de opfokperiode.*

In hoofdstuk 3 zijn de effecten van de verschillende voerstrategieën tijdens de opfok op voeropname, eettijd, eetsnelheid, gedrag, corticosterongehalte in het bloed en bevedering bij vrouwelijke vleeskuikenouderdieren gedurende de opfok- en legperiode onderzocht. Deze parameters werden onderzocht in het experiment zoals beschreven in hoofdstuk 2. Gedurende de opfokperiode kregen de HGP dieren gemiddeld 6,5% meer voer verstrekt dan de SGP dieren. De eettijd (minuten/dag) tijdens de opfokperiode was bij de HGP dieren 17% langer, terwijl de eetsnelheid (gram voer/minuut) 8% lager was ten opzichte van de SGP dieren. Dit verlengde het voeropname gedrag van de HGP dieren, maar leidde niet tot minder stereotiep object pikken en pikken naar andere dieren. De bevedering tijdens de opfok- en legperiode werd niet beïnvloed door de verschillende groeicurves. Alleen op 16 weken leeftijd werd bij de HGP dieren een lager corticosterongehalte gevonden. De HGP dieren vertoonden meer eet- en zitgedrag maar minder foerageergedrag tijdens de opfokperiode, terwijl gedurende de legperiode alleen meer loopgedrag werd waargenomen. Om hetzelfde lichaamsgewicht te bereiken was de voergift voor de CPm en CPl dieren 4,6 en 10,0% hoger ten opzichte van de CPh dieren, terwijl de eettijd respectievelijk 22 en 63% langer en de eetsnelheid 9 en 26% lager was. De langere eettijd voor de CPm en CPl dieren tijdens de opfokperiode resulteerde in meer voeropname en rustgedrag en in minder stereotiep object pikken en pikken naar andere dieren ten opzichte van de CPh dieren. Op 22 weken leeftijd werd, in tegenstelling tot de opfokperiode, de voergift en eettijd niet beïnvloed door het eiwitniveau van het voer, terwijl de eetsnelheid bij CPm en CPl dieren respectievelijk 8 en 16% hoger was in vergelijking met CPh dieren. Op 27 weken leeftijd was het effect van eiwitniveau van het voer tijdens de opfokperiode op eetsnelheid verdwenen. De corticosterongehalten tijdens de opfok- en legperiode werden niet beïnvloed door het eiwitniveau van het voer tijdens de opfokperiode. Wanneer een laag eiwitvoer werd verstrekt was de bevedering, vooral tijdens de eerste 11 weken van de opfokperiode, slechter. Geen effect van groeicurve op bevedering werd gevonden. *Het eiwitniveau van het opfokvoer heeft een positief effect op enkele gedragsuitingen tijdens de opfokperiode, terwijl deze slechts incidenteel beïnvloed worden door de groeicurves. De fysiologische parameter (corticosterongehalte bloed) werd echter niet beïnvloed.*

De effecten van verschillende voerstrategieën tijdens de opfokperiode, zoals beschreven in hoofdstuk 2, op bevruchting, uitkomst, embryonale sterfte, technische resultaten nakomelingen en kenmerken van eendagskuikens en eieren zijn bediscussieerd in hoofdstuk 4. De samenstelling van verse eieren en de orgaanontwikkeling van eendagskuikens werden vastgesteld. Vleeskuikens van de verschillende behandelingen zijn gedurende een groeiperiode van 34 dagen gevolgd waarbij voeropname, groei en slachtrendementen zijn bepaald. Broedeieren afkomstig van HGP ouderdieren van 29 weken leeftijd vertoonden ten opzichte van SGP ouderdieren een hogere bevruchting en uitkomst van ingelegde eieren. De embryonale sterfte was tussen dag 1 en 9 van het broedproces lager terwijl de uitkomst van bevruchte broedeieren niet werd beïnvloed. Broedeieren, afkomstig van ouderdieren die het CPm voer kregen, gaven ten opzichte van de CPh en CPl dieren een lagere uitkomst van de bevruchte broedeieren. Dit werd veroorzaakt door de hogere embryonale sterfte tussen dag 18 en 21 van het broedproces. Vleeskuikens afkomstig van 29 weken oude HGP ouderdieren vertoonden, ten opzichte van vleeskuikens van SGP ouderdieren, een tendens (P = 0,059) tot een hoger lichaamsgewicht op dag 34 wat samenging met een tendens (P = 0,057) tot een hogere groei. Vleeskuikens van ouderdieren die het CPm en CPl voer kregen vertoonden een hogere voeropname tussen 18 en 27 dagen leeftijd dan vleeskuikens van CPh ouderdieren. Mannelijke vleeskuikens (haantjes) afkomstig van CPl ouderdieren hadden een hoger percentage borstfilet dan haantjes afkomstig van CPh ouderdieren, terwijl het percentage borstfilet bij vrouwelijke vleeskuikens (hennetjes) niet beïnvloed werd door het eiwitniveau van het voer. *Een hogere groeicurve tijdens de opfokperiode verhoogt de bevruchting, verlaagt de embryonale sterfte en verbetert de technische resultaten van de nakomelingen van jonge ouderdieren, terwijl een lager eiwitniveau van het opfokvoer een minder duidelijk effect geeft op deze kenmerken.*

Op basis van de resultaten beschreven in de hoofdstukken 2 tot en met 4 is een experiment uitgevoerd met verschillende eiwitniveaus in het voer tijdens de opfokperiode, terwijl tijdens de legperiode voeders met verschillende energieniveaus werden toegepast. Hoofdstuk 5 beschrijft de effecten van deze voeders op lichaamssamenstelling, technische resultaten ouderdieren en broedresultaten. Het experiment werd volgens een 2 x 3 x 2 factoriele opzet uitgevoerd om de effecten van 2 eiwitniveaus voeders (hoog = CPh en laag = CPl) tijdens de opfokperiode, 3 energieniveaus voeders (3.000 = MEh1, 2.800 = MEs1 en 2.600 = MEl1, kcal/kg AME_n) tijdens de eerste fase van de legperiode en 2 energieniveaus voeders (2.800 = MEs2 en 3.000 = MEh2, kcal/kg AME_n) tijdens de tweede fase van de legperiode op lichaamssamenstelling en reproductie bij vleeskuikenouderdieren te onderzoeken. Opfok ouderdieren die het CPl voer kregen, hadden op 22 weken leeftijd een 12,8% hogere voeropname, 14% minder borstvlees en 97% meer buikvet. Het hogere buikvet en lagere borstvlees percentage bij de CPl dieren gaf, ten opzichte van de CPh dieren, door een lagere embryonale sterfte tussen dag 10 en 21 van het broedproces, een hogere uitkomst van de broedeieren, tijdens de eerste fase van de legperiode en een hogere broedeiproductie tijdens de tweede fase van de legperiode. De ouderdieren die het MEh1 en MEl1 voer kregen, vertoonden, ten opzichte van de MEs1 dieren, een tendens tot een lagere broedeiproductie. De dieren die het MEh1 voer kregen, hadden een hogere uitval in vergelijking met de dieren die het MEs1 en MEl1 voer kregen. Het MEh2 voer gaf geen verschillen in broedeiproductie, verhoogde de uitkomst van de bevruchte broedeieren, verlaagde de embryonale sterfte tussen dag 3 en 21 van het broedproces en verhoogde het aantal eerste klas kuikens. *Een laag eiwitvoer tijdens de opfokperiode verandert de lichaamssamenstelling met als gevolg positieve effecten op broedresultaten tijdens de eerste fase van de legperiode en een hogere broedeiproductie tijdens de tweede fase van de legperiode. Een hoog of laag energievoer geeft in vergelijking met een standaard energievoer tijdens de eerste fase van de legperiode een tendens tot een lager aantal eieren en broedeieren, terwijl een hoog energievoer tijdens de tweede fase van de legperiode de uitkomst verhoogt, de embryonale sterfte verlaagt en het aantal verkoopbare kuikens verhoogt.*

In hoofdstuk 6 zijn de effecten van de verschillende voerstrategieën tijdens de opfok en leg, zoals beschreven in hoofdstuk 5, op gedrag en bevedering tijdens de opfok- en legperiode onderzocht. Tijdens de opfokperiode kregen de CPl dieren 12.1% meer voer, wat resulteerde in een 137% langere eettijd en 47% lagere eetsnelheid. Dit gaf meer eet-, zit- en comfortgedrag, terwijl lopen, foerageren en stereotiep object pikken minder werden waargenomen. De bevedering was slechter tijdens de opfokperiode bij de dieren die het laag eiwitvoer kregen, terwijl dit effect tijdens de legperiode niet meer zichtbaar was. Een 7.6% lagere voergift bij de MEh1 ouderdieren resulteerde in een 21% kortere eettijd en 19% hogere eetsnelheid ten opzichte van de MEs1 ouderdieren. De ouderdieren die het MEh1 voer kregen hadden een slechtere bevedering tijdens de gehele legperiode in vergelijking met de MEs1 dieren. Een 7.7% hogere voergift bij de MEl1 ouderdieren resulteerde in een 31% langere eettijd en een 18% lagere eetsnelheid ten opzichte van de MEs1 ouderdieren. De MEl1 ouderdieren besteedden meer tijd aan voeropname en minder tijd aan foerageren, comfort en stereotiep object pikken in vergelijking met de MEs1 ouderdieren. Geen verschil werd aangetroffen in bevedering tussen de MEl1 en MEs1 ouderdieren. De MEh2 ouderdieren kregen 8.8% minder voer, wat resulteerde in een 16% kortere eettijd en 9% hogere eetsnelheid. De lagere voergift resulteerde in een kortere tijdsbesteding aan voeropname en staan en in een langere tijdsbesteding aan foerageren en comfort gedrag. De bevedering werd niet beïnvloed door het energieniveau van het voer tijdens de tweede fase van de legperiode. *Het verstrekken van een grotere hoeveelheid voer, door een hogere energie-eiwit verhouding, resulteerde in een langere eettijd en verminderd stereotiep object pikken wat een indicatie is voor verminderde honger en frustratie. Dit kwam meer tot uitdrukking tijdens de opfok- dan de legperiode. Een lagere dagelijkse eiwitopname leidde echter tijdens de opfok en eerste fase van de legperiode tot een slechtere bevedering.*

In de algemene discussie (hoofdstuk 7) wordt een evaluatie en integratie gegeven van de resultaten, zoals gerapporteerd in de hoofdstukken 2 tot en met 6. De gevonden resultaten zijn vergeleken met resultaten van andere experimenten en mogelijke verklaringen worden gegeven. Praktische en economische implicaties met betrekking tot de nieuwe voerstrategieën, met als doel het verbeteren van de reproductie en welzijn van de ouderdieren sector, worden ook in dit gedeelte geëvalueerd. Het verstrekken van een laag eiwitvoer aan opfok ouderdieren resulteerde in een 6% hogere voerwinst voor de gehele periode. Een hogere energie-eiwit verhouding van de voeders tijdens de eerste fase van de leg resulteerde in een gemiddeld 5% lagere voerwinst. Een hogere energie-eiwit verhouding van het voer tijdens de tweede fase van de leg resulteerde echter in een 3% hogere voerwinst. *Een 15 tot 20% hogere energie-eiwit verhouding in het voer van opfok ouderdieren is een succesvolle strategie om reproductiekenmerken en gedrag te verbeteren. Het advies is om de energie-eiwit verhouding tijdens de eerste fase van de leg niet te veranderen, terwijl een circa 6% hogere energie/eiwit verhouding tijdens de tweede fase van de leg betere broedresultaten geeft.*

Dankwoord

Mijn dank gaat in de eerste plaats uit naar mijn ouders (Riekert en Aaltje) die mij op de wereld hebben gezet. Helaas heeft mijn moeder dit promotietraject niet mee kunnen maken. Geboren op een boerderij in het prachtige Putten is mijn interesse voor dieren al op zeer jonge leeftijd gewekt. Als goed gemengd Veluws bedrijf hielden we daar koeien, varkens, kippen, honden, katten en pony's. Tijdens mijn jeugd ging mijn interesse vooral uit naar kippen en veel minder naar koeien en varkens. Waarom dit was weet ik niet goed, maar het zal wel iets te maken hebben gehad met de grootte en hanteerbaarheid van kippen. Grappig is wel dat de kippen die we thuis hielden, vleeskuikenouderdieren waren. En dat zijn niet echt gezellige kippen als je daar als pakweg 10-jarig jochie tussen loopt. Het rapen van de eieren (met de hand natuurlijk) deed ik dan ook meestal samen met mijn broertje. De een kon de ander dan letterlijk rugdekking geven. Zowel de hennen als hanen reageerden regelmatig behoorlijk agressief op onze aanwezigheid. In het bijzonder de "broedse" hennen verdedigden hun eieren met veel lawaai en agressief pikken naar handen. De hanen echter waren erg bedreven in het in de rug aanvallen. Tijdens mijn studietijd was het vangen van kippen (vleeskuikens en uitgelegde legkippen), samen met broers en vrienden, een zwaar maar financieel aantrekkelijke optie om mijn basisbeurs aan te vullen. Dit was natuurlijk altijd avond- en nachtwerk wat onder de naam ECCC (Emous Chicken Catch Crew) voor de nodige sterke verhalen binnen onze familie heeft gezorgd. Kortom, ik heb een groot gedeelte van mijn jeugd letterlijk tussen de kippen doorgebracht.

Gedurende mijn studie aan de CAH, en vooral tijdens mijn halfjaar stage bij De Schothorst te Lelystad, is mijn interesse voor onderzoek ontstaan. Dit resulteerde bijna logisch in mijn eerste baan bij het toenmalige COVP (Centrum voor Onderzoek en Voorlichting Pluimveehouderij) te Beekbergen. Daar zag ik dat collega's die een HBO vooropleiding hadden afgerond ook een promotietraject konden starten en afronden. Dit bracht mij op het idee dat dit voor mij in de toekomst misschien ook wel zou zijn weggelegd. Na wat uitstapjes bij andere organisaties kwam ik in 1998 toch weer bij de combinatie van mijn eerste liefde uit: onderzoek met kippen! Na jaren onderzoek met legkippen kon ik in 2003, tot mijn grote vreugde, weer aan de slag met vleeskuikenouderdieren. Ondertussen had ik in 2005 min of meer toevallig een gesprek met René Kwakkel die mij wel wilde bijstaan in een promotietraject als die mogelijkheid zich voor zou doen. Nadat ik in 2007 een project had afgerond over de bevruchtingsproblematiek bij vleeskuikenouderdieren, ontstond bij mij het idee om eens serieus naar de voeding van moderne vleeskuikenouderdieren te kijken. Ik werd in dat jaar getriggerd door de uitspraak van Joan van Haandel (vermeerderaar): "Mijn buurman slacht regelmatig vleeskuikenouderdieren en die treft tegenwoordig nauwelijks nog vet aan". Dit was vanuit mijn ervaring een bijzondere opmerking.

Verder had ik in die tijd regelmatig contact met de vermeerderaar Ad Beijer die zichzelf de vraag stelde of het voer wel aansloot bij de behoefte van de vleeskuikenouderdieren die hij in de stal had. Vanuit deze opmerkingen en aanwijzingen begon het besef te groeien dat het voer niet geschikt was voor het moderne vleeskuikenouderdier. Mijn dank gaat dan hier ook uit naar Joan en Ad die een belangrijke rol hebben gespeeld bij het begin van dit onderzoek.

Begin 2009 diende ik een projectplannetje in voor een literatuurstudie naar de voedingsbehoefte van moderne vleeskuikenouderdieren bij de Klankbordgroep vermeerdering van het Productschap Pluimvee en Eieren. Men vond dit zeker de moeite van het onderzoeken waard en daarom zijn contacten gelegd met twee andere belangrijke stakeholders voor dit onderwerp: het Productschap voor Diervoeding en Aviagen-EPI. Hierdoor werd het project flink uitgebreid met twee experimenten verdeeld over drie jaar. Het bij elkaar brengen van deze zeer verschillende partijen ging niet zonder slag of stoot. In totaal besloeg dit een traject van één jaar en vier maanden totdat uiteindelijk op 17 augustus 2010 door alle partijen groen licht werd gegeven. Alle vertegenwoordigers van dit gremium (Piet, Loek, André, Otto, Rik, Marinus, René, Machiel, Frank) worden hartelijk bedankt voor de positieve houding tijdens de totstandkoming en voortgang van het project.

De “go” voor dit project was voor twee van mijn collega’s aanleiding om onafhankelijk van elkaar mij te attenderen op deze grote kans om een promotietraject te starten. Marinus en Teun, hartelijk dank voor deze tip om deze kans te grijpen. Twee dagen bedenktijd waren genoeg om “ja” te zeggen tegen dit “once in a lifetime” moment dat voorbij kwam. Om dit promotietraject te starten heb ik meteen een afspraak gemaakt met René Kwakkel die direct enthousiast zijn medewerking toezegde. Ook Wouter Hendriks zag mogelijkheden om met dit project een promotietraject in te zetten. Waarvoor mijn grote dank.

Persoonlijk zag ik geen grote uitdagingen in het uitvoeren van de experimenten, maar het schrijven van wetenschappelijke artikelen leek mij wel een behoorlijk grote hobbel. Toch is dat helemaal goed gekomen en daar heb ik heel veel support van René voor gekregen. Mijn dank richting René voor de hulp en positieve feedback die ik kreeg op mijn papers is dan ook enorm. Het was wel regelmatig lachen als ik weer enkele fraaie staaltjes “Pengels” (Puttens Engels) aan het papier had toevertrouwd, maar jij wist dat toch weer met diverse aanpassingen om te bouwen naar wetenschappelijk Engels. Het grappige was wel dat ik na een maandje of vier ploeteren op mijn eerste paper het schrijven leuk begon te vinden. Voorafgaande aan de gebruikelijke avondsessies bij de familie Kwakkel werd er eerst genoten van voortreffelijke maaltijden. Emmy, heel hartelijk dank voor je ongekende gastvrijheid en de plezierige gesprekken die we hebben gehad. Na deze sessies werden de papers nogmaals grondig bestudeerd door mijn zeer gewaardeerde collega Marinus. Met allerlei inhoudelijke vragen over voeding, experimenten en analyses kon ik altijd bij hem terecht. Hartelijk dank Marinus! Voordat de papers echt opgestuurd konden worden, werden deze door Wouter kritisch bestudeerd en werden de puntjes op de 'i' gezet.

Uiteindelijk zorgde al dat geploeter en gezweet op die teksten en tabellen ervoor dat de eerste paper geaccepteerd werd. Dit kan zonder overdrijven een van mijn hoogtepunten in mijn leven genoemd worden. Zaterdag 11 mei 2013 lag ik met 40°C koorts op de bank en kreeg vanuit Amerika van Poultry Science een email dat mijn eerste paper geaccepteerd was! Vanaf dat moment ben ik er echt in gaan geloven dat ik het promotietraject tot een goed einde zou brengen.

Het eerste experiment is uitgevoerd op de proefaccommodatie "De Haar" (nu "Carus") in Wageningen. Mijn dank gaat in de eerste plaats uit naar Willem voor zijn coöperatieve en flexibele houding en leerzame gesprekken tijdens de werkzaamheden. Daarnaast wil ik ook Ries, Leen en Rini heel hartelijk danken voor jullie inzet bij mijn experiment. Het tweede experiment is uitgevoerd bij Schothorst Feed Research in Lelystad waar ik veel dank verschuldigd ben aan Annelies. Jij zorgde er voor dat alle eieren verzameld werden, de dieren de juiste hoeveelheid voer kregen en optimaal verzorgd werden. Verder hebben Jack en Wim de nodige arbeid verricht om het experiment perfect te laten verlopen. Joke bezorgde mij iedere week weer de nodige harde en betrouwbare data. Allemaal heel hartelijk dank voor dit intensieve en soms wat saaie, maar onmisbare werk voor het uitvoeren van grondig onderzoek en betrouwbare resultaten. Natuurlijk wil ik ook de studenten Emily, Mart en Christianne die mij hebben geholpen bij de experimenten bedanken voor hun inbreng.

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Tijdens de experimenten kon ik altijd gebruik maken van de brede vleeskuikenouderdieren kennis van Otto van Tuijl. Je hebt een belangrijke bijdrage geleverd om de experimenten prima te laten verlopen. Zelfs de hanen die uit Denemarken kwamen en dus de taal niet spraken en dachten dat het voer uit de lucht kwam vallen, gingen na wat aanpassingen volgens (jouw) plan functioneren.

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25 jaar een geweldige combinatie waarbij we bij elkaar het beste naar boven halen. Ik kan me geen leven zonder jou voorstellen en hoop nog heel lang je maatje te zijn! Ik wil tegen mijn kinderen Rosalie, Jorian en Michiel zeggen dat jullie mijn leven gekleurd hebben in de mooiste tinten. Leef het leven met alles wat je hebt en als je ergens voor wilt gaan, ga er vol en met passie voor zodat je je doel zult bereiken.

Tot slot, dankzij Uw hulp kon ik al het werk verrichten dat heeft geleid tot het klaren van deze klus.

"The miracle isn't that I finished. The miracle is that I had the courage to start" - John Bingham

Rick van Emous
Putten, 6 februari 2015

Curriculum vitae

Rikkert Aalt (Rick) van Emous was born on the 19th of December 1965 in Putten (the Netherlands), where he also spent his childhood, and where he is still living. After primary school, Rick studied at an Agricultural College. In 1985, Rick continued his study at the University of Applied Sciences (CAH) in Dronten. In 1990, he graduated as a professional Bachelor in Animal Production. He accepted a temporary job as research assistant for two years at the Center for Research and Extension for Poultry (COVP) 'Spelderholt' in Beekbergen, where he was involved in a project to reduce the ammonia emission in broiler houses. Thereafter, he joined Cobb-Europe in Putten as a technical-commercial adviser. After a job at MOBA in Barneveld, he returned in 1998 to the Applied Poultry Research 'Spelderholt' in Beekbergen. Here, he focused on the development of alternative housing systems for laying hens. This institute merged with several animal husbandry research institutes to the present institute Wageningen UR Livestock Research. From 2003 onwards, he gradually switched to research projects with broiler breeders. He introduced a new housing system for broilers breeders ('Quality Time') in which females and males are separated for several hours during the day. This innovative system was introduced to improve mating behavior and, as a consequence, fertility. In November 2010, he started his PhD at the Animal Nutrition Group of Wageningen University. At present, Rick is researcher at the department of Animal Nutrition of Wageningen UR Livestock Research in Wageningen.

Rikkert Aalt (Rick) van Emous werd geboren op 19 december 1965 in Putten (Nederland), waar hij opgroeide en tot op heden woont. Na de basisschool, lagere en middelbare landbouwschool startte hij in 1985 met de hogere landbouwschool (CAH) te Dronten. Na zijn afstuderen in 1990 (richting Veehouderij) werkte hij als onderzoeksassistent gedurende twee jaar bij het Centrum voor Onderzoek en Voorlichting voor de Pluimveehouderij (COVP) 'Spelderholt' te Beekbergen waar hij betrokken was bij een project om de ammoniakemissie bij vleeskuikens te verminderen. Hierna ging hij aan de slag als technisch-commercieel medewerker bij Cobb-Europe te Putten. Na werkzaam te zijn geweest bij de MOBA te Barneveld keerde hij in 1998 terug naar het Praktijkonderzoek Pluimveehouderij (PP) 'Spelderholt' te Beekbergen. Bij het PP 'Spelderholt' te Beekbergen lag de focus op het ontwikkelen van alternatieve huisvestingssystemen voor legkippen. Vanaf 2003 werkte hij meer en meer aan projecten met vleeskuikenouderdieren. Zo introduceerde hij een innovatief huisvestingssysteem ('Quality Time') waarbij de hennen en hanen gedurende de dag een aantal uren gescheiden worden. Dit systeem werd geïntroduceerd om het paargedrag en de daarmee samenhangende bevruchting te verbeteren. In november 2010 startte hij zijn promotieonderzoek bij de leerstoelgroep Diervoeding van Wageningen Universiteit. Anno 2014 is Rick werkzaam als onderzoeker bij de afdeling Diervoeding bij Wageningen UR Livestock Research te Wageningen.

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Van Emous, R. A., M. M. Van Krimpen, and R. P. Kwakkel. 2014. Menos proteína, más huevos. Industria Avícola, Mayo 2014, 14-17. (In Spanish).

TRAINING AND SUPERVISION PLAN

Basic package (1.5 ECTS)
WIAS Introduction Course — exemption
Ethics and Philosophy in Life Sciences — 2013

Scientific exposure (14.7 ECTS)
International conferences
2nd International Nutrition Symposium, Wageningen, The Netherlands, November 10 — 2010
18th European Symposium on Poultry Nutrition, Izmir, Turkey, October 31-November 4 — 2011
24th WPSA Poultry Congress, Salvador, Bahia, Brazil, August 5-9 — 2012
2nd International Poultry meat Congress, Antalya, Turkey, April 24-28 — 2013
9th European Symposium on Poultry Welfare, Uppsala, Sweden, June 17-20 — 2013
14th European Poultry Conference, Stavanger, Norway, June 23-27 — 2014
Seminars and workshops
Het gebruik van managementinfo in de Pluimveevleesketen, Wageningen, The Netherlands — 2012
39th Animal Nutrition Research Forum, Utrecht, The Netherlands — 2014
Presentations
Oral presentation Int. Nutrition Symposium, Wageningen, The Netherlands, November 10 — 2010
Oral presentation ESPN, Izmir, Turkey, October 31 - November 4 — 2011
Oral presentation WPSA World's Poultry Congress, Salvador, Bahia, Brazil, August 5-9 — 2012
Oral presentation Poultry Meat Congress, Antalya, Turkey, April 24-28 — 2013
Oral presentation European Symposium on Poultry Welfare, Uppsala, Sweden, June 17-20 — 2013
Oral presentation EPC, Stavanger, Norway, June 23-27 — 2014
Oral presentation 3rd Distributors Meeting Novogen, Lunteren, The Netherlands, April 16-17 — 2013
Oral presentation European Technical Managers Aviagen, Leuven, Belgium, March 19 — 2014
Oral presentation European Technical Managers Aviagen, Edinburgh, Scotland, April 2 — 2014

In-depth studies (6.3 ECTS)
PHLO cursus 'Pluimveevoeding: Nieuwe ontwikkelingen en praktijk' — 2000
Cursus 'Toegepaste statistiek' — 2001
PAOD cursus 'Rode bloedluis (Dermanyssus Gallinae) bij pluimvee' — 2003
Schothorst Feeds and Nutrition course — 2014

Statutory Courses (3.0 ECTS)
Cursus Artikel 9 functionaris — 2012

Professional Skills Support Courses (7.7 ECTS)
Cursus 'Projectmangement training vervolg' — 2008
Cursus 'Commerciele vaardigheden' — 2008
Course Writing for Academic Publication — 2011
Cursus Expert in Sales — 2014

Research Skills Training (6.0 ECTS)
Preparing own PhD research proposal — 2011
Daily experience as project leader of different research projects at WUR-LR (2010-2015) — 2015

Didactic Skills Training (11.6 ECTS)
Lecturing
Lectures Swechick Poultry meeting, Helsingborg, Sweden, September 29-30 — 2010
Lectures workshop DenHatch, Billund, Denmark, January 12-13 — 2011
Lezing workshop Vencomatic BV Sales & RD team, Eersel, The Netherlands, April 18 — 2011
Lezingen JPE Sales & RD team, Barneveld, The Netherlands, Feb-Sep (8 workshops) — 2013
Lezing studenten Veterinair Universiteit, Utrecht, The Netherlands, September 23 — 2013

Supervising theses	
Emily van Calmthout (Major MSc thesis)	2011
Roelof de Jong (Major MSc thesis)	2011
Mart Coolen (Minor BSc thesis)	2011
Christianne van Winkoop (Major MSc thesis)	2014
Management Skills Training (4.0 ECTS)	
Co-organisation Infodagen Fijnstof, The Netherlands, December (4 workshops)	2010
Co-organisation Themamiddag Welzijn, Wijchen, The Netherlands, November 24	2011
Organisation Themamiddag voetzoollaesies vleeskuikens, Wijchen, The Netherlands, May 9	2012
Organisation Themamiddag Ingrepenbesluit, Wijchen, The Netherlands, December 3	2013
Education and Training Total	**54.8**

COLOPHON

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