First week nutrition for broiler chickens

Effects on growth, metabolic status, organ development, and carcass composition

David Lamot

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Thesis

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ABSTRACT

During the first week of life, broiler chickens undergo various developmental changes that are already initiated during incubation. Ongoing development of organs such as the gastro-intestinal tract and the immune system may affect the nutritional requirements during this age period. Despite the residual yolk that is available at hatch and that may provide nutritional support during the first days after hatch, the growth performance may be affected by the time in between hatch and first feed intake. Furthermore, it remains largely unknown to what extend nutritional composition of a pre-starter diet, as well as feed availability directly after hatch have an effect on physiological development directly after hatch, but also at later age. The aim of this thesis was to determine the impact of feed availability and feed composition provided during the first week of life on short-term physiological development, as well as potential long-term effects on growth performance of broiler chickens. Especially early hatched chickens were suggested to benefit more from direct feed access compared to midterm and late hatched chickens, as they tended to have a higher body weight gain during the first week after hatch. A delay in feed access for 48 h resulted in lowered body weight gain and feed intake when compared to direct feed access, but so did a short (13 to 26 h) delay in feed access after hatch. In the latter case, delayed feed access resulted in a lower weight to length ratio of the jejunum and ileum at 4 d of age compared with chickens with direct feed access. Although delayed feed access after hatch resulted in lower body weight gain during the first week after hatch and thereafter, it can be discussed whether this is truly an impairment of long-term growth or just a delayed onset of growth. With respect to feed composition, the inclusion of fish oil and medium chain fatty acids in a pre-starter diet had minor effects on humoral immune function. Inclusion of medium chain fatty acids did result in higher body weight gain and lowered feed efficiency during the first week of life, but only during the period it was provided. Feeding increased diet densities during the first week of life, obtained by formulating diets with different dietary fat levels, resulted in an increased gain to feed ratio, whereas body weight gain and feed intake decreased. Despite the shift in dietary energy supply from carbohydrates to fat and the perceived lower fat digestibility in young broiler chickens, nitrogen metabolizability and fat digestibility were not affected in the current study by feeding increased diet densities. The relative crop, liver and pancreas weights decreased when feeding increased diet densities, whereas the length of the entire intestinal tract increased. This suggests that broiler chickens repartition visceral organ development in response to feeding more concentrated diets during the first week of life. Interestingly, protein and fat accretion were not affected. Continued feeding of increased diet densities after 7 d of age resulted in increased BW gain, G:F ratio and metabolizable energy intake, but mainly during the periods that these diets were provided. In summary, even short durations of delayed feed access already impact intestinal development of young broiler chickens. However, a delayed feed access up to 48 h after hatch does not result in impaired growth, but only a delayed onset of growth. Even though digestibility of fats and oils may be suboptimal in young broiler chickens, feeding of these diets does not have to result in lowered performance per se. Young broiler chickens appear to adapt themselves towards high density diets with high fat inclusion levels in the first week of life, enabling them to digest and metabolize these diet types despite a suboptimal capacity for fat digestion. High density diets result in higher growth performance, but only for the period these diets are provided and thus carry-over effects at later age appear to be limited.

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CHAPTER 1

General Introduction

INTRODUCTION

The first week of life of a broiler chicken has a large impact on its health, welfare, and growth performance as it matures. One of the important aspects in the first week is the nutrition for the newly-hatched chicken. To provide proper nutritional support for broiler chickens during the first week of life, it is important to have a strong understanding of the physiological status of broiler chickens at this age. The physiological status, with a particular emphasis on digestive status, determines the broiler chickens' potential for nutrient digestibility, utilization, and retention.
This introduction describes (1) the physiological status and developmental transitions of the broiler chicken during the first week of life, (2) the digestive capacity of the chicken during the first week of life, and (3) how the physiological and digestive status of the broiler chicken during the first week of life can be affected through nutrition. The general introduction concludes with the aims and outline of the thesis.

PHYSIOLOGICAL STATUS DURING THE FIRST WEEK OF LIFE

During the first week of life, the broiler chicken undergoes major physiological transitions, such as maturation of the gastrointestinal tract (GIT) and the immune system, as well as development of the thermoregulatory system (Christensen, 2009). Coinciding with these physiological transitions, the remaining residual yolk is metabolized and exogenous feed is offered as a nutrient source.

Gastrointestinal tract

Maturation of the GIT starts during the final stage of incubation, initiated by absorption of the amniotic fluid (Moran, 1985) and changes in hormones (cortisol, thyroid hormone) and growth factors (Bohórquez, 2010). Maturation of the GIT in young broiler chickens has been extensively reviewed (Wijtten et al., 2012b; Lilburn and Loeffler, 2015) and is mainly reflected by increased overall length and weight. The small intestine increases about 3 times in relative weight during the first week of life, mainly due to villi development to increase the absorptive area of the GIT. Villi development is measured by greater villi length and increased villi density, resulting in a larger surface area available for absorption of nutrients provided through feed. The surface area of a single villi develops faster in the duodenum than in the jejunum and ileum (Geyra et al., 2001b), but when combined with the number of villi per intestinal section (duodenum, jejunum, ileum), the jejunum has a larger total villus surface area compared to duodenum and ileum from approximately 3 d of age onwards. During the first days after hatch, enterocytes within these villi slightly change their functionality, moving from absorption

of macromolecules (such as immunoglobulins) during incubation towards absorption of dietary nutrients during the first days after hatch and thereafter (Moran, 2007). This change in functionality of enterocytes is already initiated during the final incubation stage, as demonstrated in turkey embryos (De Oliveira et al., 2009). By studying gene expression patterns involved with enterocyte brush border enzyme and nutrient transporter activity, it was found that the digestive capacity at hatch is limited to relatively simple molecules, such as small peptides and mono- and disaccharides. A more developed small intestine by means of length and villi number and size may stimulate nutrient absorption by both a higher absorption surface and a longer transit time of feed (Noy and Sklan, 1995). The rapid development of the GIT relative to other organ systems in the first week of life suggests that a large portion of the retained energy and protein at this age is allocated for intestinal development.

Immune system

As a first line of immunological defense, young broiler chickens mainly depend on their innate immune system and maternal antibodies that are deposited by the hen into the egg (Bar-Shira and Friedman, 2006; Hamal et al., 2006). The innate immune response consists of cellular responses by macrophages, dendritic cells, heterophils, and natural killer cells, the alternative complement pathway, and a morphologically mature intestinal tract by means of a sufficient mucus layer and developed intestinal epithelial cells. Although the innate immune system is functional at the time of hatch, its development continues during the first days after hatch. Within the GIT, goblet cells commence acidic mucin production during the final stage (>18 d) of incubation (Uni et al., 2003), functioning as a protective layer against pathogens within the intestine. Within two days after hatch, the intestinal tract is populated by matured heterophils, protecting the intestinal tract through secretion of antibacterial β-defensins (Bar-Shira and Friedman, 2006). Excretion of β-defensins by heterophils compensates for a lack of secretion of defensins from enterocytes within the gut epithelium at that age. The acquired immune system is largely represented in the gut associated lymphoid tissue (GALT) that initiates its function after the first two weeks of life: firstly by cellular responses followed by humoral responses (Bar-Shira et al., 2003). B-cells, part of the acquired immune response and responsible for antibody production, start to colonize the small intestine from 4 d of age onwards, but they only become functional at approximately 2 weeks of age (Bar-Shira et al., 2003). To compensate for this immature acquired immunological development during the first two weeks of age, a young broiler chicken has to rely on maternal antibody protection. Maternal antibody transfer consists primarily of IgY, but also limited amounts of IgM and IgA (Hamal et al., 2006). Maternal IgA is mainly transferred into the intestinal tract of a young broiler chicken, where it disappears during the first two weeks of age (Lammers et al., 2010). In addition, maternal IgY and IgM levels gradually decrease during the first 2 to 3 weeks of age (Sahin et al.,

2001). The time period during which maternal immunoglobulins decline and the acquired immune system is still developing may cause a potential gap in immune protectiveness that puts a young broiler chickens at risk.

Thermoregulatory system

Chickens act as poikilothermic during embryonic development and shortly after hatch, meaning that their body temperature may vary, depending on environmental conditions (Tazawa et al., 2001). Chickens are considered to act as homeothermic from approximately 10 d of age onwards, meaning that they can keep their body temperature relatively constant, within a range of ambient temperatures (Nichelmann and Tzschentke, 2002). The transformation from being poikilothermic to homeothermic is considered to be a combination of neural and endocrinological processes (Baarendse et al., 2007; Debonne et al., 2008). Moreover, dietary composition was found to affect thermoregulatory properties (Van den Brand et al., 2010). A pre-starter diet with and without additional animal fat inclusion resulted in the lowest rectal temperature decrease when early post-natal chickens were exposed to mild cold stress (20°C for half an hour at 2 to 3 d of age) compared to broiler chickens that were fed either dextrose, albumen, or nothing. It was hypothesized that feeding broiler chickens a complete diet instead of single ingredients would result in increased metabolism (increased heat production and body temperature) due to better intestinal development (Van den Brand et al., 2010). This increase in metabolism was not found in treatments in which single ingredients were provided (dextrose, albumen).

Residual yolk

The residual yolk functions as the primary nutrient supply for broiler chickens directly after hatch, a function that is gradually taken over by ingested feed. The nutritional role of the residual yolk is unclear, as some studies estimate that the residual yolk only contributes about 10 to 11% of the total energy and protein intake from 0 to 3 d of age (Wijtten, 2011), whereas others estimate this to be approximately 30% (Murakami et al., 1992). It is expected, though, that the relative contribution of the residual yolk is dependent on the amount and composition of the exogenous feed consumed. If the residual yolk actually functions as a backup nutrient source during the first days after hatch and when no exogenous feed is available, it may be hypothesized that the absorption rate of the residual yolk will increase during periods without feed availability. This is in line with Noy et al. (1996), who demonstrated that delayed feed access after hatch (48 to 96 h) resulted in lowered yolk weights at 96 h post-hatch compared to direct fed chickens. However, other studies with delayed feed access up to 48 to 72 h after placement in a grow-out facility found no differences in yolk utilization (Gonzales et al., 2003; Van den Brand et al., 2010). This can be explained by the assumption that feed intake results in increased metabolism and as a consequence more of the residual yolk is metabolized, thus resulting in a net equal residual

yolk absorption rate between direct and delayed fed chickens. Besides functioning as a nutrient supply, the residual yolk may also have an impact on health status of young broiler chickens, because it contains maternal immunoglobulins (mainly IgY; Ulmer-Franco et al., 2012) and other immunological constituents (e.g. cytokines), while the innate (by means of morphological development of the intestinal tract) and the acquired immune system are still under development (see above).
Concluding, the first week of life is a very dynamic period for the broiler chicken as it goes through various physiological transitions. These transitions should be taken into account to ensure proper fulfilment of the chicken's nutritional requirements.

DIGESTIVE STATUS DURING THE FIRST WEEK OF LIFE

From the first week of incubation onwards, broiler chicken embryos mainly rely on lipid oxidation for their growth and development, as the yolk primarily contains fatty acids that may function as an energy source (De Oliveira et al., 2008). At hatch, the residual yolk still consists mainly of fatty acids (Yadgary et al., 2010). Nevertheless, chickens are provided a diet rich in complex carbohydrates at placement on a broiler farm (Sklan, 2001). The switch from fats towards carbohydrates as the primary energy supply for young broiler chickens may be perceived as contradictive, given their reliance on lipid metabolism during most of the incubation process. This requires a better understanding of the digestive status of chickens during the first week of life.

Nutrient digestibility

Fulfilment of the nutritional requirements of broiler chickens during the first week of life requires proper understanding of ingredient digestibility and metabolizability, as this ultimately affects the nutrient availability for utilization. Because the GIT is not fully mature yet at the moment of hatch, it appears to be logical to suggest that digestibility and metabolizability of various nutrients are also not optimal.
For example, net enzyme secretion in the duodenum (amylase, trypsin and lipase) increases significantly as the chicken ages (Noy and Sklan, 1995), supported by age dependent activity of amylase, trypsin, lipase, and maltase in the pancreas (Sell et al., 1991). Although it is suggested that bile salt secretion is limited in a young broiler chicken (Krogdahl, 1985), bile acids are abundant from 2 d of age onwards and only decline at a later age (Green et al., 1987). Digestibility coefficients for dietary nitrogen, starch, and fat have been determined for broiler chickens at various ages in a number of studies (Table 1). Although digestibility coefficients depend on the location within the GIT where they are determined (fecal vs. ileum, with or without correction for urea N; Garcia et al., 2007), nitrogen and starch digestibility tend to be already high at hatch and remain high as the broiler chicken ages.

Table 1. Digestibility coefficients for dietary nitrogen (N), starch, fat, and fatty acids in corn, wheat, and sorghum based diets for broiler chickens at various ages.

			Age, d									
Nutrient	**Basal diet**[1]	**Type**[2]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**21**	**Ref.**[3]
N	Wheat-SBM	F, M	-	0.79	0.75	0.66	0.64	0.56	0.59	0.60	0.57	3
	Sorghum-SBM	F, M	-	-	0.76	-	0.66	-	0.61	0.64	-	3
	Corn-SBM	F, M	-	0.82	0.76	0.72	0.67	0.70	0.63	0.62	0.63	3
	Corn-SBM	I, M	-	-	-	0.78	-	-	0.80	0.9	0.93	2
Starch	Wheat-SBM	F, D	-	-	-	-	0.95	-	0.90	0.97	-	3
	Sorghum-SBM	F, D	-	-	-	-	0.95	-	0.92	0.97	-	3
	Corn-SBM	F, D	-	-	-	-	0.96	-	0.94	0.98	-	3
	Corn-SBM	F, D	0.93		0.93		-	-	0.97	0.99	0.99	1
	Corn-SBM	I, D	-	-	-	0.85	-	-	0.82	0.85	0.83	2
Fat	Wheat-SBM	F, D	-	-	-	-	0.62	-	0.42	0.76	-	3
	Sorghum-SBM	F, D	-	-	-	-	0.52	-	0.43	0.76	-	3
	Corn-SBM	F, D	-	-	-	-	0.69	-	0.65	0.78	-	3
	Corn-SBM	F, D	0.61		0.58		-	-	0.59	0.74	0.73	1

[1]SBM = soybean meal. [2]Determination type can be either ileal (I) or fecal (F) digestibility (D) or metabolizability (M). [3]Reference: 1. Batal and Parsons (2002b), 2. Noy and Sklan (1995), 3. Thomas et al. (2008).

Fat digestibility has been extensively reviewed to be age-dependent (Ravindran et al., 2016), which is also observed in Table 1 and Table 2, where fat digestibility is low during the first week of life and continues to increase from 7 and 14 d of age. Moreover, Tancharoenrat et al. (2013) reported that digestibility of fats is not only highly age-dependent, but also depends on the degree of saturated and unsaturated fatty acids (Table 2). In work with turkey poults, Turner et al. (1999) determined that, the dietary fat digestibility coefficient from 3 to 8 d of age increased approximately 29% (0.70 vs. 0.90) when a blend of animal and vegetable fat in the diet was replaced with medium chain triglycerides (76% $C_{8:0}$). Individual amino acid digestibility increases over time when measured in corn and canola based diets (Table 3), for which the average digestibility rates are comparable to the nitrogen digestibility rate reported in Noy and Sklan (1995). In addition to being age dependent, amino acid digestibility also depends on the raw material (Batal and Parsons, 2003), where processed soybean products (soy protein isolate, soy protein concentrate) appear to be better digestible at a younger age than regular soybean meal.

Energy utilization

Dietary energy utilization, defined as the amount of energy retained divided by the amount of feed intake, is known to vary depending on the energy source used (fat, carbohydrates or protein) and age of the chicken (Sulistiyanto et al., 1999; Batal and Parsons, 2002b;

Table 2. Fecal apparent fat digestibility of different fat sources for broiler chickens at various ages (modified from Tancharoenrat et al., 2013).

	U:S ratio[1]	Digestibility coefficient, DC			Relative increase in DC[2], %		
Age, d		**7 d**	**14 d**	**21 d**	**7-14 d**	**14-21 d**	**7-21 d**
Soybean oil	5.06	0.59	0.90	0.97	152	107	163
Tallow	0.80	0.37	0.65	0.74	177	113	200
Tallow:SBO[3]	2.93	0.50	0.83	0.83	166	100	166
Poultry fat	2.07	0.60	0.85	0.93	141	110	155
Palm oil	0.93	0.60	0.81	0.84	134	104	139

[1]U:S ratio: ratio between unsaturated and saturated fatty acids in the raw material.
[2]Relative difference calculated between two subsequent ages.
[3]Blend of tallow and soybean oil (at 50:50 ratio).

Thomas et al., 2008). Energy utilization may even vary among energy sources; for example, dextrose is known to have a higher ME_n value than high amylose starch (independent of broiler age), while both being carbohydrate sources (Batal and Parsons, 2004). Although dietary fat digestibility appears to be age-dependent (Batal and Parsons, 2002b; Thomas et al., 2008), energy utilization of various fat sources (coconut oil, beef tallow, safflower oil) does not, in contrast to various cereal and protein sources (Sulistiyanto et al., 1999).
Concluding, the digestive capacity of broiler chickens is both age- and ingredient-dependent. Based on current nutrient digestibility data, it may be suggested that carbohydrate sources are more easily digestible than fats during the first week of life, although this is based on a limited number of studies available during this stage of life.

FEEDING STRATEGIES DURING THE FIRST WEEK OF LIFE

Feed access after hatch

Optimally incubated chickens hatch within a time frame (hatch window) of approximately 24 h and are only removed from the incubator when the majority of the chickens have hatched. In commercial hatcheries, hatch windows may last up to 36 h (Decuypere et al., 2001). Combined with chicken handling in the hatchery and transportation, the time between hatch and first feed intake may last up to 72 h (Noy and Uni, 2010; Willemsen et al., 2010). As a result, early hatched chickens spend a relatively long period within the incubator at a relatively high ambient temperature without feed or water access compared to midterm and late hatched chickens. Consequently, they have a higher risk of dehydration and a relatively longer delay in the onset of various physiological developments as described by Christensen (2009). This suggests that broiler chickens of the same batch of eggs may experience the effect of 'delayed' feed availability on

Table 3. Digestibility coefficients for dietary amino acids in corn with either soybean meal (SBM) or canola based diets for broiler chickens at various ages.

			Age, d					
Amino Acid	**Basal diet**	**Type[1]**	**1-2**	**3-4**	**7**	**14**	**21**	**Ref.[2]**
Arginine	Corn-Canola	D	0.83	0.85	0.88	0.88	0.89	1
Arginine	Corn-SBM	D	0.86	0.86	0.90	0.91	0.91	1, 2
Arginine	Corn-SBM	M	-	0.87	0.92	-	0.93	3
Cysteine	Corn-Canola	D	0.68	0.66	0.72	0.74	0.77	1
Cysteine	Corn-SBM	D	0.66	0.61	0.73	0.78	0.80	1, 2
Cysteine	Corn-SBM	M	-	0.64	0.77	-	0.81	3
Histidine	Corn-Canola	D	0.76	0.78	0.82	0.82	0.83	1
Histidine	Corn-SBM	D	0.81	0.80	0.85	0.87	0.88	1, 2
Histidine	Corn-SBM	M	-	0.81	0.88	-	0.90	3
Isoleucine	Corn-Canola	D	0.76	0.77	0.81	0.82	0.83	1
Isoleucine	Corn-SBM	D	0.80	0.80	0.86	0.88	0.88	1, 2
Isoleucine	Corn-SBM	M	-	0.81	0.88	-	0.90	3
Leucine	Corn-Canola	D	0.80	0.81	0.84	0.85	0.85	1
Leucine	Corn-SBM	D	0.81	0.81	0.87	0.89	0.90	1, 2
Leucine	Corn-SBM	M	-	0.83	0.89	-	0.91	3
Lysine	Corn-Canola	D	0.73	0.75	0.79	0.80	0.80	1
Lysine	Corn-SBM	D	0.82	0.82	0.85	0.87	0.88	1, 2
Lysine	Corn-SBM	M	-	0.83	0.89	-	0.91	3
Methionine	Corn-Canola	D	0.80	0.74	0.80	0.81	0.81	1
Methionine	Corn-SBM	D	0.82	0.83	0.88	0.91	0.91	1, 2
Methionine	Corn-SBM	M	-	0.87	0.93	-	0.95	3
Phenylalanine	Corn-Canola	D	0.74	0.73	0.81	0.82	0.81	1
Phenylalanine	Corn-SBM	D	0.77	0.77	0.86	0.88	0.88	1, 2
Phenylalanine	Corn-SBM	M	-	0.84	0.89	-	0.91	3
Threonine	Corn-Canola	D	0.66	0.68	0.73	0.74	0.75	1
Threonine	Corn-SBM	D	0.71	0.68	0.79	0.83	0.84	1, 2
Threonine	Corn-SBM	M	-	0.71	0.80	-	0.85	3
Valine	Corn-Canola	D	0.74	0.75	0.79	0.80	0.81	1
Valine	Corn-SBM	D	0.78	0.77	0.84	0.86	0.87	1, 2
Valine	Corn-SBM	M	-	0.74	0.83	-	0.86	3
Av. AA	Corn-Canola	D	0.75	0.75	0.80	-	0.82	1
Av. AA	Corn-SBM	D	0.78	0.77	0.82	0.86	0.87	1, 2
Av. AA	Corn-SBM	M	-	0.79	0.87	-	0.89	3

[1]Digestibility (D) or metabolizability (M). [2]Reference: 1. Batal and Parsons (2002b), 2. Batal and Parsons (2002a), 3. Biggs et al. (2007).

physiological development differently due to differences in biological age. Interestingly, irrespective of their biological age at pulling, these chickens all start at 'day 0' once they arrive at a grow-out facility. Careghi et al. (2005), therefore, made a distinction between the biological and chronological age of broiler chickens. Using this differentiation, the chronological age can be similar for one batch of chickens, while early, midterm, and late hatched chickens differ in biological age.

Especially during the first days after hatch, delayed feed access may negatively affect chicken development. With respect to the GIT, this is reflected by a lower absolute weight, shorter intestinal length, lower enzymatic activity, decreased villi and crypt cell density, and lower crypt depths and villi heights (Corless and Sell, 1999; Geyra et al., 2001a; Gonzales et al., 2003; Maiorka et al., 2003). Intestinal development is not always quantified for individual parts of the intestinal tract (duodenum, jejunum, ileum, and caecum), while it appears that some parts are more sensitive for delayed feed availability than others (Geyra et al., 2001a). Unfortunately, the exact duration of delayed feed availability is not always well-defined in studies. Some studies define the time till first feed intake only after all chickens were pulled from the incubator, therewith ignoring possible interactions of delayed feed availability with moment of hatch, while in other studies these two factors interacted (Careghi et al., 2005; Van de Ven et al., 2011). An interaction between delayed feed availability and moment of hatch implies that direct feed access may have differentiated effects on broiler chickens of various biological ages (early, midterm, or late hatchers).

Feed Composition

Various studies have examined the effect of feed composition during the first week of life on short and long-term growth and development of broiler chickens: individual ingredients (Noy and Sklan, 1999; Van den Brand et al., 2010), protein levels and sources (Noy and Sklan, 2002; Longo et al., 2007; Everaert et al., 2010; Wijtten et al., 2012a), minerals (Maiorka et al., 2004; Garcia et al., 2006), cellulose (Noy and Sklan, 2002), and macro nutrient ratios (Swennen et al., 2009). Substantial attention has been given to the importance of protein level and quality in relation to intestinal development, which has been reviewed by Wijtten et al. (2012b). In general, it can be concluded that deficiencies of essential amino acids or imbalances of the amino acid profile may result in impaired intestinal development from 0 to 7 d of age, by means of reduced villi height and overall intestinal weight. On the contrary, an increase of the overall amino acid inclusion levels in feed provided from 0 to 14 d of age resulted in an increased duodenum weight at approximately 7 d of age (Wijtten et al., 2010).

Although some studies have looked into the inclusion of increased dietary fat levels in the diet (Noy and Sklan, 2002; Van den Brand et al., 2010), in general the inclusion of fats and oils in a pre-starter diet is considered undesirable due to their low digestibility in young broiler chickens compared to starch (see *Digestive status during the first week of life)*.

Based on the perception that fats and oils are generally not well digestible in young broiler chickens, diets fed during this age period are often rich in carbohydrates (Sklan, 2001). Consequently, limited research has been conducted related to the role of dietary fats and oils in diets for broiler chickens during the first week of life.

THESIS AIM

The aim of this thesis is to determine effects of the moment of first feed intake after hatch and the effect of feed composition (with special attention to dietary fat inclusion) on broiler chickens during the first week of life as well as short and long-term effects on growth, metabolic status, organ development, and carcass composition.

OUTLINE OF THE THESIS

The first part of this thesis describes effects of the moment of first feed intake after hatch on growth performance and physiological development of chickens. In Chapters 2 and 3, studies are described that focused on the effect of the spread of hatch related to the moment of first feed intake after hatch (Chapter 2), as well as the moment of first feed intake after hatch related to the inclusion of dietary fats (soybean oil, fish oil, or medium chain fatty acids; Chapter 3) on broiler chicken growth, organ development, carcass composition, and (humoral) immune status.
The second part of this thesis describes the effects of feeding increased diet densities during the first week of life, coinciding with increased dietary fat levels, on growth and metabolism (Chapter 4) as well as digestive organ size and nutrient utilization (Chapter 5) of broiler chickens. Subsequently, Chapter 6, refers to a study where the potential long-term effects of feeding increased diet densities during the first week of life on growth and carcass composition were examined. In Chapter 7, the results obtained from the studies described in Chapters 2 till 6 are combined and discussed.

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CHAPTER 2

Effects of moment of hatch and feed access on chicken development

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ABSTRACT

The current study evaluated effects of hatch moment and immediate feed and water access within a 24-h hatch window on chicken growth and development. Five hundred four male chickens obtained from a 49-wk-old Ross 308 breeder flock were assigned to 72 cages based on hatching moment (early, midterm, or late; selected during periods of 475 to 481, 483 to 487, and 489 to 493 h after onset of incubation). At the end of each hatching period, chickens were moved to the grow-out facility and one-half of the chickens received feed and water ad libitum immediately. Remaining chickens received feed and water from 504 h after onset of incubation (d 0). Body weight gain and feed intake for each cage were recorded at d 0, 1, 4, 7, 11, and 18. Chickens were sampled at d 4 and 18 for organ and carcass development. Early hatchers had lower BW at placement compared with midterm and late hatchers but compensated for this afterward, resulting in a higher BW at d 4 (112.8, 107.1, and 103.3 g, respectively). From d 0 to 18, early hatchers tended to have higher BW gain than both other groups. Relative breast meat yield at d 18, expressed as percentage of carcass weight, was higher for early (30.4%) than midterm (28.5%) and late hatchers (27.8%). Up to d 7, direct feed access resulted in higher BW gain (6.1%) and feed intake (4.2%) compared with delayed feed access. No effect of moment of feed access on feed efficiency or organ weights was found. Direct feed access resulted in a higher weight:length ratio of the jejunum (12.5%) and ileum (7.5%) at d 4 compared with delayed feed access. These results suggest that early hatchers have a different developmental and growth pattern than midterm or late hatchers within a 24-h hatch window. A mild delay in feed access after hatch affects growth and development during the first week after hatch.

Key words: first week nutrition, hatch window, physiological development, broiler chicken

INTRODUCTION

Optimal growth and development of a chicken can only be achieved when these are supported from the moment of hatch onward. The first week after hatch counts for approximately 20% of the lifespan of modern broilers, emphasizing the importance of this period for subsequent performance. In current practice, time between hatch and moment of first feed intake (**FI**; holding time) may take up to 72 h because of variation in hatch time, chick handling, and transportation time (Noy and Uni, 2010; Willemsen et al., 2010). As a result of the delayed time between hatch and moment of first feed intake, early hatched chickens spend a relatively long time within the incubator without feed or water access, being at risk for dehydration as well as impaired or delayed physiological development. This puts the chick at risk because, as Christensen (2009) described, especially around hatch the chicken undergoes major physiological transitions, such as maturation of the immune and thermoregulatory systems, and the gastrointestinal **(GI)** tract. Maturation of the GI tract can be reflected by overall length and weight as well as villi length and area (Uni et al., 1999). Feed deprivation, especially during the first days after hatch, depresses intestinal development on the short and long term, reflected by a lower intestinal weight, shorter length, lower enzymatic activity, altered villi and crypt cell density, and lower crypt depths and villi heights (Corless and Sell, 1999; Geyra et al., 2001a; Gonzales et al., 2003; Maiorka et al., 2003).

Unfortunately, the duration and timing of feed deprivation is not always well-defined in literature. Some studies vary the time till first FI only after all chickens were pulled simultaneously from the incubator, thereby ignoring possible interactions of delayed feed access with moment of hatch (i.e., time spent in the incubator until pulling). A study by Careghi et al. (2005) found that these two factors (age at hatch and feed + water availability) interacted when trying to quantify the effect of hatch moment (i.e., holding time inside the incubator) on development and growth during the grow-out period. It appeared that especially late hatched chickens may benefit from direct feed access, in contrast to early hatched chickens. The relative organ growth from hatch until regular pulling age was significantly delayed in chickens withheld from feed and water for 32 h after hatch compared with those with direct feed and water access from hatch onward (Van de Ven et al., 2013). Maturation of the GI tract was significantly lower for withheld chickens compared with chickens with direct feed and water access, reflected in a shorter intestinal length (-20%). Also, relative spleen (-44%) and liver (-31%) weights were reduced as a consequence of delayed feed and water access until pulling. In the same study, early hatched chickens were found to have lower glucose and thyroxine levels directly after hatch compared with midterm and late hatched chickens (Van de Ven et al., 2013). Moreover, these physiological and metabolic differences between groups of early, midterm, and late hatched chickens directly after hatch had disappeared when measured at pulling (515 h after onset of incubation).

In studies conducted until now, the hatch window was often longer than 30 h. It is unknown if physiological differences between early, midterm, and late hatched chickens remain when the hatch window is shorter. In addition, studies to quantify the physiological and metabolic effects of hatch moment and feed access mainly focus on the first week after hatch. As a consequence, potential crossover effects of hatch moment and moment of feed access on organ growth or carcass composition at a later age are rarely considered.
The objective of this study was to examine the effect of direct versus delayed feed and water access immediately after hatch on growth, organ development, and carcass composition of chickens hatched at different intervals within a short (24 h) hatch window until d 18 of age.

MATERIALS AND METHODS

All procedures in this study were approved by the Ethical Committee of the Animal Sciences group of Wageningen University and Research Centre, Lelystad, the Netherlands.

Experimental design

In this study, the effects of hatch moment within a 24-h hatch window and moment of feed and water access after hatch were studied using a 3 × 2 factorial design. Treatments were hatch moment (475 to 481, 483 to 487, and 489 to 493 h after onset of incubation) and moment of feed and water access after hatch. The latter was either direct (within 0.5 h from the moment of pulling; 481.5, 487.5, and 493.5 h after onset of incubation) or delayed (504 h after onset of incubation). Per hatch moment, 168 male chickens were evenly distributed over 24 cages (n = 72 cages total). The average biological age of early, midterm, and late hatched chickens at placement in the grow-out unit was 478, 485, and 491 h after onset of incubation, respectively. One-half of the cages received ad libitum feed and water directly after placement. In the remaining cages, ad libitum feed and water were provided from 504 h after onset of incubation (d 0) onward. Early, midterm, and late hatched chickens with delayed feed access had approximately 26, 19, and 13 h of no feed and water access after hatch, respectively.

Incubation

Hatching eggs (n = 3,218; weight range 56 to 79 g) of a Ross 308 breeder flock (49 wk of age) were incubated from embryonic day (**E**) 0 to E18 at an eggshell temperature of 37.8°C and 55.0% RH using 2 NatureForm NMC 2340 incubators (NatureForm Inc., Jacksonville, FL). Eggs with cracked, dirty, or deformed shells were excluded from the study at the moment of allocation. At E14, eggs were candled and at E18 eggs were transferred into hatching baskets within the same incubators. From E18 onward, both incubator temperatures were fixed at 32.7°C.

The number of hatched chickens and sex ratio were recorded at a 2-h interval from 469 h of incubation onwards to determine the actual hatch window and to mark male chickens as being early, midterm, or late hatched chickens. Male chickens were only marked when cleared from the shell.

Newly hatched chickens were colored on the head using felt pens having different colors, meaning that the moment of hatch could be taken into account while allocating chickens in the grow-out unit. The room temperature in which the incubators were located was increased until approximately the same temperature as the set incubator temperature to minimize temperature fluctuations and to reduce the potential effect on hatchability of the nonhatched eggs. At the end of each hatch period (early, midterm, or late), the male chickens were directly transferred to a unit for the grow-out period until d 18 posthatch.

Grow-out housing and management

For the grow-out period, a unit was available containing 72 cages (50 × 50 cm) with a housing capacity of 7 chickens per cage. The 6 treatments were randomly assigned to cages within 12 blocks, resulting in 12 replicates per treatment. Cages had a raised wire floor covered with a rubber pad. Artificial lighting was set for 23 h/d from d 0 to 2, 16 h/d from d 2 to 6, 21 h/d from d 6 to 7, and 18 h/d from d 7 to 18. Temperature was set to gradually decrease by 0.5°C per day during the first 14 d, from 34°C at d 0. From d 14 onward, the temperature was set to gradually decrease until a final temperature of 26°C was reached at d 18. At the start of the study, feed was supplied using a small feeder placed within the cage, positioned on top of a paper cover, which in turn was placed on top of the wire floor and rubber pad. From d 4 onward, the feed was supplied in a metal feeder trough placed in front of the cage. Each cage was equipped with two nipple drinkers adjustable in height. A commercially available prestarter diet (2.0 mm pellet; 3,100 kcal of $AME_{n,}$ 1.30% true fecal digestible lysine) was provided from d 0 to 4, followed by a commercially available starter diet (2.5 mm pellet; 2,750 kcal AME_n, 1.06% true fecal digestible lysine) from d 4 to 18.

Data collection

At 504 h after onset of incubation (d 0), hatchability was calculated as percentage of total placed eggs and as a percentage of fertile eggs (eggs which contained living embryos after E14). Feed consumption and BW were determined at placement and d 0, 1, 4, 7, 11, and 18 during the grow-out period. Individual BW were collected at placement and d 18, whereas for the remaining BW weighings only cage weights were collected. The FI measurements were recorded per cage.

The G:F ratio was calculated based on calculated BW gain and FI. At d 4 and 18, 1 randomly chosen chicken was weighed, killed and dissected per cage to determine organ weights of heart, proventriculus plus gizzard, liver, pancreas, bursa, residual yolk (d 4 only), and breast fillet (d 18 only). Chickens were killed by cervical dislocation at d 4 and by carbon dioxide

at d 18. Chickens were not feed deprived in advance of dissection and organ weights were calculated as a percentage of the fed BW, except for breast meat yield, which was calculated as a percentage of empty carcass weight. Carcass weight included feathers, head, blood, and legs. For the intestinal tract, the empty weight and length of the duodenum (duodenal loop excluding pancreas), jejunum (end duodenum to Meckel's diverticulum), ileum (Meckel's diverticulum to ileal-caecal junction), and cecum were measured. Intestines were emptied by gentle squeezing. The midsection (1 cm) of each intestinal part was collected at d 4 and stored in formaldehyde solution. Cross-sections of each part were made and stained using haematoxyline-eosine (GD Deventer, Deventer, the Netherlands). Samples were subjected to histological analysis (villi length and width) using digital microscopy software (Motic Images Plus 2.0, Motic Instruments Inc., Richmond, Canada).

Statistical analysis

Data were subjected to mixed model analysis using the PROC MIXED procedure in SAS (Version 9.3, 2011, SAS Institute Inc., Cary, NC) according to the statistical model:

$$Y_{ijk} = \mu + \alpha_i + \gamma_j + \alpha \times \gamma_{ij} + b_k + \varepsilon_{ijk}$$

where Y_{ijk} = dependent variable, μ = overall mean, α_i = fixed effect of hatch moment (i = early, midterm, or late), γ_j = fixed effect of moment of feed access after hatch (direct or delayed), b_k = random block effect inside grow-out unit (k = 1, 2, ..., 12), and ε_{ijk} = residual error. Data were analyzed, using cage as the experimental unit. Data are expressed as least square means. Effects were considered to be significant when $P \leq 0.05$.

RESULTS

General

Average fresh egg weight was 68.1 g. At candling (E14), 434 eggs (13.5% of total) were removed. Overall hatchability was 97% of fertile eggs. The majority of the chickens hatched within a window of 24 h. Of all placed eggs, 6.3% hatched either before the early hatch period or after the late hatch period.

Hatch moment × feed access

At d 0, direct feed access increased BW for early hatched chickens, whereas it did not for midterm and late hatched chickens ($P = 0.001$; Table 1). Direct fed early hatched chickens had a higher BW compared with early hatched chickens witheld from feed and water (5.3%), whereas this effect was smaller for midterm (1.6%) and late hatched chickens (0.2%). Relative heart weight at d 4 in delayed fed late hatched chickens was higher compared with

Table 1. The effect of hatch moment and moment of feed access after hatch on BW, BW gain, feed intake, and G:F ratio of chickens, expressed as least square means[1]

		BW, g				BW gain, g/d				Feed intake, g/d					Gain to feed ratio				
	Feed access	Placement[2]	0 d	4 d	18 d	0-4 d	4-7 d	7-11 d	11-18d	0-18 d	0-4 d	4-7 d	7-11 d	11-18d	0-18 d	0-4 d	4-7 d	7-11 d	11-18d	0-18 d
Hatch moment																				
Early	Direct	50.2	49.2^{a}	115.9	777.7	16.7	18.0	40.3	63.8	40.5	12.8	25.7	50.4	86.2	51.9	1.304	0.699	0.800	0.740	0.781
	Delayed	50.4	46.6^{c}	109.7	748.0	15.8	16.7	38.1	62.3	39.0	12.1	24.1	47.8	84.1	50.0	1.308	0.692	0.798	0.740	0.779
Midterm	Direct	51.0	48.7^{ab}	107.5	746.8	14.7	16.9	38.5	62.1	38.8	11.1	24.4	47.5	83.7	49.6	1.325	0.693	0.811	0.741	0.781
	Delayed	50.6	47.9^{b}	106.7	750.3	14.7	15.7	39.4	62.7	39.0	11.0	23.4	48.0	85.4	50.3	1.333	0.669	0.808	0.745	0.782
Late	Direct	51.2	49.4^{a}	105.0	741.9	13.9	17.0	38.0	62.0	38.5	10.3	24.2	47.5	83.9	49.5	1.351	0.702	0.800	0.739	0.777
	Delayed	51.7	49.3^{a}	101.5	738.2	13.1	16.5	37.2	62.6	38.3	10.0	23.4	46.5	83.8	49.1	1.302	0.701	0.801	0.748	0.780
SEM (n = 12)		0.3	0.3	1.1	10.3	0.3	0.4	0.7	1.0	0.6	0.2	0.4	0.8	1.4	0.8	0.013	0.009	0.004	0.004	0.003
Hatch moment																				
Early		50.3^{a}	47.9	112.8^{a}	762.9	16.2^{a}	17.3	39.2	63.0	39.7	12.4^{a}	24.9^{a}	49.1^{a}	85.2	50.9	1.306	0.696	0.799^{a}	0.740	0.780
Midterm		50.8^{ab}	48.3	107.1^{b}	748.5	14.7^{b}	16.3	39.0	62.4	38.9	11.1^{b}	23.9^{b}	47.8^{ab}	84.6	50.0	1.329	0.681	0.809^{b}	0.743	0.781
Late		51.4^{b}	49.3	103.3^{c}	740.0	13.5^{c}	16.7	37.6	62.3	38.4	10.2^{c}	23.8^{b}	47.0^{b}	83.8	49.3	1.326	0.702	0.800^{a}	0.743	0.779
SEM (n = 24)		0.2	0.2	0.8	7.5	0.2	0.3	0.5	0.8	0.4	0.1	0.3	0.6	1.0	0.5	0.010	0.006	0.003	0.003	0.002
Feed access																				
Direct		50.8	49.1	109.5^{a}	755.5	15.1^{a}	17.3^{a}	38.9	62.6	39.2	11.4^{a}	24.7^{a}	48.4	84.6	50.3	1.327	0.698	0.804	0.740	0.780
Delayed		50.9	47.9	106.0^{b}	745.5	14.5^{b}	16.3^{b}	38.3	62.5	38.8	11.0^{b}	23.6^{b}	47.4	84.5	49.8	1.314	0.688	0.802	0.744	0.780
SEM (n = 36)		0.2	0.2	0.6	6.2	0.2	0.3	0.4	0.6	0.3	0.1	0.3	0.5	0.8	0.4	0.008	0.005	0.002	0.002	0.002
***P*-values**																				
Hatch moment x Feed access		0.403	0.001	0.052	0.231	0.151	0.595	0.096	0.484	0.268	0.146	0.537	0.142	0.361	0.242	0.055	0.364	0.871	0.608	0.708
Hatch moment		0.004	<0.001	<0.001	0.081	<0.001	0.056	0.054	0.754	0.058	<0.001	0.022	0.030	0.592	0.077	0.160	0.054	0.020	0.686	0.686
Feed access		0.708	<0.001	<0.001	0.231	0.008	0.006	0.245	0.910	0.286	0.016	0.002	0.126	0.884	0.378	0.237	0.135	0.598	0.242	0.820

$^{a-c}$Effect means within columns with no common superscript differ significantly ($P \leq 0.05$). [1]Each cage contained 7 male broilers at the start of the study. [2]Moment of placement in the grow-out unit.

directly fed late hatched chickens (0.13%), whereas this was not found for early (-0.05%) and midterm (-0.02%) hatched chickens ($P = 0.047$; Table 2). At d 18, the relative liver weight was higher (0.14%) for direct fed early hatched chickens compared with delayed fed early hatched chickens, whereas for midterm hatched chickens this was found to be the other way around ($P = 0.042$; Table 2). Midterm hatched chickens with direct feed access had larger relative bursa weights at d 18 compared with midterm hatched chickens with delayed feed access (0.04%), whereas this was opposite in early (-0.03%) and late (-0.01%) hatched chickens ($P = 0.014$; Table 2).

Hatch moment

At placement in the grow-out facility, early hatched chickens had lower BW compared with late hatched chickens, with midterm hatched chickens in between ($P < 0.001$; Table 1). At d 4, early hatched chickens had a higher BW compared with midterm (5.3%) and late (9.2%) hatched chickens, and midterm hatched chickens had a higher BW (3.7%) compared with late hatched chickens ($P < 0.001$). From d 0 to 4, the early hatched chickens had a higher FI and BW gain compared with midterm and late hatched chickens, and midterm hatched chickens had a higher FI and BW gain compared with late hatched chickens ($P < 0.001$; Table 1). The higher FI for early hatched chickens persisted until d 11 ($P = 0.022$). Midterm hatched chickens had a higher G:F ratio from d 7 to 11 compared with early (0.010) and late (0.009) hatched chickens ($P = 0.020$; Table 1). For the overall period (d 0 to 18), the absolute BW gain and FI tended ($P = 0.058$ and $P = 0.077$) to be higher for early and late hatched chickens, with midterm hatched chickens in between (Table 1). Absolute breast meat yield at d 18, expressed as a percentage of carcass weight, was higher for early (30.4%) versus midterm (28.5%) and late (27.8%) hatched chickens ($P < 0.001$; Table 2). Midterm hatched chickens were found to have lower duodenum villi width at d 4 compared with early (-19.7%) and late (-16.3%) hatched chickens ($P = 0.048$; Table 4). In addition, the ileum and cecum weight to length ratio at d 18 were higher for early versus midterm and late hatched chickens ($P = 0.006$ and 0.001; Table 5), mainly caused by a higher intestinal weight. No effect of hatch moment on other organ size was found, including the residual yolk.

Feed access

At d 4, chickens with direct feed access had a higher BW (3.3%) compared with chickens with delayed feed access ($P < 0.001$; Table 1). From d 0 to 4, the direct fed chickens had a higher FI (3.6%) and BW gain (4.1%) compared with delayed fed chickens ($P < 0.001$; Table 1). Moment of feed access after hatch did not affect organ weights (including the residual yolk), whereas direct feed access resulted in a higher weight:length ratio of jejunum (12.5%) and ileum (7.5%) at d 4 compared with delayed feed access ($P = 0.002$ and $P = 0.029$), mainly caused by a shortened ileum length (20.6 cm/kg of BW; $P = 0.020$) and higher

Table 2. The effect of hatch moment and moment of feed access after hatch on relative carcass and organ development of chickens at d 4 and 18, expressed as least square means[1]

		Organ development, % of BW													
		Day 4							Day 18						
	Feed access	Carcass[2]	Heart	Stomach[3]	Liver	Pancreas	Spleen	Bursa	Carcass[2]	Breastfillet[4]	Heart	Stomach[3]	Liver	Pancreas	Spleen	Bursa
Hatch moment																
Early	Direct	68.3	0.76^{ab}	5.52	4.13	0.45	0.06	0.14	61.5	30.5	0.65	2.52	3.40^{b}	0.39	0.10	0.23^{a}
	Delayed	68.5	0.71^{b}	5.56	3.87	0.44	0.08	0.18	62.4	30.3	0.69	2.39	3.26^{c}	0.36	0.09	0.20^{ab}
Midterm	Direct	68.7	0.73^{ab}	5.65	3.92	0.48	0.06	0.15	62.7	29.0	0.67	2.47	3.22^{c}	0.40	0.09	0.19^{b}
	Delayed	69.1	0.71^{ab}	5.41	3.77	0.48	0.07	0.14	62.5	28.0	0.63	2.54	3.49^{a}	0.40	0.09	0.23^{a}
Late	Direct	67.9	0.69^{b}	5.63	3.99	0.46	0.08	0.14	63.1	27.8	0.68	2.44	3.25^{c}	0.38	0.09	0.20^{ab}
	Delayed	68.1	0.82^{a}	5.85	3.91	0.48	0.07	0.15	63.3	27.7	0.68	2.43	3.25^{c}	0.39	0.09	0.19^{b}
SEM (n = 12)		0.6	0.04	0.15	0.14	0.02	0.01	0.01	0.3	0.6	0.02	0.09	0.08	0.02	0.01	0.01
Hatch moment																
Early		68.4	0.74	5.54	4.00	0.45	0.07	0.16	62.0^{b}	30.4^{a}	0.67	2.45	3.33	0.38	0.10	0.21
Midterm		68.9	0.72	5.53	3.84	0.48	0.06	0.14	62.6^{ab}	28.5^{b}	0.65	2.51	3.35	0.40	0.09	0.21
Late		68.0	0.76	5.74	3.95	0.47	0.07	0.15	63.2^{a}	27.8^{b}	0.68	2.44	3.25	0.39	0.09	0.20
SEM (n = 24)		0.4	0.03	0.11	0.10	0.02	0.00	0.01	0.2	0.5	0.02	0.06	0.06	0.01	0.00	0.01
Feed access																
Direct		68.3	0.73	5.60	4.01	0.46	0.07	0.14	62.4	29.1	0.67	2.47	3.29	0.39	0.09	0.21
Delayed		68.6	0.75	5.61	3.85	0.47	0.07	0.16	62.7	28.7	0.67	2.45	3.33	0.39	0.09	0.21
SEM (n = 36)		0.3	0.02	0.09	0.08	0.01	0.00	0.01	0.2	0.4	0.01	0.05	0.05	0.01	0.00	0.01
***P*-values**																
Hatch moment x Feed access		0.986	0.047	0.220	0.756	0.697	0.115	0.143	0.275	0.740	0.255	0.548	0.042	0.495	0.818	0.014
Hatch moment		0.227	0.609	0.233	0.441	0.334	0.182	0.289	0.003	<0.001	0.351	0.716	0.412	0.389	0.244	0.333
Feed access		0.572	0.475	0.958	0.117	0.754	0.287	0.118	0.243	0.418	0.992	0.763	0.493	0.617	0.912	0.999

$^{a-c}$Effect means within columns with no common superscript differ significantly ($P \leq 0.05$). [1]One bird dissected per cage. [2]Carcass: including feathers, head, blood, and legs. [3]Stomach: proventriculus plus gizzard. [4]Breast meat yield. Expressed as percentage of carcass weight.

relative jejunum weight (0.19% of BW, respectively; $P = 0.017$; Table 3). Furthermore, villi width of the duodenum at d 4 was smaller for direct fed chickens versus delayed fed chickens (83 vs. 94 μm; $P = 0.043$; Table 4). At d 18, delayed feed access increased jejunum weight to length ratio compared with direct fed chickens (26.92 vs. 25.25 mg/mm, respectively; $P = 0.030$; Table 5). The relative duodenum weight was higher for delayed fed chickens (0.07% of BW; $P = 0.025$), whereas the cecum weight was higher for direct fed chickens (0.06% of BW; $P = 0.010$).

DISCUSSION

Hatch moment × feed access

The interaction found at d 0 for hatch moment and feed access shows that especially early hatched chickens benefit from direct feed access compared with midterm and late hatched chickens. This corresponds with previous research (Sklan et al., 2000; Van de Ven et al., 2013) and can be explained by early hatched chickens spending the longest time without feed and water when subjected to delayed feed and water access compared with midterm and late hatched chickens (in the current study, 26 vs. 19 and 13 h, respectively). This interaction is inherent to the experimental design and it is hypothesized that it (most likely) does not occur when chickens from different hatch moments receive an identical period of delayed feed access after hatch. It has even been suggested that when using such a design, especially late hatched chickens appear to benefit from direct feed access after hatch (Careghi et al., 2005; Wang et al., 2014). This can be explained by late hatched chickens being more mature in development at the moment of hatch. To differentiate between studies and time intervals used for moment of first feed and water intake, as well as measurements on BW gain and FI intake after hatch, Careghi et al. (2005) made a distinction for chickens in biological and chronological age. This study, in which chickens from various biological ages (defined by hatch moment) were weighed at and fed for a standardized chronological age, reflects current practice in which eggs are hatched within housing systems that provide direct feed and water access.

Chickens in this study suffered from weight loss between placement and d 0 irrespective of hatch moment and feed access directly after hatch. This might be due to the metabolic status directly after hatch in which glycogen reserves are depleted and plasma lactate levels increased (De Oliveira et al., 2008; Molenaar et al., 2011) and the chicken requires some time to recover from this. Another explaination can be that chickens continue to develop after hatch by investment in organ development and growth of the digestive system, irrespective of feed access. When no exogenous feed and water is provided, the residual yolk contents are primarily used as an energy supply. Previous research has shown that conversion of residual yolk into body tissue is not 1:1 (Molenaar et al., 2010), resulting in

Table 3. The effect of hatch moment and moment of feed access after hatch on intestinal development of chickens at d 4, expressed as least square means[1]

		Intestinal length, cm / kg of BW				Intestinal weight, % of BW				Weight to length ratio intestines, mg/mm			
	Feed access	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum
Hatch moment													
Early	Direct	141.8	318.3	303.0	128.4	1.82	2.60	1.90	0.77	12.80	8.25	6.28	5.96
	Delayed	157.8	343.8	337.2	133.5	1.87	2.44	1.98	0.76	11.89	7.16	5.90	5.69
Midterm	Direct	154.2	340.1	331.8	132.9	1.83	2.52	1.86	0.80	11.91	7.42	5.68	6.04
	Delayed	150.0	338.8	343.3	138.1	1.71	2.22	1.83	0.81	11.33	6.58	5.38	5.83
Late	Direct	153.8	334.5	334.3	136.4	1.83	2.50	2.04	0.81	11.98	7.54	6.15	5.99
	Delayed	164.6	348.6	350.2	144.8	1.91	2.39	1.92	0.80	11.66	6.91	5.57	5.52
SEM (n = 12)		4.8	9.2	11.2	5.1	0.09	0.12	0.09	0.04	0.54	0.41	0.28	0.28
Hatch moment													
Early		149.8	331.0	320.1	131.0	1.84	2.52	1.94	0.76	12.34	7.71	6.09	5.83
Midterm		152.1	339.5	337.5	135.5	1.77	2.37	1.85	0.80	11.62	7.00	5.53	5.94
Late		159.2	341.6	342.3	140.6	1.87	2.45	1.98	0.81	11.82	7.23	5.86	5.75
SEM (n = 24)		3.3	6.5	7.9	3.5	0.07	0.10	0.06	0.03	0.446	0.327	0.226	0.206
Feed access													
Direct		150.0[b]	331.0	323.0[b]	132.6	1.83	2.54[a]	1.94	0.79	12.23	7.74[a]	6.04[a]	6.00
Delayed		157.5[a]	343.7	343.6[a]	138.8	1.83	2.35[b]	1.91	0.79	11.62	6.88[b]	5.62[b]	5.68
SEM (n = 36)		2.7	5.3	6.6	2.9	0.07	0.09	0.05	0.03	0.410	0.296	0.204	0.177
***P*-values**													
Hatch moment x Feed access		0.087	0.307	0.519	0.927	0.277	0.580	0.443	0.934	0.767	0.769	0.827	0.866
Hatch moment		0.110	0.433	0.089	0.152	0.315	0.295	0.234	0.471	0.203	0.101	0.058	0.768
Feed access		0.050	0.076	0.020	0.126	0.953	0.017	0.675	0.884	0.078	0.002	0.029	0.133

[a,b]Effect means within columns with no common superscript differ significantly ($P \leq 0.05$). [1]One bird dissected per cage.

BW loss. In case of direct feed and water availability after hatch, the relative low feed and water intake during the first hours after hatch cannot compensate for the absolute BW weight loss due to residual yolk consumption and potential dehydration due to the environmental temperature in which the chicken hatches. Although direct feed and water access can partially compensate for BW loss, it appears from this study that it will not do so entirely. Lastly, BW loss between hatch and d 0 may depend on management conditions (Van de Ven et al., 2013). In the study of van de Ven et al (2013), chickens hatched in a traditional incubator suffered from BW loss between hatch and pulling but not when in a combined hatching and grow-out facility. This difference might be explained by chick handling after pulling including additional stress, which is avoided when chickens hatch directly in a grow-out facility.

Table 4. The effect of hatch moment and moment of feed access after hatch on intestinal villi length and width development of chickens at d 4, expressed as least square means[1]

		Intestinal villi length, µm			Intestinal villi width, µm		
Hatch moment	**Feed access**	**Duodenum**	**Jejunum**	**Ileum**	**Duodenum**	**Jejunum**	**Ileum**
Early	Direct	486	410	373	91	69	72
	Delayed	568	481	274	101	61	69
Midterm	Direct	520	414	377	73	84	52
	Delayed	419	436	316	82	86	66
Late	Direct	626	368	334	83	80	69
	Delayed	462	329	321	101	64	72
SEM (n = 12)		132	59	36	8	11	42
Hatch moment							
Early		527	445	324	96[a]	65	70
Midterm		469	425	347	77[b]	85	59
Late		544	348	327	92[a]	72	70
SEM (n = 24)		76	34	24	5	9	27
Feed access							
Direct		544	397	361	83[b]	78	64
Delayed		483	415	304	94[a]	70	69
SEM (n = 36)		57	28	24	4	9	31
***P*-values**							
Hatch moment x Feed access		0.377	0.511	0.422	0.565	0.555	0.869
Hatch moment		0.746	0.130	0.739	0.048	0.112	0.921
Feed access		0.449	0.623	0.112	0.043	0.377	0.902

[a,b]Effect means within columns with no common superscript differ significantly ($P \leq 0.05$).
[1]One bird dissected per cage.

Table 5. The effect of hatch moment and moment of feed access after hatch on intestinal development of chickens at d 18, expressed as least square means[1]

		Intestinal length, cm/kg of BW				Intestinal weight, % of BW				Weight to length ratio intestines, mg/mm			
	Feed access	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum
Hatch moment													
Early	Direct	32.4	80.0	76.6	33.9	1.09	2.05	1.51	0.57	33.77	25.60	19.88	16.80
	Delayed	33.1	79.9	82.0	34.3	1.16	2.15	1.51	0.52	35.18	27.07	18.50	15.11
Midterm	Direct	33.5	84.4	86.4	37.1	1.05	2.05	1.40	0.53	31.69	24.40	16.40	14.38
	Delayed	33.2	81.7	81.4	33.4	1.15	2.14	1.43	0.48	34.82	26.29	17.61	14.25
Late	Direct	33.5	83.1	82.3	35.9	1.09	2.11	1.45	0.52	32.86	25.75	17.84	14.59
	Delayed	34.3	81.7	80.4	34.0	1.15	2.22	1.49	0.49	33.80	27.41	18.83	14.40
SEM (n = 12)		1.0	2.6	2.9	1.2	0.04	0.07	0.04	0.02	1.38	0.93	0.67	0.46
Hatch moment													
Early		32.7	80.0	79.3	34.1	1.13	2.10	1.51	0.54	34.47	26.33	19.19[a]	15.96[a]
Midterm		33.4	83.1	83.9	35.3	1.10	2.09	1.42	0.50	33.26	25.34	17.01[c]	14.31[b]
Late		33.9	82.4	81.3	35.0	1.12	2.16	1.47	0.51	33.33	26.58	18.34[b]	14.50[b]
SEM (n = 24)		0.7	1.8	2.1	0.9	0.03	0.05	0.03	0.02	0.98	0.66	0.49	0.32
Feed access													
Direct		33.1	82.5	81.8	35.6	1.08[b]	2.07	1.45	0.54[a]	32.77	25.25[b]	18.04	15.26
Delayed		33.5	81.1	81.3	33.9	1.15[a]	2.17	1.48	0.49[b]	34.60	26.92[a]	18.31	14.59
SEM (n = 36)		0.6	1.5	1.7	0.7	0.03	0.04	0.03	0.01	0.804	0.548	0.412	0.265
***P*-values**													
Hatch moment x Feed access		0.835	0.879	0.179	0.272	0.832	0.991	0.833	0.867	0.707	0.974	0.096	0.163
Hatch moment		0.512	0.465	0.270	0.631	0.848	0.533	0.093	0.154	0.616	0.369	0.006	0.001
Feed access		0.618	0.503	0.832	0.093	0.025	0.070	0.496	0.010	0.110	0.030	0.613	0.077

[a-c]Effect means within columns with no common superscript differ significantly ($P \leq 0.05$).
[1]One bird dissected per cage.

The interaction for hatch moment and feed access in the present study on bursa weight at d 18 does not correspond with previous research in which delayed feed access after hatch seemed to result in smaller relative bursa weights compared with direct fed chickens (Dibner et al., 1998; Bar-Shira et al., 2005). In this study, only a numeric reduction in bursa weight was observed in early hatched chickens.

This can partially be explained due to the extended periods of delayed feed access after hatch (24 to 72 h) in previous studies, whereas in this study this period was a maximum of 26 h for the early hatched chickens. The difference in bursa size was only observed at d 18, although it could be expected that this difference also would occur at an earlier age (d 4) as demonstrated by Dibner et al. (1998) and Bar-Shira et al. (2005) in case of a longer period of delayed feed access after hatch. The lower bursa weight as such can be explained by the delayed appearance of biliary IgA and germinal centers due to delayed feed access, after which reduced lymphocyte proliferation then results in a less mature state of the bursa (Dibner et al., 1998) and delayed increased of B and T cell populations (Bar-Shira et al., 2005).

Although early feed access and hatch window did not interact for BW gain d 0 to 18 (P = 0.268), the effect of feed access on weight gain from d 0 to 18 seemed to be almost entirely explained by the treatment of early hatched chickens (3.8 vs. -0.5 vs. 1.3% difference in BW gain d 0 to 18, for early, midterm, and late hatched chickens, respectively, having direct vs. delayed feed access). This can be explained by early hatched chickens having relatively more time to consume feed between placement in the grow-out facility and the measurement on d 0 compared with midterm and late hatched chickens.

Hatch moment

At placement, BW of early hatched chickens was lower compared with midterm and late hatched chickens, whereas this was found to be the other way around at d 4. This effect has not been observed in previous studies focusing on hatch moment within the hatch window (Careghi et al., 2005; Van de Ven et al., 2011). This can be explained by an increased FI and growth time (increased relative growth) of early hatched chickens from d 0 to 4 compared with midterm and late hatched chickens. However, in previous research, it was found that not early but late hatched chickens have increased relative growth compared with chickens hatched at other hatch moments. The observed effect in BW gain d 0 to 4 may again also be caused by the different periods in delayed feed access between early (26 h), midterm (19 h), and late (13 h) hatched groups of chickens combined with BW measurements at chronological age (interaction hatch moment × feed access).

At d 18, the BW of early hatched chickens was only numerically higher compared with midterm and late hatched chickens. Although in this study the trend remained, the effect of hatch moment within the hatch window on long-term growth seems to disappear as the chicken ages. It is hypothesized that this is related to the relatively small time differences

between hatch moments. Breast meat yield at d 18 was higher for early hatched chickens compared with midterm and late hatched chickens. Thus far, differences in carcass development (e.g., breast meat percentage) have only been linked with the effect of feed acces and not with the effect of hatch moment. It is clearly described that delayed feed access results in delayed satellite cell proliferation, involved in muscle development (Halevy et al., 2003; Moore et al., 2005). To the authors' knowledge, the effect of hatch moment on carcass composition at moment of slaughter has never been the primary subject of research, although several studies looked at BW at slaughter age. A previous study determined the proportional breast muscle weight at hatch and at d 5 for chickens hatched at different moments in time (Wang et al., 2014), whereas again long-term effects (>14 d of age) were not considered.

Results suggest that early hatched chickens physiologically differ from midterm and late hatched chickens, with a tendency for long-term differences. Recent literature by van der Ven et al (2013) also suggests that early hatched chickens differ from later hatched chickens from a metabolic point of view. Early hatched chickens were found to have lower metabolite concentrations (glucose) in blood serum directly after hatch compared with midterm and late hatched chickens. Although the differences in glucose concentration were not confirmed, in the study of Wang et al. (2014) glycogen tended to be lower in early hatched chickens compared with midterm and late hatched chickens. The described metabolic differences at hatch might not explain differences found in carcass composition at later age though. In this study, no effects of hatch window on organ growth (besides the heart) were found. This is in contrast to other studies, in which for example lungs, bursa and stomach weights were already different among chickens obtained from different hatch moments directly after hatch (Van de Ven et al., 2013). This might be related to differences in the overall hatch window, which was relatively short in this study compared with others, reducing the potential differences between treatment groups. It should be noted that in this study, chickens (due to practical reasons) were only kept till d 18 and therefore it remains unknown if the altered carcass composition remains until slaughter.

Feed access

Early, midterm, and late hatched chickens with delayed feed access spent approximately 26, 19, and 13 h after hatch, respectively, without feed and water access. Except for BW at d 0, the effect of feed access did not interact with hatch moment on growth performance. The beneficial effect of direct feed access on BW gain and FI during the first week after hatch corresponds with earlier research (Bigot et al., 2003; Juul-Madsen et al., 2004), although in many of those studies the applied delayed feed access was often much longer (19 h average in current study vs. 24 to 48 h in previous studies). These results suggest that the effect of delayed feed access after hatch might be underestimated, especially during suboptimal conditions, considering that a feed deprivation period of less than 24 h can

already have an effect on short-term growth performance. However, chickens seem to be able to compensate for a relatively short period of delayed feed access after hatch, as in this study the differences in growth performance did not persist until 18 d of age.

The increased growth performance of direct fed chickens cannot be explained by more rapid relative organ development when comparing organ size of direct versus delayed fed chickens at d 4. Therefore, it seems more plausible to explain differences in performance by considering the detrimental effect of delayed feed access on development and functioning of the GI tract during the first days after hatch. This is further supported by earlier findings in which peak development of the intestinal tract in chickens was found to occur around 3 to 8 d of age, suggesting a high nutritional demand to facilitate development from the moment of hatch onward (Noy and Sklan, 1997). Intestinal development is not always quantified for individual parts of the intestinal tract (duodenum, jejunum, ileum, cecum), whereas it appears that some parts, such as the duodenum and jejunum, are more sensitive for feed deprivation compared with the ileum by means of affected cellular proliferation (Geyra et al., 2001a). This is not confirmed in the current study, despite that delayed feed access resulted in a lower weight to length ratio of jejunum and ileum at d 4 compared with the direct feed access group. It can be hypothesized that a lower weight to length ratio is the result of a less developed intestine as measured by villi growth (length, width, and density) and this is supported by earlier findings in which delayed feed access directly after hatch (48 h) resulted in reduced villi development (Uni et al., 2003).

However, in this study no differences were found in villi length and width in the jejunum and ileum. The higher weight to length ratio of the jejunum for direct fed chickens was mainly related to a higher relative weight and no differences were found in villi development. This suggests that instead of more rapid villi growth (size) and density, the weight increase can be related to another form of intestinal development. For example, proliferation of cells during the first days after hatch is not only limited to the crypt base, but covers all epithelial cells (reviewed by Wijtten et al., 2012) and delayed feed access is known to negatively affect this proliferation rate (Uni, 1999). Although speculative, another suggestion is that direct feed acecss facilitates immunological development by faster formation of a microbial commensal population, affecting immune cellular development (such as goblet cells) in the gut and mucus dynamics (Deplancke and Gaskins, 2001; Dibner et al., 2008). Although it remains difficult to draw any solid conclusions on the (long-term) effect of direct or delayed feed access on intestinal development, there is definitely an effect of delayed feed access.

Results suggest that early hatched chicken appear to have a different developmental and growth pattern compared with midterm and late hatched chicken within a 24-h hatch window. Furthermore, direct feed access after hatch positively affects growth and development during the first week after hatch. Even a short delay in feed access after hatch decreases growth and development during the 4 d after hatch, although to some extend this is compensated for in the long-term by means of compensatory growth.

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CHAPTER 3

Effects of feed access after hatch and inclusion of fish oil and medium chain fatty acids in a pre-starter diet on broiler chicken growth performance and humoral immunity

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ABSTRACT

Delayed feed and water access is known to impair growth performance of day old broiler chickens. Although effects of feed access on growth performance and immune function of broilers have been examined before, effects of dietary composition and its potential interaction with feed access are hardly investigated. This experiment aimed to determine whether moment of first feed and water access after hatch and pre-starter composition (0 to 7 days) affect growth rate and humoral immune function in broiler chickens. Direct fed chickens received feed and water directly after placement in the grow-out facility, whilst delayed fed chickens only after 48 h. Direct and delayed fed chickens received a control pre-starter diet, or a diet containing medium chain fatty acids (**MCFA**) or fish oil. At 21 days, chickens were immunized by injection of sheep red blood cells. The mortalility rate depended on an interaction between feed access and pre-starter compisition ($P = 0.014$). Chickens with direct feed access fed the control pre-starter diet had a higher risk for mortality than chickens with delayed feed access fed the control pre-starter diet (16.4 v. 4.2%) whereas the other treatment groups were in-between. BW gain and feed intake till 25 days in direct fed chickens were higher compared with delayed fed chickens, whilst gain to feed ratio was lower. Within the direct fed chickens, the control pre-starter diet resulted in the highest BW at 28 days and the MCFA pre-starter diet the lowest ($\Delta = 2.4\%$), whereas this was opposite for delayed fed chickens ($\Delta = 3.0\%$; $P = 0.033$). Provision of MCFA resulted in a 4.6% higher BW gain and a higher gain to feed ratio compared with other pre-starter diets, but only during the period it was provided (2 to 7 days). Minor treatment effects were found for humoral immune response by measuring immunoglobulins, agglutination titers, interferon gamma (**IFN- γ**), and complement activity. Concluding, current inclusion levels of fish oil (5 g/kg) and MFCA (30 g/kg) in the pre-starter diet appear to have limited (carryover) effects on growth and development, as well as on humoral immune function.

Keywords: first week nutrition, fish oil, medium chain fatty acids, humoral immunity

IMPLICATIONS

Delayed feed intake after hatch for broiler chickens resulted in lower BW gain. Thus, changing hatchery and farm management towards direct feeding after hatch can shorten the growth period. Feeding medium chain fatty acids (MCFA) during the first week after hatch resulted in higher BW gain and improved feed efficiency during the first week after hatch. A shortened growth period, as well as improved growth performance due to feeding MCFA, may result in economic benefits for the farmer.

INTRODUCTION

Although commonly accepted in modern broiler farming, a delayed first feed and water intake for chickens due to spread of hatch, handling at the hatchery and transport to the grow-out facility, may impair BW gain and organ growth (Van de Ven et al., 2013; Lamot et al., 2014). Additionally, delayed feed intake was found to result in relatively smaller immune organ sizes up to at least 2 weeks of age (Bar-Shira et al., 2005). Although delayed feed intake seems to have limited effect on (mucosal) immune function in healthy broiler chickens (Simon et al., 2014), it does seem to affect the specific antibody response to an immune challenge in later life and colonization of T and B lymphocytes in the cecum and colon (Bar-Shira et al., 2005).

The effect of delayed feeding on BW gain and immune function may also interact with diet composition. Direct and delayed fed chickens may have different dietary requirements due to their metabolic state and as such diets may skew immune function differently when fed immediately or delayed. Diet composition is also important during the first days after hatch as the gastrointestinal tract is still immature (Christensen, 2009) and enzyme secretion is not fully functional. Thereby, nutrient digestibility during the first week after hatch is not optimal yet and needs to be accounted for.

While the role of protein and amino acids in pre-starter diets has been studied extensively (Wijtten et al., 2010), studies on the use of dietary fat are limited due to the general perception that fats are not well digested at that age (Batal and Parsons, 2002; Thomas et al., 2008). However, these fats and oils might have an effect on immune function. Two profound examples are fish oil (rich in n-3 long chain polyunsaturated fatty acids) and medium chain fatty acids (MCFA). Fish oil has been found to reduce inflammatory responses at 2 weeks of age when fed to broilers (Korver and Klasing, 1997). On the contrary, MCFA (especially lauric acid; C12:0) were found to increase IL-1α expression *in vitro* in a dose response manner through toll-like receptor (TRL-4) activation (Lee et al., 2001). Although effects of nutrition on immune function in poultry have been extensively reviewed (Korver, 2012), most studies do not focus on effects of nutrition in the 1st after hatch. Moreover, mostly general effects of feed and water access on immune function and function are studied, ignoring effects of diet composition (Walstra, 2011; Simon et al., 2014).

The current experiment aimed to determine whether (1) moment of feed and water access after hatch and (2) pre-starter composition affected growth rate and innate and specific humoral immune function in broiler chickens until 28 days. It is hypothesized that inclusion of fish oil or MCFA in a pre-starter diet will result in higher BW gain and feed efficiency and altered humoral immune function.

MATERIALS AND METHODS

The procedures applied in this experiment were approved by the Animal Care and Use Committee of the Animal Sciences Group of Wageningen University and Research Centre, the Netherlands.

Experimental design and diets

Effects of moment of first feed and water access (for the remainder referred to as 'feed access'), and pre-starter composition were studied using a 2 x 3 factorial arrangement of treatments. Direct fed chickens received feed access directly after placement in the grow-out facility, whereas delayed fed chickens were withheld from feed access for 48 h after placement onward. Pre-starter diets contained either soybean oil (control), fish oil at 5 g/kg diet, or a MCFA blend at 30 g/kg diet (3 g/kg diet C10:0 and 27 g/kg diet C12:0; Sigma-Aldrich Chemie B.V., the Netherlands). Fish oil and MCFA were exchanged against soybean oil to keep the diets isocaloric. Pre-starter diets (2.0 mm pellet; 0 to 7 days) were followed by a starter (2.5 mm pellet; 7 to 14 days) and grower diet (3.0 mm pellet; 14 to 28 days) that were similar for all treatment groups. Diets were formulated based on digestibility and nutrient data provided by CVB (2007), the compositions of the pre-starter, starter and grower diets are provided in Table 1. Pre-starter diets were manufactured using one basal diet and together with starter and grower diets produced and pelleted by Research Diet Services (the Netherlands). All chicken were fed *ad libitum* from the moment of feed access till the end of the experiment. At 21 days, all chickens were immunized by injection in the *pectoralis major* with 1.0 ml of 25% volume-based sheep red blood cells (**SRBC**) suspension in phosphate-buffered saline.

Housing and management

Hatching eggs (n = 2160; weight range 58 to 69 g) of a Ross 308 breeder flock (42 weeks of age) were incubated at a temperature of 37.5°C and 54.0% relative humidity. After removal from the incubator, 576 male chickens were kept in a grow-out facility for 28 days. Female chickens were excluded from the experiment. The facility contained 96 cages (50 x 50 cm) with six chickens per cage. Treatments were randomly assigned to cages within 16 blocks, resulting in 16 replications per treatment. Cage floors were covered with 2 cm of wood shavings. Each cage was equipped with two nipple drinkers adjustable in height. Artificial lighting was set to 23 h/day from 0 to 2 days, 20 h/day from 2 to 6 days and 18 h/day from 6 days onwards. Temperature was set at 34°C at the start and set to decrease gradually until a final temperature of 22.1°C was reached at 18 days. Temperature was fixed at this level for the remainder of the experiment.

Table 1. Ingredient and nutritional composition of the experimental diets

Item	Pre-starter control 0-7 d	Pre-starter fish oil 0-7 d	Pre-starter MCFA[1] 0-7 d	Starter diet 7-14 d	Grower diet 14-28 d
Ingredient composition, g/kg (as fed)					
Corn	401.3	400.8	403.0	427.9	447.7
Wheat	100.0	100.0	100.0	200.0	200.0
Soybean meal (>48% CP)	341.8	341.9	341.5	302.8	279.7
Potato protein (77% CP)	37.5	37.5	37.5	-	-
Corn gluten meal	37.5	37.5	37.5	-	-
Soybean oil	31.4	26.8	0.02	20.5	30.1
C10:0[2]	-	-	3.0	-	-
C12:0[2]	-	-	27.0	-	-
Fish oil	-	5.0	-	-	-
Limestone	16.6	16.6	16.6	17.0	13.5
Monocalciumphosphate	16.4	16.4	16.4	13.6	11.5
Salt	4.26	4.26	4.26	2.17	2.13
Sodiumbicarbonate	0.96	0.96	0.97	2.46	1.81
DL-Methionine	1.96	1.96	1.96	2.11	1.95
L-Lysine HCL	0.34	0.34	0.35	1.43	1.53
L-Threonine	-	-	-	0.18	0.18
Premix starter[3]	10.0	10.0	10.0	10.0	-
Premix grower[4]	-	-	-	-	10.0
Calculated chemical composition, g/kg (as fed)					
Ash	68	68	68	62	55
AME, MJ/kg	11.62	11.62	11.62	11.51	11.93
Lys	14.6	14.6	14.6	11.8	11.3
Sodium	2.0	2.0	2.0	1.6	1.4
Potassium	8.5	8.5	8.5	8.2	7.8
Chloride	3.0	3.0	3.0	2.0	2.0
Analyzed chemical composition, g/kg					
Crude protein	265	271	277	218	208
Crude fat	58	57	54	46	56
Crude fiber	23	23	17	25	25
Dry matter	886	888	893	875	874
Calcium	10.1	9.6	9.9	10.0	7.7
Phorphorous	7.1	7.2	7.4	6.9	5.9

[1] Medium-chain fatty acids. [2] Sigma-Aldrich Chemie B.V., the Netherlands. [3] Contributed per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 5,000 IU; vitamin E, 30 mg; vitamin K3, 2.3 mg; vitamin B1, 1.0 mg; vitamin B2, 4.5 mg; vitamin B6, 2.7 mg; vitamin B12, 20 µg; niacine, 40 mg; D-pantothenic acid, 9 mg; choline chloride, 500 mg; folic acid, 0.5 mg; biotin, 100 µg; $FeSO_4.H_2O$, 150 mg; $CuSO_4.5H_2O$, 40 mg; MnO, 100 mg; $ZnSo_4.H_2O$, 145 mg; ; KJ, 2.0 mg; Na_2SeO_3, 0.56 mg; antioxidant (oxytrap PXN), 125 mg. [4] Supplied per kg diet: vitamin A, 10,000 IU; vitamin D3, 2,000 IU; vitamin E, 20 mg; vitamin K3, 2.3 mg; vitamin B1, 0.8 mg; vitamin B2, 4.5 mg; vitamin B6, 1.9 mg; vitamin B12, 20 µg; niacine, 30 mg; D-pantothenic acid, 8 mg; choline chloride, 400 mg; folic acid, 0.5 mg; biotin, 50 µg; $FeSO_4.H_2O$, 150 mg; $CuSO_4.5H_2O$, 40 mg; MnO, 100 mg; $ZnSo_4.H_2O$, 145 mg; KJ, 1.9 mg; Na_2SeO_3, 0.50 mg; antioxidant (oxytrap PXN), 125 mg.

Growth performance

Feed intake and BW were determined at 0, 2, 7, 14, 21, 25 and 28 days during the grow-out period. Feed intake was recorded per cage, whilst BW was recorded individually. Gain to feed ratio was calculated based on calculated BW gain and feed intake per cage.

Immunological analysis

At 2 days (in advance of feed access for delayed fed broiler chickens), one randomly chosen chicken per cage was killed by decapitation for blood collection. From 7 days onward, one chicken per cage was selected for weekly blood sampling from the brachial vein. In addition, a sample was collected at 4 days after immunization (day 25). Blood samples were taken before switching to a new feeding phase and in advance of immunization. Serum was stored after decantation at -80˚C for further analysis.

Immunoglobulins. Serum samples were analyzed to detect natural antibodies (**NAb**), IgM and IgY titers against Keyhole Limpet Hemocyanin (**KLH**) using similar methodology as described by Lammers et al. (2004). Antibody titers measured against KLH were expressed as the ^{2}log values of the dilutions that gave an extinction closest to 50% of the highest mean extinction of a standard positive present on every microtiter plate.

Agglutination titer. Total serum antibody titers to SRBC were only determined at 21, 25 and 28 days (0, 3 and 7 days post immunization). SRBC antibody titers were assessed by agglutination according to methodology previously described by Van der Zijpp and Leenstra (1980). Antibody titers measured against SRBC were expressed as the ^{2}log of the reciprocal of the highest serum dilution giving complete agglutination.

IFN-γ. Serum was analyzed using a commercial sandwich type ELISA kit (Chicken IFN-γ CytoSetTM; cat. no. CAC1233; Invitrogen, CA, USA) and expressed as the amount of picogram per mililiter.

Complement activity. Complement activity was not measured at day 2. Activity was measured using a hemolytic technique as described by Parmentier et al. (2002) with an adapted light-scattering method. Serum was diluted and incubated with either sensitized sheep erythrocytes (Hemolysins, ref. no. 72202; Biomérieux, the Netherlands) to measure activity of the classical pathway (**CPW**) or rabbit erythrocytes to measure activity of the alternative pathway (APW). Hemolytic complement activity was scored and expressed as 'yes' or 'no' based on lysis of erythrocytes.

Statistical analysis

Data were analyzed in SAS (Version 9.3, 2011, SAS Institute Inc., Cary, NC, USA) using cage as the experimental unit. The dietary treatment period (0 to 7 days), grow-out period (7 to 21 days) and immunization period (21 to 28 days) were separately analyzed as the chicken underwent different physiological processes during these periods.

Growth performance data (BW gain, feed intake, and gain to feed ratio) and immune data until 2 days were subjected to mixed model analysis using the PROC MIXED procedure. The following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha \times \beta_{ij} + b_k + e_{ijk}$$

where Y_{ijk} is the dependent variable, μ the overall mean, α_i the fixed effect of moment of feed access (i = direct or delayed), β_j the fixed effect of pre-starter composition (j = control, fish oil, or MCFA), b_k the random block effect (k = 1, 2, 3, …, 16), and e_{ijk} the residual error term. For data from 0 to 2 days, effects of feed access and the interaction with pre-starter composition were excluded from the model. Mortality data were analyzed as survival data to test homogeneity of survival curves, using the PROC LIFETEST procedure.

Immunological data were considered repeated measures from 7 days onwards and therefore subjected to mixed model analysis using the repeated lines statement. Hemolytic complement activity was analyzed using the PROC GLIMMIX procedure including the repeated lines statement. The three-way interaction between day, feed access and pre-starter composition did not solve and was therefore omitted from the model. Output is expressed as the chance for a complement response.

As the applied dietary treatments had only limited effects on humoral immune function, outcomes of the immunological analysis are only described in the Results section. However, a tabular overview is provided in Supplementary Tables S1 to S4 of Supplementary material S1. Although not the aim of the experiment, the current experiment also provided insight in general humoral immune responses in time (0 to 28 days) of broiler chickens. These results are described in Supplementary material S1 and presented in Supplementary Tables S1 to S4 and Supplementary Figure S1.

Data are reported as least square means and differences between treatment means are assumed to be significant when $P \leq 0.05$.

RESULTS

General

For mortality from 0 to 28 days, an interaction was found for feed access and pre-starter composition ($P = 0.014$; Figure 1). Chickens with direct feed access fed the control pre-starter diet had higher risk for mortality than chickens with delayed feed access fed the control pre-starter diet (16.4% v. 4.2%).

Dietary treatment period (0 to 7 days)

No interaction was found between feed access and pre-starter composition for any of the performance and immune variables from 2 to 7 days. BW gain and feed intake from 2 to 7 days of direct fed chickens were 34.9 and 54.0% higher compared with fed chickens (both $P < 0.001$; Table 2). Gain to feed ratio was lower for direct fed chickens compared to delayed fed chickens (-0.149; $P < 0.001$; Table 2).

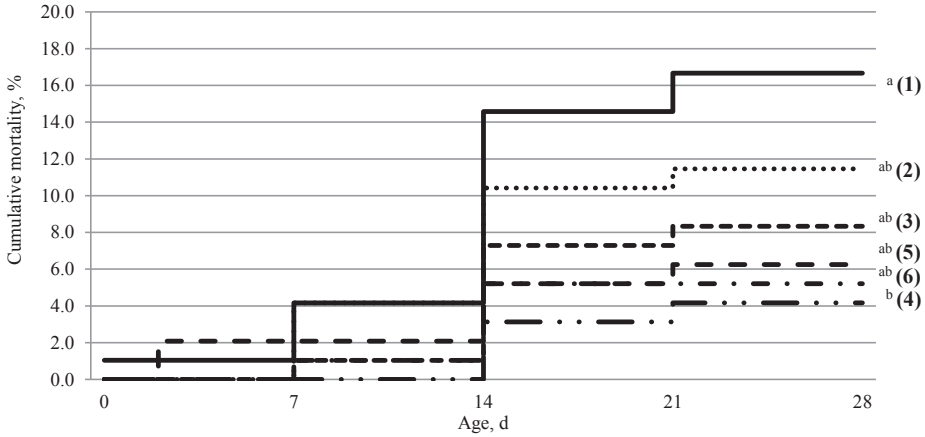
Provision of MCFA in the pre-starter diet resulted in a higher BW gain from 2 to 7 days (4.8% on average; $P = 0.010$), and a higher gain to feed ratio (1.132 v. 1.102 and 1.094, respectively; $P = 0.004$) compared with the control and fish oil pre-starter diets (Table 2). Feed access and pre-starter composition did not affect KLH specific NAb levels, IFN-γ, and the complement CPW and APW response during the pre-starter period.

Grow-out period (7 to 21 days)

A tendency for interaction between feed access and pre-starter composition was found for BW gain from 7 to 21 days ($P = 0.097$; Table 2). Within the direct fed chickens, provision of MCFA in the pre-starter diet resulted in a 1.6% lower BW gain compared with the control and fish oil containing pre-starter diets, whereas this was opposite for delayed fed chickens (3.4% higher BW gain for the MCFA pre-starter diet). BW gain and feed intake from 7 to 21 days of direct fed chickens were, respectively, 18.5 and 20.3% higher compared with delayed fed chickens (both $P < 0.001$; Table 2). Gain to feed ratio was lower for direct fed chickens compared with delayed fed chickens (0.736 v. 0.747; $P = 0.005$; Table 2). Starter diet composition tended to affect the alternative complement pathway response from 7 to 21 days ($P = 0.093$), where MCFA and fish oil containing starter diets had a lower chance for an APW complement response compared with the control starter diet. Feed access and pre-starter composition did not affect KLH specific NAb levels, IFN-γ, and complement CPW response during the grow-out period.

Immunization period (21 to 28 days)

The results are reported in Tables 3 and 4. An interaction was found between feed access and pre-starter composition for BW at 28 days ($P = 0.033$; Table 4). Within the direct fed chickens, the control pre-starter diet had the highest BW and the MCFA containing pre-



	0	2	7	14	21	28
———	1.0	1.0	4.2	14.6	16.7	16.7
.....	0.0	0.0	4.2	10.4	11.5	11.5
– – –	0.0	0.0	1.0	7.3	8.3	8.3
——— .	0.0	0.0	0.0	3.1	4.2	4.2
– –	0.0	2.1	2.1	5.2	6.3	6.3
– . –	0.0	0.0	1.0	5.2	5.2	5.2

Figure 1. Mortality rate of broiler chickens, expressed per treatment. (1) Direct feed access & control pre-starter, (2) direct feed access & fish oil pre-starter, (3) direct feed access & medium chain fatty acids (**MCFA**) pre-starter, (4) delayed (48h) feed access & control pre-starter, (5) delayed (48h) feed access & fish oil pre-starter, (6) delayed (48h) feed access & MCFA pre-starter. Feed access effect: $P = 0.002$, Diet effect: $P = 0.428$. Moment of feed access x diet interaction effect: $P = 0.014$. Cumulative mortality rates with different superscripts differ significantly at $P \leq 0.05$.

starter diet the lowest (Δ = 2.4%), whereas this was the opposite for the delayed fed chickens (Δ = 3.0%). Furthermore, a tendency for interaction between feed access and pre-starter diet was found for feed intake between 21 and 25 days ($P = 0.066$; Table 4). Within the direct fed chickens, the control pre-starter diet had a higher feed intake than the MCFA pre-starter diet (Δ = 3.5%), whereas this was opposite in the delayed fed chickens (Δ = 2.4%). BW gain between 21 and 25 days for direct fed chickens was 7.3% higher compared with delayed fed chickens ($P < 0.001$; Table 3), but no treatment effect was found on BW gain from 25 to 28 days. Feed intake from 21 to 28 days of direct fed chickens was 8.3% higher compared with delayed fed chickens ($P < 0.001$; Table 3). Gain to feed ratio from 21 to 25 days, as well as from 25 to 28 days, was lower for direct fed chickens compared to delayed fed chickens ($P = 0.001$ and $P = 0.005$; Table 3).

Direct fed chickens had higher serum IFN-γ concentrations compared with delayed fed chickens (on average 96.9 v. 80.5 pg/ml; $P = 0.017$). Furthermore, provision of MCFA in the pre-starter diet tended to result in higher IFN-γ concentrations from 21 to 28 days

compared with the control and fish oil containing pre-starter diets (98.5 v. 84.0 and 83.5 pg/ml on average from 21 to 28 days; $P = 0.074$). Feed access and pre-starter composition did not affect KLH specific NAb levels, agglutination titers, and the complement CPW and APW response during the immunization period.

Table 2. Effects of moment of feed access and pre-tarter composition on BW, BW gain, feed intake and gain to feed ratio of broiler chickens 0 to 21 days (LSmeans)[1]

	FA			DIET				*P* values		
	DIR	DEL	SEM	CONT	FISH	MCFA	SEM	FA	DIET	FA x DIET
n	48	48		32	32	32				
BW (g)										
0 d	46.1	46.1	0.1	46	46.2	46.1	0.2	0.967	0.519	0.455
21 d	1061^{A}	867^{B}	7	958	964	969	8	<0.001	0.616	0.068
BW gain (g/d)										
0-2	-	-	-	9.6	10.2	10.1	0.2	-	0.263	-
2-7	25.5^{A}	18.9^{B}	0.3	21.9^{A}	21.8^{A}	22.9^{B}	0.3	<0.001	0.010	0.101
7-21	62.0^{A}	52.3^{B}	0.4	56.9	57.3	57.3	0.5	<0.001	0.806	0.097
Feed intake (g/d)										
0-2	-	-	-	7.7	8	7.8	0.1	-	0.362	-
2-7	24.7^{A}	16.0^{B}	0.2	20.2	20.3	20.5	0.3	<0.001	0.636	0.189
7-21	84.2^{A}	70.0^{B}	0.6	76.6	77.5	77.3	0.7	<0.001	0.63	0.180
Gain to feed ratio										
0-2 d	-	-	-	1.246	1.272	1.283	0.016	-	0.208	-
2-7 d	1.035^{A}	1.184^{B}	0.007	1.102^{a}	1.094^{a}	1.132^{b}	0.008	<0.001	0.004	0.401
7-21 d	0.736^{A}	0.747^{B}	0.003	0.743	0.74	0.741	0.003	0.005	0.839	0.563

FA = Feed and water access; DIR = Direct access; DEL = Delayed access (48h); DIET = Pre-starter diet; CONT = Control; FISH = Fish Oil; MCFA = Medium chain fatty acids. [1] Each cage contained 6 male broilers at the start of the experiment. For analysis of data collected from 0 to 2 days, effects of feed and water access and the interaction with pre-starter composition were excluded from the model. [A,B] Values within a row with different superscripts differ significantly at $P \leq 0.01$.

Table 3. Effects of moment of feed access and pre-starter composition on BW, BW gain, feed intake and gain to feed ratio of broiler chickens 21 to 28 days (LSmeans)[1]

	FA			DIET				*P* values	
	DIR	DEL	SEM	CONT	FISH	MCFA	SEM	FA	DIET
n	48	48		32	32	32			
BW (g)									
21 d	1061[A]	867[B]	7	958	964	969	8	<0.001	0.616
28 d	1760	1531	10	1645	1645	1647	13	<0.001	0.993
BW gain (g/d)									
21-25 d	92.4[A]	86.1[B]	1.0	90	89.1	88.6	1.2	<0.001	0.676
25-28 d	109.7	107.0	1.3	108.9	108.5	107.6	1.6	0.109	0.808
Feed intake (g/d)									
21-25 d	141.2[A]	127.1[B]	1.0	134.3	134.7	133.4	1.2	<0.001	0.741
25-28 d	167.9[A]	159.2[B]	1.4	164.6	163.4	162.6	1.7	<0.001	0.621
Gain to feed ratio									
21-25 d	0.654[A]	0.677[B]	0.005	0.670	0.662	0.665	0.006	0.001	0.577
25-28 d	0.652[A]	0.673[B]	0.006	0.662	0.664	0.661	0.007	0.005	0.943

FA = Feed and water access; DIR = Direct access; DEL = Delayed access (48h); DIET = Pre-starter diet; CONT = Control; FISH = Fish Oil; MCFA = Medium chain fatty acids. [1] Each cage contained 6 male broilers at the start of the experiment. [A,B] Values within a row with different superscripts differ significantly at $P \leq 0.01$.

Table 4. Interaction effects of moment of feed access and pre-starter composition on BW, BW gain, feed intake and gain to feed ratio of broiler chickens 21 to 28 days (LSmeans)[1]

	DIR			DEL				*P* values
	CONT	FISH	MCFA	CONT	FISH	MCFA	SEM	FA x DIET
n	16	16	16	16	16	16		
BW (g)								
21 d	1065	1068	1051	852	861	887	11	0.068
28 d	1778[a]	1764[a]	1736[a]	1511[b]	1525[b]	1557[b]	17	0.033
BW gain (g/d)								
21-25 d	94.6	92.7	89.9	85.4	85.5	87.4	1.6	0.089
25-28 d	111.9	108.8	108.4	106	108.1	106.8	2.2	0.409
Feed intake (g/d)								
21-25 d	143.1	142.1	138.3	125.5	127.2	128.5	1.7	0.066
25-28 d	171.2	166.8	165.8	158.1	160.1	159.5	2.3	0.195
Gain to feed ratio								
21-25 d	0.66	0.652	0.651	0.68	0.672	0.68	0.008	0.790
25-28 d	0.653	0.652	0.653	0.671	0.677	0.67	0.009	0.882

FA = Feed and water access; DIR = Direct access; DEL = Delayed access (48h); DIET = Pre-starter diet; CONT = Control; FISH = Fish Oil; MCFA = Medium chain fatty acids. [1] Each cage contained 6 male broilers at the start of the experiment. [a,b] Values within a row with different superscripts differ significantly at $P \leq 0.01$.

DISCUSSION

Moment of feed access

Delayed feed access resulted in lower BW gain from 0 to 25 days compared with direct fed chickens. A lower BW gain might be explained by impaired intestinal development, which is a direct consequence of delayed feed access as demonstrated by Noy et al. (2001). Impaired intestinal development has been described by a lowered relative weight of the intestinal tract, shortened intestinal length, changed villi density, lowered villi height and a lowered number of goblet cells per villus (Maiorka et al., 2003). A too long period of feed withdrawal may cause a permanent impairment of growth from which chickens do not recover by means of compensatory growth and performance. In the current experiment, chickens were indeed not capable to recover from delayed feed access for 48 h, although differences became smaller and non-significant from 25 days onwards. In the current experiment, the partial compensatory growth was reflected by chickens with delayed feed access having a higher gain to feed ratio throughout the experimental period than those with direct feed access. An improved feed efficiency of broiler chickens from 0 to 21 days after a period of feed restriction was found not to be related to a lowered maintenance requirement nor continued lower metabolic rate as a consequence of feed restriction. However, a higher metabolizable energy intake as a result of a higher feed intake relative to body size, does improve feed efficiency (Zubair and Leeson, 1994).

In the current experiment, an interaction was found for mortality rate from 0 to 28 days between feed access and pre-starter composition, although this was primarily caused by differences between direct and delayed fed chickens that were fed the control pre-starter diet. Differences in mortality might relate to the metabolic status of the chicken during the first week after hatch. Various studies have suggested a positive relationship between restricted feeding and a lowered mortality risk due to lower occurrence of ascites (Balog, 2003; Wijtten et al., 2010). Delayed feed access after hatch can be considered as a form of feed restriction too and as such may cause a similar effect. Feed restriction has been related to a lowered metabolic rate and subsequently a lower oxygen requirement, possibly resulting in less ascites associated health problems (Wijtten et al., 2010). Therefore, delayed feed access after hatch may result in a physiological benefit over direct feeding by means of a lowered mortality risk. However, in other research where chickens were delayed fed for 1 or 2 days, the mortality risk was not affected (Corless and Sell, 1999). In chickens that were infected with the malabsorption syndrome, inclusion of MCFA resulted in a lower mortality risk (Gutierrez Del Alamo et al., 2007) of which the effect was contributed to the antimicrobial properties of MCFA (Van Immerseel et al., 2004). Inclusion of MCFA in a pre-starter diet can be hypothesized to have similar antimicrobial properties, possibly preventing the colonization of pathogenic microflora associated with increased mortality risk. It remains up for discussion then why a similar effect of MCFA on mortality risk was

not found in delayed fed broiler chickens and if this for example is related to the amount of feed intake or timing of feeding.
Chickens with direct feed access had higher concentrations of IFN-γ 21 to 28 days compared with chickens with delayed feed access for 48 h. IFN-γ is predominantly produced by natural killer cells as part of the innate immune response, followed by $CD4^+$ and $CD8^+$ T cells during the acquired immune response (Schoenborn and Wilson, 2007). This advocates that also T cells are capable of producing IFN-γ when stimulated by antigens, next to natural killer cells. Delayed feed access may result in delayed colonization of the cecum and colon by T cells, as well as expression of IL-2 mRNA in hindgut T cells (Bar-Shira et al., 2005). The latter might suggest that chickens with delayed feed access are less able to produce IFN-γ around the gastrointestinal tract compared with direct fed chickens, as T cells produce IFN-γ in response to IL-2 stimulation.
In the current experiment, delayed feed access did not strongly affect the measured parameters for humoral immune function and response after a SRBC challenge, while in other experiments the immune response was impaired when exposed to an immunological challenge in later life (Bar-Shira et al., 2005; Simon et al., 2015). This suggests that the measured immune responses that are initially related to a dietary treatment, may also strongly depend on the type of immunological challenge used.

Pre-starter composition

Inclusion of MCFA in the pre-starter diet resulted in higher BW gain and gain to feed ratio (not feed intake) compared with the control and fish oil containing pre-starter diets, but only during the period the pre-starter diet was provided (till 7 days). However, a tendency for interaction between moment of feed access and pre-starter composition was found as well for BW gain and feed intake from 7 to 25 days. It appears that a MCFA pre-starter diet is most effective for chickens with delayed feed access and it might be hypothesized that inclusion of MCFA in a pre-starter diet had a beneficial effect on the recovery of the gastrointestinal tract after an initial period of feed deprivation. MCFA are known to increase tight junction permeability of mucosal tissue (Lindmark et al., 1998). Increased tight junction permeability may impair the intestinal barrier functionality and may thus result in increased susceptibility to pathogens and a transfer of larger toxic molecules. However, the disadvantage of increased tight junction permeability seems to be outweighed in an intestine that is already damaged, because it may also result in a more rapid absorption of nutrients, such as glucose (Ballard et al., 1995). These nutrients may be required as an energy source for intestinal recovery. Besides increasing tight junction permeability of mucosal tissue, MCFA by themselves may also function as an easily accessible energy supply for enterocytes and thus cell recovery due to their relatively high digestibility coefficient at hatch compared with other fats (0.86 for polyunsaturated fatty acids v. 0.61 for soybean oil; Noy and Sklan, 1995, Turner et al., 1999, Batal and Parsons, 2002). Hence, MCFA might

have a beneficial effect in the intestine of delayed fed chickens only during a restoration phase, meaning that in direct fed chickens that do not need to restore their intestinal integrity, MCFA lack this beneficial effect.

Inclusion of fish oil in the pre-starter diet did not affect natural anti-KLH IgY titers. However, Wang et al. (2000) found increased IgY levels (+30%) when feeding layer chickens from 0 to 8 weeks of age fed a diet containing 5% fish oil v. sunflower oil, animal oil blend or linseed oil. Therefore, the current inclusion level of fish oil (0.5%) may have been too low to find effects on the humoral immune system.

In summary, delayed feed access for day old chicks resulted in lower growth performance compared with direct access. Based on partial compensatory growth, it can be suggested that the current period of delayed feed access did not result in a permanent impairment of the chickens' growth capacity. Feeding MCFA in the pre-starter diet resulted in higher BW gain and gain to feed ratio, but only during the period the diets were provided. Feeding a MCFA containing pre-starter diet tends to be most effective for delayed fed broiler chickens by means of higher growth performance, but most effective for direct fed chickens with respect to a lowered mortality risk. Current inclusion levels of fish oil and MCFA in the pre-starter diet appear to have limited (long-term) effects on growth and development, as well as on humoral immune function.

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CONFLICTS OF INTEREST

The Authors declare that there is no conflict of interest.

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SUPPLEMENTARY MATERIAL

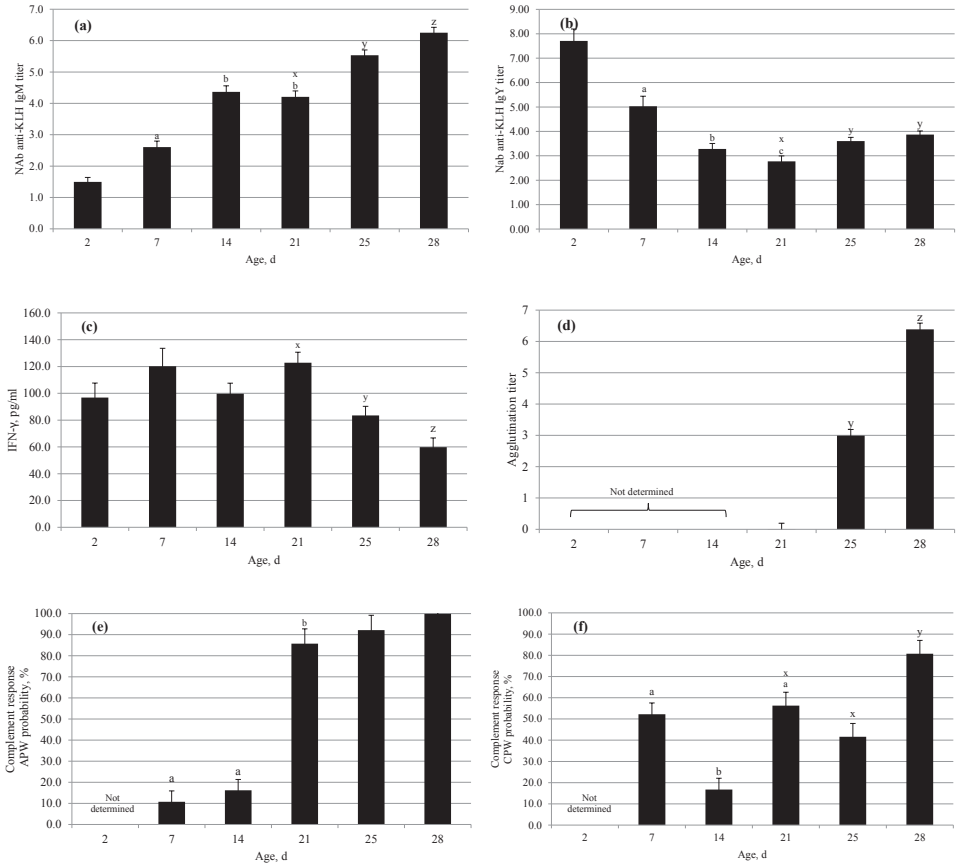
RESULTS

Regardless of treatments, expression of natural antibodies (**NAb**) titers against Keyhole Limpet Hemocyanin (**KLH**) IgM increased as the chicken aged from 7 to 28 days (P<0.001; Supplementary Table S2, S3, and Supplementary Figure S1a). NAb anti-KLH IgY was also age dependent from 7 to 28 days (*P*<0.001; Supplementary Table S2, S3, and Supplementary Figure S1b), but its expression initially declined from 7 days onwards, reached a lowest point at 21 days, increasing again at 25 days and then remaining stable until 28 days. Interferon gamma (**IFN-γ**) concentrations declined from 21 days onwards (*P*<0.001; Supplementary Table S3 and Supplementary Figure S1c). The agglutination titer increased from 21 to 28 days of age as a result of sheep red blood cell (**SRBC**) immunization at 21 days of age (*P*<0.001; Supplementary Table S4 and Supplementary Figure S1d). The classical (**CPW**) and alternative (**APW**) complement pathway were found to be age dependent until 21 days of age, whereas only the CPW remained age dependent until 28 days of age (*P*<0.001; Supplementary Figures S1e and S1f).

Summary Table S1. Effects of moment of feed and water access after placement in the grow-out facility and pre-starter composition on natural antibody (**NAb**) Keyhole Limpet Hemocyanin (**KLH**) IgM and IgY and interferon gamma (**IFN-γ**) in broiler chickens at 2 days of age (LSmeans)[1]

	DIET				
	CONT	FISH	MCFA	**SEM**	***P* values**
n	32	32	32		
IgM (NAb anti-KLH titer)	1.6	1.5	1.5	0.1	0.762
IgY (NAb anti-KLH titer)	7.8	7.8	7.7	0.5	0.971
IFN-γ (pg/ml)	100	99.6	90.8	10.9	0.766

DIET = Starter diet; CONT = Control; FISH = Fish Oil; MCFA = Medium chain fatty acids. [1] One broiler chicken sampled per cage. For analysis of data collected from 0 to 2 days of age, effects of feed and water access and the interaction with starter diet composition were excluded from the model.



Supplementary Figure S1 Natural antibody (NAb) anti-Keyhole Limpet Hemocyanin (KLH) IgM (a) and IgY titers (b), interferon gamma (IFN-γ) concentrations (c), agglutination titers (d), and complement activity (the chance for a response at that age, expressed as percentage) based on the alternative *(APW)* (e) and classic (CPW) (f) complement pathway in broiler chickens from 2 to 28 days in direct fed broiler chickens. Chickens received a control, fish oil or medium chain fatty acids (MCFA) containing starter diet from 0 to 7 days. Chickens were sensitized with sheep red blood cells at 21 days. Levels that differ within the period from 7 to 21 days (grow-out period) are indicated by superscripts labelled a-c, differences within the period from 21 to 28 days (immunization period) are indicated by superscripts labelled x-z. Columns with different superscripts differ significantly at $P \leq 0.05$.

Supplementary Table S2. Effects of moment of feed and water access after placement in the grow-out facility and pre-starter composition on natural antibody (**NAb**) keyhole Limpet Hemocyanin (**KLH**) IgM and IgY, interferon gamma (**IFN-γ**), and complement activity based on the classical (**CPW**) and alternative (**APW**) complement pathway in broiler chickens 7 to 21 days (LSmeans)[1]

Item		IgM (NAb anti-KLH titer)			IgY (NAb anti-KLH titer)			IFN-γ (pg/ml)			Complement CPW[2] (%)			Complement APW[2] (%)		
		7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
DIR	CONT	2.9	4.5	3.9	5.0	3.5	2.7	110.3	106.7	153.4	-	-	-	-	-	-
	FISH	2.8	4.1	4.1	4.6	2.5	2.2	139.3	104.1	112.9	-	-	-	-	-	-
	MCFA[2]	2.9	4.5	4.2	4.8	3.5	3.0	102.0	97.1	155.7	-	-	-	-	-	-
DEL	CONT	2.8	4.2	3.7	4.7	2.8	2.8	112.9	96.7	86.9	-	-	-	-	-	-
	FISH	2.3	4.2	5.1	6.0	3.7	2.9	129.4	96.6	117.4	-	-	-	-	-	-
	MCFA	2.2	4.7	4.2	4.9	3.7	3.1	126.8	96.9	110.4	-	-	-	-	-	-
	SEM (n = 16)		0.5			0.6			19.7			-			-	
FA	DIR	2.9	4.4	4.1	4.8	3.2	2.6	117.2	102.6	140.7	47.6	19.0	60.1	12.5	25.6	80.7
	DEL	2.4	4.4	4.3	5.2	3.4	2.9	123.0	96.7	104.9	56.9	14.7	52.5	9.2	9.8	89.6
	SEM (n = 48)		0.3			0.3			11.3			7.7			7.3	
DIET	CONT	2.8	4.4	3.8	4.9	3.2	2.7	111.6	101.7	120.2	53.1	28.2	62.9	15.5	19.1	96.5
	FISH	2.6	4.2	4.6	5.3	3.1	2.6	134.4	100.3	115.2	59.6	22.7	45.1	10.0	15.9	75.0
	MCFA	2.5	4.6	4.2	4.8	3.6	3.0	114.4	97.0	133.0	43.9	6.6	60.7	8.0	13.9	72.6
	SEM (n = 32)		0.4			0.4			13.7			9.5			8.7	
DAY		2.6^{A}	4.4^{B}	4.2^{B}	5.0^{A}	3.3^{B}	2.8^{C}	120.1	99.7	122.8	52.2^{A}	16.8^{B}	56.3^{A}	10.8^{A}	16.2^{A}	85.7^{B}
	SEM		0.2			0.2			7.9			5.3			5.1	

Supplementary Table S2. *Continued*

P values					
FA	0.850	0.296	0.279	0.807	0.482
DIET	0.905	0.816	0.882	0.275	0.093
DAY	<0.001	<0.001	0.510	<0.001	<0.001
FA x DIET	0.824	0.134	0.439	0.746	0.187
FA x DAY	0.414	0.941	0.307	0.394	0.127
DIET x DAY	0.337	0.621	0.907	0.153	0.636
FA x DIET x DAY	0.634	0.875	0.669	-	-

FA = Feed and water access; DIR = Direct access; DEL = Delayed access (48h); DIET = Starter diet; CONT = Control; FISH = Fish Oil; MCFA = Medium chain fatty acids. [1] One broiler chicken sampled per cage. [2] The three-way interaction between day, moment of feed and water access and starter diet composition was excluded from the model, as it did not solve for that term and therefore had to be omitted. Results expressed as the chance (percentage) for a response at that age. [A,B,C] Values within a column with different superscripts differ significantly at $P \leq 0.05$.

Supplementary Table S3. Effects of moment of feed and water access after placement in the grow-out facility and pre-starter composition on natural antibody (**NAb**) keyhole Limpet Hemocyanin (**KLH**) IgM and IgY, and interferon gamma (**IFN-γ**) in broiler chickens 21 to 28 days (LSmeans)[1]

		IgM (NAb anti-KLH titer)			IgY (NAb anti-KLH titer)			IFN-γ (pg/ml)		
		21 d	**25 d**	**28 d**	**21 d**	**25 d**	**28 d**	**21 d**	**25 d**	**28 d**
DIR	CONT	3.8	5.9	6.3	2.7	3.9	3.9	153.4	79.3	58.8
	FISH	4.1	5.9	6.3	2.3	3.7	4.3	113.3	78.8	55.4
	MCFA	4.2	5.5	6.6	2.9	3.2	3.8	155.8	105.5	71.9
DEL	CONT	3.7	5.2	6.2	2.7	3.5	3.8	86.8	73.2	52.5
	FISH	5.2	5.3	6.0	3.0	3.8	3.7	117.0	79.7	57.3
	MCFA	4.3	5.5	6.1	3.1	3.5	3.8	110.4	84.1	63.2
	SEM (n = 16)		0.4			0.4			17.0	
FA	DIR	4.0	5.8	6.4	2.6	3.6	4.0	140.8	87.9	62.0
	DEL (48 h)	4.4	5.3	6.1	2.9	3.6	3.8	104.7	79.0	57.7
	SEM (n = 48)		0.2			0.2			9.7	
DIET	CONT	3.8	5.5	6.3	2.7	3.7	3.8	120.1	76.2	55.6
	FISH	4.6	5.6	6.2	2.6	3.8	4.0	115.1	79.2	56.3
	MCFA	4.2	5.5	6.3	3.0	3.3	3.8	133.1	94.8	67.6
	SEM (n = 16)		0.3			0.3			11.8	
DAY		4.2^{A}	5.5^{B}	6.3^{C}	2.8^{A}	3.6^{B}	3.9^{B}	122.8^{A}	83.4^{B}	59.8^{C}
	SEM		0.2			0.2			6.8	
P values										
FA		0.490			0.886			0.017		
DIET		0.586			0.957			0.074		
DAY		<0.001			<0.001			<0.001		
FA x DIET		0.704			0.845			0.260		
FA x DAY		0.127			0.389			0.357		
DIET x DAY		0.365			0.250			0.961		
FA x DIET x DAY		0.359			0.615			0.654		

FA = Feed and water access; DIR = Direct access; DEL = Delayed access (48h); DIET = Starter diet; CONT = Control; FISH = Fish Oil; MCFA = Medium chain fatty acids. [1] One broiler chicken sampled per cage. [A,B,C] Values within a column with different superscripts differ significantly at $P < 0.01$.

Supplementary Table S4. Effects of moment of feed and water access after placement in the grow-out facility and pre-starter composition on complement activity based on the classical (CPW) and alternative (APW) complement pathway, and agglutination titer in broiler chickens 21 to 28 days (LSmeans)1

		Agglutination titer			Complement CPW[2] (%)			Complement APW[2] (%)		
		21 d	**25 d**	**28 d**	**21 d**	**25 d**	**28 d**	**21 d**	**25 d**	**28 d**
DIR	CONT	0.0	4.4	6.9	-	-	-	-	-	-
	FISH	0.0	3.0	5.7	-	-	-	-	-	-
	MCFA	0.0	2.8	7.2	-	-	-	-	-	-
DEL	CONT	0.0	2.9	6.2	-	-	-	-	-	-
	FISH	0.0	2.5	6.4	-	-	-	-	-	-
	MCFA	0.0	2.3	5.9	-	-	-	-	-	-
	SEM (n = 16)		0.5			-			-	
FA	DIR	0.0	3.4	6.6	60.0	54.2	77.7	82.6	91.7	99.7
	DEL (48 h)	0.0	2.6	6.2	52.3	30.0	83.5	86.9	92.6	99.9
	SEM (n = 48)		0.3			9.3			15.3	
DIET	CONT	0.0	3.7	6.6	63.4	63.4	81.3	95.6	95.4	100.0
	FISH	0.0	2.7	6.0	44.7	44.7	74.7	75.3	91.0	97.6
	MCFA	0.0	2.5	6.6	60.1	60.1	85.2	72.5	88.6	90.7
	SEM (n = 16)		0.3			11.9			8.2	
DAY		0.0^{A}	3.0^{B}	6.4^{C}	56.2^{A}	41.6^{A}	80.7^{B}	84.9	92.2	99.9
	SEM		0.2			6.3			7.0	
P values										
FA			0.549			0.311			0.297	
DIET			0.114			0.356			0.542	
DAY			<0.001			<0.001			0.444	
FA x DIET			0.604			0.380			0.368	
FA x DAY			0.144			0.241			0.596	
DIET x DAY			0.593			0.865			0.808	
FA x DIET x DAY			0.180			-			-	

FA = Feed and water access; DIR = Direct access; DEL = Delayed access (48h); DIET = Starter diet; CONT = Control; FISH = Fish Oil; MCFA = Medium chain fatty acids. [1] One broiler chicken sampled per cage. [2] The three-way interaction between day, moment of feed and water access and starter diet composition was excluded from the model, as it did not solve for that term and therefore had to be omitted. Results expressed as the chance (percentage) for a response at that age. [A,B,C] Values within a column with different superscripts differ significantly at $P \leq 0.01$.

DISCUSSION

Considering age dependent immune development in the chicken, the NAb anti-KLH IgM titers increased over time, while for NAb anti-KLH IgY the level decreased directly after hatch and remained low thereafter. These findings correspond with earlier research (Simon et al., 2014). Maternal transfer of NAb IgM from broiler breeders into the egg was found to be very limited (Hamal et al., 2006). Therefore, the measured NAb anti-KLH IgM levels in the current experiment are supposed to be of endogenous origin. However, there is major transfer of maternal NAb IgY to the yolk (Hamal et al., 2006), which is directly absorbed from the residual yolk by the chicken at the moment of hatch (Tesar et al., 2008).

While current results suggest that IFN-γ is already available at a very young age (from 2 days of age onwards), this does not match earlier research where IFN-γ mRNA expression was only marginal in similar aged chickens with a similar period of feed withdrawal (Simon et al., 2014). The observed differences might be explained by the fact that in earlier research IFN-γ mRNA expression was determined in intestinal tissue (mucosal response), whilst in the current study IFN-γ concentrations at the protein level in blood samples were analyzed (systemic response). Because IFN-γ was determined in healthy chicken a strong systemic response is not likely, whereas direct exposure of intestinal immune cells to bacteria may induce stronger pro-inflammatory responses. Moreover, the lack of endogenous sIgA production at 21 days could be the reason for enhanced pro-inflammatory cytokine induction, because sIgA prevents bacterial translocation and has anti-inflammatory properties (Lammers et al., 2010).

In the current experiment the higher CPW complement activity at 7 days compared to 14 days of age (Supplementary Figure 1) may be the result of the presence of maternal antibodies, because the CPW is antibody dependent (Janeway et al., 2001). At 14 days of age maternal antibodies are no longer present, whereas endogenous immunoglobulin production is still suboptimal. However, CPW activity between 7 and 21 days of age may also be influenced by endogenous produced IgM, because production of this isotype precedes IgY production. APW related complement activity only increased from 21 days of age onwards (Supplementary Figure 1), but is antibody independent.

The age dependent increase in agglutination titer is most likely a consequence of SRBC immunization. Until now, to our knowledge no literature is available demonstrating age related dynamics in complement activity of broiler chickens.

Concluding, the humoral immune status by means of NAb anti-KLH IgM and IgY, IFN- γ, and complement activity (CPW and APW) is strongly age dependent, while the agglutination titer is strongly challenge dependent.

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CHAPTER 4

Diet density during the first week of life: Effects on energy and nitrogen balance characteristics of broiler chickens

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ABSTRACT

This study aimed to determine effects of diet density on growth performance, energy balance, and nitrogen **(N)** balance characteristics of broiler chickens during the first wk of life. Effects of diet density were studied using a dose-response design consisting of 5 dietary fat levels (3.5, 7.0, 10.5, 14.0, and 17.5%). The relative difference in dietary energy level was used to increase amino acid levels, mineral levels, and the premix inclusion level at the same ratio. Chickens were housed in open-circuit climate respiration chambers from d 0 to 7 after hatch. Body weight was measured on d 0 and 7, whereas feed intake was determined daily. For calculation of energy balances, O_2 and CO_2 exchange were measured continuously and all excreta from d 0 to 7 was collected and analyzed at d 7. Average daily gain (ADG) and average daily feed intake (ADFI) decreased linearly ($P = 0.047$ and $P < 0.001$, respectively), whereas gain to feed ratio increased ($P < 0.001$) with increasing diet density. Gross energy **(**GE**)** intake and metabolizable energy (ME) intake were not affected by diet density, but the ratio between ME and GE intake decreased linearly with increasing diet density ($P = 0.006$). Fat, N, and GE efficiencies (expressed as gain per unit of nutrient intake), heat production, and respiratory exchange ratio (CO_2 to O_2 ratio) decreased linearly ($P < 0.001$) as diet density increased. Energy retention, N intake and N retention were not affected by diet density. We conclude that a higher diet density in the first wk of life of broiler chickens did not affect protein and fat retention, whereas the ME to GE ratio decreased linearly with increased diet density. This suggests that diet density appears to affect digestibility rather than utilization of nutrients.

Key words: broiler chickens, dietary fat, diet density, energy balance, indirect calorimetry

INTRODUCTION

Studies to determine the maintenance and growth requirements of broiler chickens often focus on their overall life span without differentiating for different age periods (Sakomura et al., 2005; Lopez and Leeson, 2008). These studies ignore that requirements may be age-related, depending on the physiological status of the chicken. For young broilers, requirements may depend on the availability of the residual yolk **(RY)**, the poikilothermic nature of the chicken at early age (Tazawa et al., 1988), and the ongoing maturation of organs, particularly the intestinal tract (Fan et al., 1997). This means that requirements and metabolic processes during the first wk of life, and how these may be affected by diet composition, are hardly investigated.

Metabolic processes require energy input. During the first few days after hatch, the RY functions as energy supply for initial growth and development, containing about 14 to 26% fat and only about 2.3 to 2.7% of carbohydrates (Yadgary et al., 2010). This suggests that lipids are the major contributor to the energy supply for chickens not only during embryonic development, but also directly after hatch. Beside energy from the RY, the chick also requires energy from exogenous energy sources (feed and water) shortly after hatch. Next to fats and oils, carbohydrates function as the major energy source in modern broiler chicken diets (Sklan, 2001). Feeding of carbohydrates results in a shift from mainly lipid oxidation during the late stage of incubation (Noble and Cocchi, 1990) towards mainly oxidation of glucose and starch post-hatch. Simultaneously, hormonal and nutrient-signaling pathways induce a shift from hepatic storage of lipids obtained from the yolk towards storage of lipids obtained through exogenous feed, as well as *de novo* production of lipids through lipogenesis (Richards et al., 2010). Even though the process of lipogenesis is considered to be functional shortly after hatch (Noble and Cocchi, 1990), digestibility of dietary fats is perceived to be age dependent and relatively low for broiler chickens during the first wk of life (Ravindran et al., 2016). The perception that fat digestibility is relatively low (relative to digestibility of carbohydrates and protein) in young compared to old broiler chickens has resulted in limited research into dietary fat inclusion during the first wk of life. However, it was already demonstrated that feeding broiler chickens fat levels up to 15% (stabilized animal grease) and 15.9% (soybean oil) from either 0 to 21 or 0 to 28 d of age resulted in maintained or even increased average daily gain (ADG) and gain to feed ratio (G:F) compared to feeding low diet densities, as long as the CP to energy ratio was kept constant (Donaldson et al., 1957; Waldroup et al., 1976). Whereas this suggests that high inclusion levels of fats and oils do not affect performance of young broiler chickens, it is not known which physiological mechanisms are involved in the oxidation and utilization of high nutrient density and dietary fat levels.

The objective of this study was to determine effects of diet density on growth performance, energy, and nitrogen **(N)** metabolism of broiler chickens during the first wk of life, using a dose-response design.

MATERIALS AND METHODS

All procedures in this study were approved by the Animal Use and Care Committee of Wageningen University & Research, the Netherlands.

Experimental design

This study used a dose-response design with 5 diet density levels through increased dietary fat levels (3.5, 7.0, 10.5, 14.0, and 17.5%), using the relative difference in dietary energy level to increase amino acid levels, mineral levels, and the premix inclusion level as well. In 8 consecutive batches, treatments were randomly distributed over 4 climate respiration chambers **(CRC)**. Depending on their size (267 or 1,800 L), each CRC contained 12 or 24 chickens, resulting in 6 replicates per treatment with 108 (3 × 12 and 3 × 24) broiler chickens per treatment and 540 broiler chickens in total.

Animals and housing

A total of 540 day-old Ross 308 male chickens (average body weight [BW], 45.8 g; range 44.8 to 47.3g) were obtained from a commercial hatchery (Lagerwey, the Netherlands). To reduce potential effects of hatch window, only chickens that were not entirely dry in the neck region (freshly hatched) were selected for this study. Chickens were placed in one of 4 open-circuit CRC that provided 1,000 × 800 mm or 800 × 500 mm (length by width) animal floor space (Verstegen et al., 1987). A CO_2 recovery test was performed immediately prior to the start of the experiment, according to procedures described by Heetkamp et al. (2015). In the 4 CRC, 99.3, 98.9, 99.8, and 99.7% of the CO_2 released was recovered. In the 2 smaller CRC, an additional ethanol oxidation test was performed to test air tightness of the CRC, according to Heetkamp et al. (2015), yielding a respiratory quotient (**RQ**) of 0.66 in both chambers. The latter is in line with the theoretical RQ of 0.667 when ethanol is completely burned, thus proving that there are no air leakages in the CRC. Either 24 or 12 chickens were used per CRC, depending on the minimum metabolic mass requirements for both CRC types to determine an accurate energy and N balance. Chickens were housed on a coated wire floor to facilitate quantitative excreta collection. Artificial lighting was set for 23h/d throughout the study period (0 to 7 d of age). Temperature was set to gradually decrease by 0.5°C per day during the study period, starting from 33°C at d 0. Chickens had ad libitum access to feed and water via nipple drinkers.

Experimental diets

Diets containing the lowest (3.5% dietary fat) and highest (17.5% dietary fat) diet densities were formulated using soybean oil as the primary fat source to increase the dietary fat level among treatments. The relative difference in energy level between the 2 diets was subsequently used to increase amino acid levels and mineral levels, as well as the premix

inclusion rate at the same ratio. Consequently, diets were not isocaloric nor isonitrogenous. Starter diets with 3.5, 7.0, 10.5, 14.0, and 17.5% dietary fat contained 2,870, 3,030, 3,190, 3,350, and 3,510 kcal/kg AME_n, and 11.6, 12.2, 12.9, 13.5, and 14.2 g/kg digestible lysine, respectively (Table 1). Diets were formulated based on digestibility and nutrient data provided by CVB (2007). Diet densities in between were obtained by blending the 2 basal diets in different ratios. All diets were produced and pelleted (2.0 mm) by Research Diet Services (the Netherlands). All diets were analyzed for N (ISO 5983-2, 2009), crude fat by acid hydrolysis (ISO 6492, 1999), dry matter (DM; ISO 6496, 1999), ash (ISO 5984, 2002), gross energy (**GE**) by adiabatic bomb calorimetry (ISO 9831, 1998), and calcium and phosphorous content by ICP-AES (ISO 27085, 2009).

Data collection

Individual BW was measured on d 0 and 7, whereas average daily feed intake (ADFI) was measured daily at the same moment of the day. G:F (g:g) was calculated from d 0 to 7, based on BW differences and feed intake (FI). The average ADG and metabolic BW ($kg^{0.75}$) between d 0 and 7 were calculated per CRC. Excreta produced from d 0 to 7 was quantitatively collected at d 7 and analyzed for N (ISO 5983-2, 2009), crude fat by acid hydrolysis (ISO 6492, 1999), DM (ISO 6496, 1999), and GE by adiabatic bomb calorimetry (ISO 9831, 1998).

The O_2 and CO_2 exchange (in L) was measured for each group of chickens in the CRC at 7.5 minute intervals. Based on these measurements, the respiratory exchange ratio **(RER)** was defined as the ratio of CO_2 produced (L) to O_2 consumed (L). Heat production **(HP)** was calculated using O_2 and CO_2 as inputs (Romijn and Lokhorst, 1961):

$$HP\ (kJ) = 16.20 \times O_2\ (L) + 5.00 \times CO_2\ (L)$$

ME intake ($kJ/kg^{0.75}/d$) was calculated from the GE content of feed and excreta:

$$ME_{INTAKE}\ (kJ/kg^{0.75}/d) = GE_{FEED}\ (kJ/kg^{0.75}/d) - GE_{EXCRETA}\ (kJ/kg^{0.75}/d)$$

The ratio between ME and GE intake **(ME:GE)** was calculated to determine the metabolizability of energy:

$$ME{:}GE = ME_{INTAKE} / GE_{INTAKE}$$

Table 1. Ingredient and nutritional composition of the experimental diets

Diet density, % dietary fat	3.5	7.0	10.5	14.0	17.5
Ingredient composition, g/kg					
Corn	663.6	596.7	529.9	463.1	396.3
Soybean meal (>48% CP)	164.2	184.2	204.1	224.1	244.1
Soy protein isolate	50.0	52.8	55.6	58.4	61.1
Potato protein (77% CP)	50.0	52.8	55.6	58.4	61.1
Corn gluten meal	20.0	21.1	22.2	23.3	24.5
Soybean oil	2.3	39.3	76.4	113.5	150.6
Monocalcium phosphate	17.0	18.2	19.4	20.6	21.8
Limestone	16.1	17.0	18.0	18.9	19.9
Salt	1.2	1.4	1.6	1.8	2.0
Sodium bicarbonate	2.0	1.9	1.8	1.7	1.6
DL-Methionine	2.2	2.5	2.8	3.1	3.4
L-Lysine HCL	1.5	1.5	1.5	1.4	1.4
Premix starter[1]	10.0	10.6	11.1	11.7	12.2
Calculated chemical composition, g/kg (unless otherwise stated)					
CP	229	238	248	258	268
Crude fat	35	70	105	140	175
Ash	57	60	63	65	68
DM	886	892	897	902	907
AME_n[2], kcal/kg	2,870	3,030	3,190	3,350	3,510
Dig. lys[3]	11.6	12.2	12.9	13.5	14.2
Ca	10.0	10.6	11.1	11.7	12.2
P	7.1	7.4	7.6	7.9	8.2
Analyzed chemical composition, g/kg (unless otherwise stated)					
CP	224	232	243	253	265
Crude fat	41.2	75.7	109.8	140.6	165.2
Crude ash	50.4	52.5	54.0	57.0	59.7
DM	889	889	897	901	902
Ca	9.3	9.7	10.3	11.7	11.0
P	6.8	6.9	7.3	8.2	7.8
Gross energy, kcal/kg	4,013	4,228	4,395	4,610	4,801

[1] Contributed per kilogram of diet: thiamine, 1.0 mg; riboflavin, 4.5 mg; niacinamide, 40 mg; D-pantothenic acid, 9 mg; pyridoxine-HCL, 2.7 mg; choline chloride, 500 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopherol), 33 IU; menadione, 2.3 mg; vitamin A (retinyl-acetate), 12,000 IU; cholecalciferol, 5,000 IU; biotin, 100 µg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 150 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSo_4 \cdot H_2O$, 145 mg; Na_2SeO_3, 0.56 mg; KI, 2.0 mg; antioxidant (oxytrap PXN), 125 mg. [2] AME_n, calculated according to CVB (2007). [3]Calculated according to CVB (2007).

Energy retention **(ER)** was calculated as the difference between ME_{INTAKE} and HP. With respect to energy retention, a distinction was made between N and fat retention. N retention was determined by measuring the level of N in feed, excreta, ventilation air (NH_3 trapped in sulphuric acid), and condensed water (NH_4^+) on the heat exchanger (ISO 5983-2, 2009). ER as protein **(ER_p)** was subsequently calculated as:

$$ER_p \text{ (kJ/kg}^{0.75}\text{/d)} = 23.6 \times 6.25 \times \text{N retention}$$

where 23.6 kJ/g is the energetic value of body protein (Larbier and Leclercq, 1994). Energy retained as fat **(ER_f)** was calculated as the difference between ER and ER_p.

Efficiencies of energy and N retention were calculated by expressing retention as a percentage of metabolizable intake. Nutrient efficiencies for N, fat, and GE were calculated as gain per unit of nutrient intake from d 0 to 7.

Statistical analysis

Data were subjected to mixed model analysis using the PROC MIXED procedure in SAS (Version 9.3, 2011, SAS Institute Inc., Cary, NC, United States), using the model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + b_k + \varepsilon_{ijk}$$

where Y_{ij} = dependent variable, μ = overall mean, α_i = fixed treatment effect (i = 3.5, 7.0, …, 17.5% dietary fat), β_j = fixed CRC type effect (j = 12 or 24 broiler chickens), b_k = random batch effect (k = 1, 2, ..., 8), and ε_{ijk} = residual error term. Contrasts were used to determine significant relationships for linear and quadratic effects of diet density. Data were analyzed using a group of animals in one CRC as the experimental unit. Data are expressed as least square **(LS)** means. LSmeans were compared after correcting with Tukey 's test for multiple comparisons, and effects were considered to be significant when $P \leq 0.05$.

RESULTS

Growth performance

From d 0 to 7, ADG (average 18.7 g/d) and ADFI (average 16.7 g/d) decreased linearly as the diet density increased (Δ = -6.8% ADG and Δ = -18.7% ADFI between 3.5 and 17.5% dietary fat; P = 0.047 and P < 0.001, respectively; Table 2). G:F ratio (average 1.124) from d 0 to 7 increased linearly as diet density increased (Δ = 14.4% between 3.5 and 17.5% dietary fat; P < 0.001; Table 2). On the contrary, nutrient efficiencies of fat, N, and GE linearly decreased as diet density increased (Δ = -71%, -3.4% and -4.7% between 3.5 and 17.5% dietary fat; P < 0.05; Table 2). For nutrient efficiency of fat, also a quadratic response was found (P < 0.001; Table 2).

Table 2. Effects of diet density on BW, ADG, ADFI, gain to feed (G:F) ratio, and nutrient efficiencies (η) for fat, nitrogen (N) and GE (expressed as g gain per unit of nutrient intake) of broiler chickens of 0 to 7 d of age, expressed as least square means[1]

		BW, g		ADG, g/d	ADFI, g/d	G:F, g:g	Fat_{η}, g gain / g fat	N_{η}, g gain / g N	GE_{η}, g gain / MJ GE
Item		**0 d**	**7 d**	**0-7 d**	**0-7 d**	**0-7 d**	**0-7 d**	**0-7 d**	**0-7 d**
Diet density, % fat	3.5	45.7	180.7	19.3	18.2^{c}	1.059^{c}	25.8^{d}	29.6^{b}	63.2^{d}
	7.0	45.7	176.0	18.6	17.2^{bc}	1.081^{bc}	14.2^{c}	29.1^{b}	61.1^{bd}
	10.5	45.7	176.5	18.7	16.4^{b}	1.140^{b}	10.4^{b}	29.3^{b}	62.1^{cd}
	14.0	45.8	177.3	18.8	16.7^{b}	1.128^{b}	8.0^{a}	27.8^{a}	58.4^{ac}
	17.5	45.7	171.6	18.0	14.8^{a}	1.212^{a}	7.4^{a}	28.6^{ab}	60.2^{ab}
SEM (n = 6)		0.3	3.0	0.4	0.3	0.017	0.3	0.5	1.0
P-value	Fixed	0.232	0.193	0.199	<0.001	<0.001	<0.001	0.044	0.008
	Linear	0.331	0.047	0.047	<0.001	<0.001	<0.001	0.019	0.004
	Quadratic	0.269	0.849	0.877	0.570	0.348	<0.001	0.568	0.319

[1] For each treatment, replicates consisted of 3 × 12 and 3 × 24 male broiler chickens at the start of the experiment.
[a-d] Effect means within columns with no common superscript differ significantly ($P \leq 0.05$).

Table 3. Effects of diet density on the gross energy (GE) intake, ME intake, the ratio between ME and GE intake (ME:GE), heat production (HP), the respiratory exchange ratio (RER) and energy retention (ER) as protein (ER_p) and fat (ER_f) of broiler chickens of 0 to 7 d of age, expressed as least square means[1]

		GE intake, kJ/ $kg^{0.75}$/d	ME intake, kJ/ $kg^{0.75}$/d	ME:GE, %	HP, kJ/ $kg^{0.75}$/d	RER	ER, kJ/ $kg^{0.75}$/d	ER_p, kJ/ $kg^{0.75}$/d	ER_f, kJ/ $kg^{0.75}$/d
Diet density, % fat	3.5	1686.5^{b}	1287.3	76.6^{bc}	557.5^{a}	1.052^{a}	730.5	349.6	381.1
	7.0	1705.1^{b}	1314.3	76.9^{c}	535.4^{bc}	1.015^{b}	777.5	355.6	421.7
	10.5	1682.4^{b}	1250.5	74.4^{b}	527.7^{c}	0.978^{c}	724.0	346.1	377.9
	14.0	1794.2^{a}	1331.1	74.2^{ab}	540.0^{b}	0.940^{d}	792.0	370.7	421.1
	17.5	1700.2^{b}	1252.1	73.9^{a}	531.7^{bc}	0.907^{e}	719.5	354.0	365.8
SEM (n = 6)		25.2	25.7	1.0	4.6	0.003	24.6	6.9	18.3
P-value	Fixed	0.015	0.131	0.042	<0.001	<0.001	0.148	0.139	0.114
	Linear	0.121	0.506	0.006	<0.001	<0.001	0.921	0.272	0.578
	Quadratic	0.296	0.476	0.711	0.001	0.511	0.200	0.656	0.123

[1] For each treatment, replicates consisted of 3 × 12 and 3 × 24 male broiler chickens at the start of the experiment.
[a-e] Effect means within columns with no common superscript differ significantly ($P \leq 0.05$).

Energy and nitrogen balance

GE intake and ME intake were not affected by diet density, but a linear decrease was found for the ratio between ME and GE (average 75.2%; Δ = -3.5% between 3.5 and 17.5% dietary fat; $P = 0.006$; Table 3). The RER (average 0.98) decreased linearly with increasing diet density (Δ = -13.8% between 3.5 and 17.5% dietary fat; $P < 0.001$; Table 3). Linear and quadratic responses were found for HP (average 538.4 kJ/kg$^{0.75}$/d) with increasing diet density (Δ = -4.6% between 3.5 and 17.5% dietary fat; $P \leq 0.001$; Table 3). This was particularly marked for the lowest diet density (3.5% dietary fat) that had higher HP compared to higher diet densities from d 4 onward. Energy retention (average 748.7 kJ/kg$^{0.75}$/d) was not affected by diet density (Table 3), neither were N intake (average 0.67 g/d) and N retention (average 2.40 g/kg$^{0.75}$/d; Table 4).

Table 4. Effects of diet density on nitrogen (N) intake, digestible N intake, N retention (NR), and efficiency (η) of N retention of broiler chickens of 0 to 7 d of age, expressed as least square means[1]

		N intake, g/day	Dig. N intake, g/kg$^{0.75}$/d	NR, g/kg$^{0.75}$/d	NR_{η}, % of N intake	NR_{η}, % of dig. N intake
Diet density, % fat	3.5	0.68	2.37	2.36	65.6	99.56
	7.0	0.66	2.41	2.40	67.0	99.60
	10.5	0.66	2.35	2.34	65.5	99.53
	14.0	0.70	2.51	2.50	66.5	99.59
	17.5	0.65	2.40	2.39	66.9	99.54
SEM (n = 6)		0.01	0.05	0.05	0.9	0.04
P-value	Fixed	0.080	0.140	0.140	0.480	0.680
	Linear	0.826	0.267	0.272	0.394	0.616
	Quadratic	0.587	0.657	0.656	0.840	0.680

[1]For each treatment, replicates consisted of 3 × 12 and 3 × 24 male broiler chickens at the start of the experiment.

DISCUSSION

The current study aimed to determine if diet density, using soybean oil as primary energy source and maintaining a constant amino acid-to-energy ratio, affected growth performance, as well as energy and N metabolism characteristics of broiler chickens during the first wk of life. The lowered ADFI and ADG as a result of the increased diet density, as found in the current study, might be explained by metabolic regulatory mechanisms, as well as by dietary physical form. Broiler chickens are known for being able to control their FI in order to

maintain a constant energy intake (Leeson et al., 1996). However, this appears to be only the case for older broilers, as Brickett et al. (2007) found that broiler chickens up to 2 wk of age are not able to maintain a constant energy intake when diet density was changed. These findings are in contrast with current results, as in the current study a constant ME intake and N intake amongst treatments were found during the first wk of life.

Because of these findings, current results suggest that energy (originating from fat and carbohydrates) or N, or a combination of both, have a regulatory role in FI already in the first wk of life.

FI regulation is considered the result of a complex interplay between various endogenous mechanisms, where the central nervous system is driven by nutrient signaling, neuroendocrine signaling, and peripheral tissue signaling within the gastrointestinal tract (Richards and Proszkowiec-Weglarz, 2007). FI regulation through nutrient signaling can be differentiated into glucostatic, lipostatic, ionostatic, and aminostatic theories (reviewed by Decuypere et al., 2007). The current study was not designed to determine whether the lower FI at higher diet densities is either glucostatic, lipostatic, ionostatic, or aminostatic driven, as not only dietary fat, but also CP and mineral levels were increased in the higher density diets.

The lowered ADFI and ADG with increased diet density found in the current trial may also be explained by the lowered nutrient efficiencies for N and energy with increased diet density. Gain per unit of N and GE consumed decreased linearly as diet density increased. Ravindran et al. (2016) reported that the energetic availability of fats and oils for an animal tend to decrease as the inclusion level increases. This might be explained by increased soap formation by free fatty acids and calcium (Ravindran et al., 2016). In the current study, increased diet density included an increased dietary fat (from 3.5 to 17.5%) and calcium (from 1.00 to 1.22%) inclusion level. This potentially increases the risk for saponification that subsequently may have resulted in lowered digestibility.

The reduction in N efficiency corresponds with earlier research where increased CP consumption actually resulted in reduced protein utilization (Swennen et al., 2007). The reduced efficiency is mainly attributed to the energetic costs associated with the removal of excessive N from the body through uric acid formation. This suggests that a constant amino acid-to-energy ratio, as used in the current study, can be further optimized in order to minimize the metabolic costs for excessive N removal from the body.

Current effects of increased diet density on ADG, ADFI, and G:F may also be explained by diet physical form. Findings with respect to increased G:F are supported by Donaldson et al. (1957) and Waldroup et al. (1976), who demonstrated that fat inclusion levels up to 15% (stabilized animal grease) or 15.9% (soybean oil) with a maintained protein to energy ratio, fed to chickens from 0 to 3 wk of age, also resulted in an increased G:F. However, in the current study the ADG was linearly lowered, whereas it maintained or even increased in previous work. Though not explicitly stated, it is very likely that with dietary fat levels as high as 23.4%, the diets used by Donaldson et al. (1957) and Waldroup et al. (1976) may

have been fed as mash. Earlier research found an interaction between diet density and feed form (mash vs. pelleted diets) with respect to ADG (Brickett et al., 2007). Even though increased diet densities (300 kcal/kg ME difference) resulted in higher ADG from 0 to 6 d when fed as mash diets, ADG was not affected when broilers were fed pelleted diets. This suggests that chickens initially try to maximize FI in order to achieve maximum growth, which can be easier achieved in pelleted than in mash diets. Although this does not explain in full the reduced ADG in the current study, it appears that feed form may contribute partially to the results observed. In addition, the observed response for ADFI might be partially biased by pellet quality. High fat levels may result in lower pellet quality in terms of pellet durability (Abdollahi et al., 2013).

The RER was highly treatment-dependent and decreased with increased diet density. This appears to be logical, as the RQ for glucose is approximately 1, whereas it is approximately 0.80 for protein and 0.70 for fat (Ferrannini, 1988). Increasing levels of diet density contain more fat at the expense of carbohydrates (glucose), resulting in relative higher fat oxidation. The authors are aware that the RER provides only limited insight into the biochemical reactions associated with ongoing metabolic processes, as it is a simple measurement of O_2 consumption and CO_2 production (Ferrannini, 1988). Therefore, it is more interesting to discuss treatments effects in relation to ME:GE ratio and retained energy.

ME is considered to be the amount of dietary energy that is readily available to support broiler metabolism. Differences in ME:GE (also known as metabolizability) can be explained by differences in how well diets are digested and utilized. In general, the ME:GE in the current study was slightly higher than has been found in earlier research (0.752 vs. 0.689; Apeldoorn et al., 1999). Differences among studies might be explained by broiler age and diet composition. Comparing treatments, the decrease in ME:GE with increases in diet density is likely the result of lowered diet digestibility due to the increasing dietary fat content. Digestibility coefficients for dietary amino acids, carbohydrates (starch), and fat in a typical corn and soybean meal-based diet fed to broiler chickens at 7 d of age were found to be 0.83, 0.97, and 0.59, respectively (Batal and Parsons, 2002). In the same study, fat digestibility was found to increase from 0.59 to 0.74 from 7 to 14 d of age. A lowered fat digestibility for young broiler chickens, combined with increased dietary fat levels at the expense of carbohydrates as provided in the current study, may explain the linear decrease in ME:GE as the metabolizability of these diets decreases.

Based on the lowered ADG, ME:GE and the increased excreta losses with increased diet density, higher dietary fat levels do not seem favorable for young broiler chickens. However, this study shows that ME is not fully used for energy retention, as part of the ME is lost as heat, which is the result of maintenance costs and inefficiencies in the deposition of protein and fat. In the current study, approximately 41 to 43% of the ME intake was lost as heat. These losses are relatively low compared to earlier studies, where average ME losses due to heat production were calculated to be 54 to 60% in broilers ranging from 0.7 to 2.0 kg BW

(Apeldoorn et al., 1999; Labussiere et al., 2015). In general, it is perceived that larger animals have a smaller surface to body mass ratio, thus resulting in reduced heat losses. This principle does not apply for current results, suggesting that differences in heat loss might be explained by other factors. In the current study, overall HP decreased both linear and quadratic as diet density increased. This might be explained by differences in nutrient efficiencies for energy retention between protein, fat, and carbohydrates, resulting in altered diet-induced thermogenesis. This is in contrast with previous studies in which changes in macro nutrients did not affect diet-induced thermogenesis in chickens (Swennen et al., 2007).

RE, RE_f, and RE_p were not affected by treatment. RE was deposited almost equally as either protein (47.4%) or fat (52.6%). This is in agreement with earlier research where retained energy was evenly (50:50) deposited as protein and fat (Apeldoorn et al., 1999). Current results on RE, RE_f, and RE_p may appear to be contradictory to calculated N, fat, and GE nutrient efficiencies, as discussed earlier. However, a distinction can be made between the bioavailability of nutrients in feed ingredients (i.e., pre-absorption) and the post-absorptive nutrient utilization (De Lange et al., 2013). Whilst calculated nutrient efficiencies are based on the pre-absorptive state, the RE, RE_f, and RE_p refer to a post-absorptive state. It might be suggested that in current broiler chickens, the nutrient intake capacity is too limited to reach the genetic production potential (De Lange et al., 2013). For the current study this implies that the digestive status of the chicken is probably the limiting factor, whereas metabolic processes related to protein and fat deposition within the chicken were not affected.

Concluding, feeding of higher diet densities in the first wk of life of broiler chickens resulted in linearly lowered ADG and ADFI, whereas G:F increased. However, protein and fat retention was not affected, whereas the ME:GE ratio decreased linearly with increased diet density. Results suggest that the absorptive capacity of the chicken might become a limiting factor when diet density is increased.

CONFLICT OF INTEREST

D.M. Lamot and P.J.A. Wijtten are employed at the Cargill Animal Nutrition Innovation Center Velddriel, the Netherlands, but do not have any conflict of interest regarding the topic described in this article. The other authors do not have any conflict of interest.

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CHAPTER 5

Diet density during the first week of life: Effects on growth performance, digestive organ weight, and nutrient digestion of broiler chickens

Poultry Science: submitted

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ABSTRACT

The current study aimed to investigate whether diet density affects growth performance and nutrient digestion during the first wk after hatch and digestive organ weight at 7 d of age. Effects were studied using a dose-response design that comprised of 5 inclusion levels of dietary fat (3.5, 7.0, 10.5, 14.0, and 17.5%). The dietary fat level was increased through soybean oil inclusion. Amino acids, minerals, and the premix were increased at the same ratio as dietary fat. Consequently, diets were neither kept isocaloric nor isonitrogenous. Broiler chickens were weighed on d 0 and 7 after hatch, whereas feed intake was measured daily. Excreta produced from d 0 to 7 were collected at d 7. Dietary DM and nitrogen metabolizability, as well as fat digestibility were calculated as an average over 7 d. Broiler chickens were sampled at d 7 to determine carcass yield, breast meat yield, and organs weights. ADG (Δ = -6.8% between 3.5 and 17.5% dietary fat; $P = 0.047$) and ADFI (Δ = -18.7% between 3.5 and 17.5% dietary fat; $P < 0.001$) decreased linearly as diet density increased, whilst G:F increased linearly (Δ = 14.4% between 3.5 and 17.5% dietary fat; $P < 0.001$). An increased diet density resulted in a linear decrease of crop, liver, and pancreas weight relative to BW ($P < 0.05$). Duodenum, jejunum, ileum, and cecum length (expressed as cm/kg of BW) and empty weight (as % of BW) increased linearly with increased diet density ($P < 0.05$). Dietary DM metabolizability decreased linearly as diet density increased (Δ = -4.1% between 3.5 and 17.5% dietary fat; $P < 0.001$), whereas fat digestibility and nitrogen metabolizability were not affected ($P > 0.05$). In conclusion, one-wk-old broiler chickens respond to increased diet densities by increasing intestinal weight and length, meanwhile decreasing liver and pancreas weight. This may be an adaptive response to cope with an increased nutrient concentration in the diet.

Key words: broiler chickens, dietary fat, diet density, organ size, digestive status

INTRODUCTION

In current broiler farming, chickens are fed diets rich in carbohydrates from the moment of hatch onwards (Sklan, 2001). Carbohydrates, such as glucose and starch, function as the main energy supply in diets, despite that they have a lower energetic value than dietary fats and oils. Dietary fats and oils are less used as main energy supplier in chicken diets because fats and oils are considered less digestible, especially during the first wk of life (Batal and Parsons, 2002b; Thomas et al., 2008; Ravindran et al., 2016). Based on these considerations, limited data are available to study whether broiler growth and development are affected by high inclusion levels of dietary fat in the first wk of life. However, during the late incubation phase, the developing chicken embryo relies on yolk lipids as a primary energy supply (Noble and Cocchi, 1990), suggesting that the developing embryo is able to metabolize lipids from a very young age onward.

Digestion and absorption of lipids in the gut is largely facilitated by enzymatic hydrolysis. Secretion of digestive enzymes, such as lipase, trypsin, and amylase continues to increase during the first three wk after hatch and is therefore considered sub-optimal during the first wk of life (Noy and Sklan, 1995), although Nitsan et al. (1991) found that lipase reached the maximum activity level in the small intestine at four days of age. Secretion of digestive enzymes, although not maximal during the first wk of life, might not limit digestion and metabolism, as feed intake **(**FI**)** and, therefore, the amount of feed to be processed are also relatively low in the first wk of life. Moreover, there may be adaptive responses in the gut that counteract the relative shortage of digestive enzymes. This assumption is based on Scott (2002), where feeding of diets with increased nutritional densities to broiler chickens resulted in altered digestive organ length and weight.

The objective of this study was to determine effects of diet density on growth performance, digestive organ weights and lengths, and nutrient utilization of broiler chickens during the first wk of life, using a dose-response design. Effects of increased diet density on post-absorptive characteristics, such as nitrogen **(N)** and energy retention within the body, are described elsewhere (**Chapter 4**).

MATERIALS AND METHODS

All procedures in this study were approved by the Animal Use and Care Committee of Wageningen University & Research, the Netherlands.

Experimental design

A dose-response design with 5 diet density levels, obtained through increased dietary fat levels (3.5, 7.0, 10.5, 14.0, and 17.5%), was applied to study their effects on growth performance, digestive organ weights and lengths, and nutrient metabolizability of broiler chickens during the first wk of life. In addition to dietary energy level, also amino acid levels, mineral levels, and the premix inclusion level were increased as diet density increased. Treatments were randomly distributed over 4 climate respiration chambers **(CRC)**. This resulted in eight consecutive batches and 6 replicates per treatment, with 108 broiler chickens per treatment and 540 broiler chickens in total.

Animals and housing

A total of 540 day-old Ross 308 male broiler chickens with an average BW of 45.8 g (range 44.8 to47.3 g) were obtained from a commercial hatchery (Lagerwey, the Netherlands). To prevent a wide range in hatch time, which is known to affect the metabolic status of the broiler chicken (Van de Ven et al., 2013; Lamot et al., 2014), only broiler chickens that were not entirely dry in the neck region were used in the current study. Chickens were housed in one of 4 CRC at the experimental facilities of Wageningen University. Per CRC either 12 or 24 broiler chickens were used, depending on the size of the CRC (**Chapter 4**). For quantitative excreta collection, broiler chickens were kept on a coated wire floor. Chickens received artificial lighting for 23 h/d throughout the study period (0 to 7 d of age). Temperature was set at 33°C on d 0 and gradually decreased by 0.5°C per day during the study period. Chickens had ad libitum access to feed and water (via nipple drinkers).

Experimental diets

Diets were formulated and produced as described in **Chapter 4**. In short, diets with low (3.5%) and high (17.5%) dietary fat content were formulated. Soybean oil was used as a fat source to vary the dietary fat level among treatments. Amino acid levels and mineral levels, as well as the premix inclusion level increased at the same ratio in which dietary ME level increased due to increased dietary fat levels. Consequently, diets were either isocaloric nor isonitrogenous. Starter diets with 3.5, 7.0, 10.5, 14.0, and 17.5% dietary fat contained 2,870, 3,030, 3,190, 3,330, and 3,510 kcal/kg AME_n, respectively, and 11.6, 12.2, 12.9, 13.5, and 14.2 g/kg digestible lysine, respectively. Diets were formulated based on digestibility and nutrient data provided by CVB (2007).

Measurements

Chickens were individually weighed on d 0 and 7, whereas feed intake (FI) was measured daily at the same time of the day. Gain to feed ratio (G:F) was calculated for 0 to 7 d, based on ADG and ADFI. The average ADG from 0 to 7 d of age was calculated per CRC. Excreta produced from 0 to 7 d of age was collected quantitatively at d 7 and analyzed afterwards.

Feed and excreta were analyzed for DM (ISO 6496, 1999), N (ISO 5983-2, 2009), and crude fat by acid hydrolysis (ISO 6492, 1999) content.

At 7 d of age, 12 broiler chickens per CRC were weighed, sacrificed, and dissected to determine carcass weight and organ weights of heart, crop, proventriculus plus gizzard, liver, pancreas, bursa, spleen, and breast meat yield (pectoralis major, pectoralis minor, sternum, and clavicle). Carcass weight excluded blood and organ weights. Broiler chickens were not feed-deprived before dissection and were killed by cervical dislocation. Organ weights were calculated as a percentage of BW, except for breast meat yield, which was expressed as a percentage of carcass weight. Digestive organs, including crop, proventriculus plus gizzard, small intestines, and cecum were emptied by gentle brushing and squeezing to determine empty weights. For the intestinal tract, the length of the duodenum (duodenal loop excluding pancreas), jejunum (end duodenum to Meckel's diverticulum), ileum (Meckel's diverticulum to ileal-cecal junction), and cecum were also measured.

As no distinction was made between fecal and urinary DM and N excretion, the dietary DM and N metabolizability coefficients **($Nutrient_{MET}$)** were calculated as follows:

$$Nutrient_{MET} = (Nutrient_{FEED} - Nutrient_{EXCRETA}) / Nutrient_{FEED}$$

The dietary fat digestibility coefficient **(Fat_{DC})** was calculated as follows:

$$Fat_{DC} = (Fat_{FEED} - Fat_{EXCRETA}) / Fat_{FEED}$$

Statistical analysis

Data were subjected to mixed model analysis, using the PROC MIXED procedure in SAS (Version 9.3, 2011, SAS Institute Inc., Cary, NC, United States) with the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + b_k + \varepsilon_{ijk}$$

where Y_{ij} = dependent variable, μ = overall mean, α_i = fixed treatment effect (i = 3.5, 7.0, …, 17.5% dietary fat), β_j = fixed CRC type effect (j = 12 or 24 broiler chickens), b_k = random batch effect (k = 1, 2, ..., 8), and ε_{ijk} = residual error term. Contrasts were used to determine significant relationships for linear and quadratic effects of diet density. Data were analyzed using a group of animals in one CRC as the experimental unit. Data are expressed as least square **(LS)** means. LSmeans were compared after correcting with Tukey 's test for multiple comparisons, and effects were considered to be significant when $P \leq 0.05$.

RESULTS

Growth performance

From d 0 to 7 the ADG was on average 18.7 g/d, and increased diet density resulted in a linear decrease in ADG (Δ = -6.8% between 3.5 and 17.5% dietary fat; $P = 0.047$; Table 1). Furthermore, except from d 0 to 1, ADFI decreased linearly as diet density increased ($P < 0.05$; Table 1). On average over the first wk of life, ADFI decreased with 3.4 g/d as diet density increased (Δ = -18.7% between 3.5 and 17.5% dietary fat; $P < 0.001$; Table 1). Average G:F from d 0 to 7 was 1.124 and increased linearly as diet density increased (Δ = 14.4% between 3.5 and 17.5% dietary fat; $P < 0.001$; Table 1). DM metabolizability was on average 0.73 from d 0 to 7 and was linearly lowered with increased diet density (Δ = -4.1% between 3.5 and 17.5% dietary fat; P = 0.001; Table 2), whereas N metabolizability (average $N_{MET} = 0.67$) and fat digestibility (average $Fat_{DC} = 0.72$) were not affected as diet density increased.

Organ weight, intestinal length, and weight

Crop, liver, and pancreas weights, expressed as a percentage of BW, decreased linearly with increased diet density (Δ = -4.6% between 3.5 and 17.5% dietary fat, $P = 0.015$; Δ = -19.4% between 3.5 and 17.5% dietary fat, $P < 0.001$; and Δ = -11.5% between 3.5 and 17.5% dietary fat, $P = 0.001$, respectively; Table 3). For absolute crop, liver, and pancreas weights, a similar response was observed ($P \leq 0.002$; data not shown). No effects of diet density were found on heart, stomach, spleen, and bursa weights at 7 d of age. Length of the duodenum (Δ = 13.9%), jejunum (Δ = 10.0%), ileum (Δ = 4.8%), and cecum (Δ = 8.3%), expressed as cm per kg of BW, increased linearly as diet density increased from 3.5 to 17.5% dietary fat ($P < 0.05$; Table 4). In addition, empty weight of the duodenum (Δ = 22.2%), jejunum (Δ = 12.1%), ileum (Δ = 17.0%), and cecum (Δ = 18.0%), expressed as a percentage of BW, linearly increased as diet density increased from 3.5 and 17.5% dietary fat ($P < 0.05$; Table 4). The calculated weight to length ratio of the intestines increased linearly for the ileum and cecum as diet density increased (Δ = 10.8 and 9.5%, respectively; $P < 0.05$; Table 4).

Table 1. Effects of diet density fed from 0 to 7 d of age on ADG, ADFI, and gain to feed ratio (G:F) of broiler chickens, expressed as least square means[1]

		ADG, g/d		ADFI, g/d						G:F, g:g
Item		**0-7 d**	**0-1 d**	**1-2 d**	**2-3 d**	**3-4 d**	**4-5 d**	**5-6 d**	**6-7 d**	**0-7 d**	**0-7 d**
Dietary fat level, %	3.5	19.3	2.1	7.7^{b}	12.5^{b}	18.5^{b}	22.9^{ab}	29.5^{c}	34.0^{c}	18.2^{c}	1.059^{c}
	7.0	18.6	1.7	7.1^{ab}	11.3^{a}	16.5^{a}	23.2^{b}	27.9^{bc}	32.9^{bc}	17.2^{bc}	1.081^{bc}
	10.5	18.7	1.6	6.5^{a}	10.9^{a}	16.1^{a}	21.7^{ab}	26.7^{b}	31.0^{bc}	16.4^{b}	1.140^{b}
	14.0	18.8	2.0	6.4^{a}	11.1^{a}	17.0^{a}	23.8^{b}	26.2^{ab}	30.1^{ab}	16.7^{b}	1.128^{b}
	17.5	18.0	1.8	6.4^{a}	10.4^{a}	15.9^{a}	18.7^{a}	23.6^{a}	27.2^{a}	14.8^{a}	1.212^{a}
SEM (n = 6)		0.4	0.2	0.4	0.5	0.4	1.0	0.6	0.9	0.3	0.017
P-value	Fixed	0.199	0.343	0.002	<0.001	<0.001	0.015	<0.001	<0.001	<0.001	<0.001
	Linear	0.047	0.572	<0.001	<0.001	<0.001	0.024	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.877	0.256	0.035	0.089	0.026	0.066	0.603	0.353	0.570	0.348

[1]For each treatment, replicates consisted of 3 × 12 and 3 × 24 male broiler chickens at the start of the study.
[a-d] LSmeans within columns lacking a common superscript differ ($P \leq 0.05$).

Table 2. Effects of diet density fed from 0 to 7 d of age on DM metabolizability (DM_{MET}), nitrogen metabolizability (N_{MET}), and fat digestibility (Fat_{DC}) coefficients of broiler chickens, expressed as least square means[1]

		0-7 d of age		
Item		**DM_{MET}**	**N_{MET}**	**Fat_{DC}**
Dietary fat level, %	3.5	0.751^{bc}	0.659	0.726
	7.0	0.756^{c}	0.673	0.738
	10.5	0.733^{ab}	0.658	0.719
	14.0	0.729^{a}	0.667	0.713
	17.5	0.720^{a}	0.672	0.725
SEM (n = 6)		0.009	0.009	0.024
	Fixed	0.012	0.495	0.865
P-value	Linear	0.001	0.384	0.612
	Quadratic	0.780	0.838	0.854

[1]For each treatment, replicates consisted of 3 × 12 and 3 × 24 male broiler chickens at the start of the study.
[a-d] LSmeans within columns lacking a common superscript differ ($P \leq 0.05$).

Table 3. Effects of diet density fed from 0 to 7 d of age on relative carcass and organ weight of broiler chickens at 7 d of age, expressed as least square means[1]

		Live BW, g	Carcass[2], %	Breast meat yield[3], %	Organ weight d 7, % of BW						
Item					**Heart**	**Crop**	**Stomach[4]**	**Liver**	**Spleen**	**Bursa**	**Pancreas**
Dietary fat level, %	3.5	182.1	59.0	19.0	0.90	0.87^{ab}	4.21	4.27^{a}	0.076	0.16	0.52^{b}
	7.0	178.7	58.4	19.3	0.89	0.92^{b}	4.22	3.84^{b}	0.079	0.16	0.53^{b}
	10.5	177.7	58.6	19.2	0.89	0.81^{a}	4.29	3.64^{bc}	0.079	0.18	0.50^{ab}
	14.0	181.1	58.4	19.5	0.93	0.81^{a}	4.14	3.66^{bc}	0.082	0.18	0.47^{ab}
	17.5	175.3	58.8	18.9	0.86	0.83^{ab}	4.23	3.44^{c}	0.077	0.16	0.46^{a}
SEM (n = 6)		2.6	0.4	0.4	0.02	0.03	0.08	0.09	0.004	0.01	0.02
P-value	Fixed	0.124	0.641	0.733	0.233	0.010	0.735	<0.001	0.872	0.408	0.010
	Linear	0.221	0.576	0.915	0.550	0.015	0.824	<0.001	0.654	0.482	0.001
	Quadratic	0.362	0.226	0.306	0.272	0.569	0.849	0.077	0.447	0.128	0.715

[1]For each treatment, replicates consisted of 3 × 12 and 3 × 24 male broiler chickens at the start of the study. [2]Carcass: whole body excluding blood and organ weights. [3]Breast meat yield. Expressed as percentage of carcass weight. [4]Stomach: proventriculus plus gizzard. [a-c] LSmeans within columns lacking a common superscript differ ($P \leq 0.05$).

Table 4. Effects of diet density fed from 0 to 7 d of age on intestinal length, weight, and weight to length ratio of broiler chickens at 7 days of age, expressed as least square means[1]

		Intestinal length, cm/kg of BW				Intestinal weight, % of BW				Weight to length ratio of intestine, mg/mm			
Item		Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum
Dietary fat level, %	3.5	97.6^{b}	204.6^{b}	195.8^{a}	77.2	1.58	2.64^{b}	1.76^{a}	0.50^{a}	16.09	12.98	9.07^{b}	6.50^{c}
	7.0	104.4^{ab}	213.1^{ab}	206.7^{b}	80.9	1.81	2.74^{ab}	1.96^{b}	0.54^{ab}	17.39	12.94	9.56^{ab}	6.64^{bc}
	10.5	104.7^{ab}	223.5^{a}	211.0^{b}	81.5	1.81	2.85^{ab}	1.99^{b}	0.54^{ab}	17.37	12.87	9.56^{ab}	6.75^{abc}
	14.0	104.5^{ab}	217.2^{ab}	203.6^{ab}	80.0	1.88	2.95^{ab}	2.06^{b}	0.58^{ab}	18.10	13.74	10.21^{a}	7.13^{a}
	17.5	111.2^{a}	225.2^{a}	205.2^{ab}	83.6	1.93	2.96^{a}	2.06^{b}	0.59^{b}	17.31	13.23	10.05^{a}	7.12^{ab}
SEM (n = 6)		2.2	4.3	3.6	2.7	0.13	0.10	0.05	0.02	1.11	0.49	0.31	0.12
P-value	Fixed	0.001	0.004	0.003	0.169	0.057	0.027	<0.001	0.021	0.455	0.521	0.006	0.002
	Linear	<0.001	0.001	0.040	0.042	0.007	0.002	<0.001	0.001	0.193	0.312	0.001	<0.001
	Quadratic	0.918	0.183	0.002	0.736	0.327	0.499	0.032	0.723	0.227	0.99	0.385	0.920

[1]For each treatment, replicates consisted of 3 × 12 and 3 × 24 male broiler chickens at the start of the study. [a-c] LSmeans within columns lacking a common superscript differ ($P \leq 0.05$).

DISCUSSION

In the same study as the current one (**Chapter 4**), it was concluded that increasing diet density in the first wk of life of broiler chickens did not affect ME intake, protein, and fat retention. The ME to gross energy ratio **(ME:GE)** linearly decreased with increased diet density. Based on these results, it was suggested that increased diet density affects nutrient digestibility or metabolizability rather than nutrient utilization. The aim of the current study was to determine whether increased diet density, using soybean oil as the primary energy source, affected growth performance, nutrient utilization, and (digestive) organ length and weight of broiler chickens during the first wk of life.

The average dietary N metabolizability coefficient in the current study was lower than the N metabolizability coefficients found in previous research (0.67 vs. 0.72 and 0.79), where similar corn-soybean meal based diets were fed until 7 d of age (Noy and Sklan, 1995; Thomas et al., 2008). In the current study, as well as in previous research, no distinction was made between fecal and urinary N excretion for calculation of the N metabolizability. Therefore, the calculated N_{MET} is a combined value for dietary CP digestion and catabolized protein (Terpstra and de Hart, 1973). This might result in underestimation of the digestibility (N_{DC}), as it was already hypothesized that increased diet densities result in lower N efficiency, which is very likely related to removal of excessive N from the body through increased uric acid formation (**Chapter 4**). The latter also suggests that the N_{DC} is dependent on diet density, whereas in the current study no effect of diet density was found on N_{MET}. Therefore, it is important in further research to differentiate between fecal and urinary N, determining the ratio between N excretion via urine and feces.

Whereas fats and oils are considered less digestible during the first wk of life, the average dietary fat digestibility coefficient in the current study was higher than in previous research (0.72 vs. 0.59 and 0.67), where a similar diet was fed until 7 d of age (Batal and Parsons, 2002; Thomas et al., 2008). Tancharoenrat et al. (2013) derived a fat digestibility for soybean oil of 0.60 at 7 d of age. Furthermore, in the current study, fat digestibility did not depend on dietary fat level, even though the inclusion level of soybean oil increased 6.5-fold from 23 to 150 g/kg combined with a reduced carbohydrate fraction in the diet (from 565 to 396 g/kg). Ravindran et al. (2016) reviewed that increased inclusion levels of fats and oils in poultry diets tend to result in lowered digestibility. However, the same review also stated that this phenomenon may especially occur in young chickens when fed saturated fats and oils, whereas soybean oil contains relatively low amounts of saturated fatty acids compared to other fats and oils.

As the dietary N metabolizability and fat digestibility were not affected by diet density, a lowered ME:GE ratio as a result of increased diet density, as found previously (**Chapter 4**) may be attributed to a lowered DM metabolizability. A lowered ME:GE ratio reflects an increase in fecal and urinary losses, meaning that the metabolizability of nutrients

is impaired. The lowered DM metabolizability as a result of increased diet density might be explained by an increase of minerals and trace components in the premix as diet density increased. This has not only resulted in an increased (trace) mineral content of the diet compared to other ingredients (4.6 vs. 5.8% for 3.5 and 17.5% dietary fat diets, respectively), but also an absolute increase (1.3%) in dietary DM content. As these minerals and trace components are not fully digestible by the broiler chicken, it will probably lower DM metabolizability. Lastly, the altered ME:GE ratio may also be explained by altered carbohydrate digestibility, but this was not determined in the current study.

The relative high fat digestibility in the current study, unaffected by dietary fat level, suggests that there is a discrepancy between the current study and previous research that advocates that enzyme secretion for fat digestion is considered sub-optimal during the first wk of life (Noy and Sklan, 1995). This might be explained by chickens having lower (but still sufficient) enzyme (lipase) secretion for fat digestion at that age, or that these chickens have a strategy to adapt their digestive system towards increased diet densities.

Changes in length and weight of digestive organs may be a result of changes in the amount of feed consumed (portion size), ingredient composition, or nutrient density in the diet. A distinction can be made between digestive organs that are primarily involved in the process of feed ingestion, digestion, and absorption (esophagus, crop, stomach, and intestines) and organs that have a supportive role in digestion or metabolization of nutrients, such as the pancreas (enzyme secretion) or the liver (metabolic processing of nutrients).

In this study, increased diet density resulted in a lower relative crop weight. It can be hypothesized that in ad libitum fed broiler chickens the crop has a redundant function because consumed feed directly passes into the gizzard (Svihus, 2014). In the current study, broiler chickens were also fed ad libitum and crop weight appeared to decrease with increasing energy density. Increasing energy density also resulted in a lower amount of feed consumed. It seems, therefore, that even in situations of continuous feed access, the crop may be used for temporary feed storage and that its weight is actually adjusted toward the amount of feed (portion size) consumed.

Whereas the relative crop weight was reduced as a result of an increased diet density, intestinal length, and weight increased. Scott (2002) found shorter small intestines at 20 d of age after feeding diets with increased density (+0.29 MJ ME /kg), whereas Wang et al. (2015) did not find an effect of diet density (+0.42 MJ ME /kg) on small intestine length at 12 d of age. In the latter study, small intestine morphology by means of villi height, width, and surface area, intestinal muscle thickness, crypt depth, and goblet cell size and density were also not affected. However, in the current study the increase in diet density was much higher (+3 MJ ME /kg) than in Scott (2002) and Wang et al. (2015), which may partly explain the difference between the current study and the literature.

Increased intestinal length and weight as a result of increased diet density might be due to differences in the absolute FI level, nutrient intake levels, and diet ingredient composition. Increased intestinal length and weight as a result of lower FI seems unlikely, as it can be hypothesized that a lowered FI may result in a reduced need for surface area of the intestines for digestion and absorption of nutrients. However, lowered FI as a consequence of increased diet density coincided with an increased nutrient intake per gram of diet, resulting in a constant metabolizable energy intake (**Chapter 4**). Consequently, the intestine has to adapt to smaller FI amounts, but higher nutrient availability. Thus, it can be speculated that nutrient concentration per gram of diet may drive intestinal growth rather than the absolute amount of FI. Larger intestines likely increase villi surface area for absorption of nutrients, as nutrients are absorbed either actively (protein, carbohydrates) or passively (fats and oils) by transport via the enterocytes located in these villi (Denbow, 2015).

Lastly, it can also be hypothesized that increased intestinal length and weight affects transit time of the digesta through the gut. However, in young broiler chickens (7 to 42 d of age), the transit time was not affected by dietary fat inclusion up to 20% (Golian and Maurice, 1992). Therefore, it seems unlikely that increased intestinal length and weight affect transit time through the gut. Summarizing, it might be that the broiler chicken is able to alter its absorptive capacity in the intestinal tract by means of increased intestinal length and weight and adapt to increased nutrient availability as a result of increased diet density.

As a supportive digestive organ, the pancreas secretes amylase, lipase, and trypsin for carbohydrate, fat, and protein digestion (Denbow, 2015), as well as hormones, such as insulin and glucagon that have a regulatory role in carbohydrate metabolism (Dupont et al., 2015). In the current study, both the absolute and relative pancreas weight reduced as diet density increased. This might be explained by the carbohydrate content of the diet, which was reduced with approximately 28% as the dietary fat level increased. On the contrary, dietary CP and fat fractions increased to approximately 17 and 42%, respectively as diet density increased. Combined with the lowered FI (portion size), it could be hypothesized that there is a lower requirement for metabolic processing of carbohydrates, facilitiated by the pancreas, thus resulting in a smaller pancreas weight.

In the current study, it was also found that at 7 d of age both the absolute and relative liver weight decreased as dietary nutrient density increased. This corresponds with earlier work of Scott (2002), where increased diet density (+0.29 MJ ME /kg) also resulted in relative lower liver weights from 12 d of age onwards compared to a regular diet density. The liver is responsible for bile secretion that is used in fat digestion (Denbow, 2015) and it could be hypothesized that increased dietary fat levels in diets with increased nutrient density require increased bile secretion due to its function as an emulsifier of fat (not measured in this study). However, in the current study this did not result in increased liver weight.

A lowered liver weight could be due to its metabolic function to facilitate the increased growth rate that is associated with feeding increased diet densities. Young broiler chickens use approximately half of their retained energy for protein synthesis and retention (Buyse et al., 2004; **Chapter 4**). Obviously, protein synthesis requires energy, and its costs were found to range from 3 to 13 kJ per gram of protein synthesized (Aoyagi et al., 1988). The liver facilitates the processes of glycolysis (using glucose as input) and beta oxidation (using fatty acids as input) for energy yielding purposes (Salway, 2004). The glucose used for glycolysis is primarily of endogenous origin in young broiler chickens (Buyse et al., 2004). Whereas in mammals, the production of endogenous glucose would be facilitated through gluconeogenesis in the liver; in avian species this primarily occurs in the kidneys (Watford et al., 1981). The latter suggests that a higher energy requirement for growth and protein retention may result in increased glycolysis in the liver but neither increased gluconeogenesis (taking place elsewhere) nor storage of glycogen in the liver, as most of it will likely be used as fuel for growth, thus partly explaining a reduced liver weight. Fatty acids that are not used for energy yielding purposes through β-oxidation can be stored in the liver as triglycerides. However, due to the relatively high protein retention rate and synthesis in young broiler chickens, it is expected that fatty acids are (in addition to glucose) mainly used for energy yielding purposes (Buyse et al., 2004). Thus, reduced storage of fatty acids in the liver may also explicate to some extent a reduced liver weight.

In conclusion, during the first wk of life, feeding higher diet densities resulted in linearly lowered ADG and ADFI, whereas G:F increased. Broiler chickens respond to high density diets by increasing intestinal weight and length while decreasing liver and pancreas weight. The altered organ lengths and weights may be an adaptive response to cope with reduced FI, but increased nutrient concentration. Increased diet densities did not affect N metabolizability or fat digestibility coefficients.

CONFLICT OF INTEREST

D.M. Lamot and P.J.A. Wijtten are employed at the Cargill Animal Nutrition Innovation Center Velddriel, the Netherlands, but do not have any conflict of interest regarding the topic described in this article. The other authors do not have any conflict of interest.

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CHAPTER 6

Diet density provided during different feeding phases: Effects on growth performance and carcass composition of broiler chickens

Poultry Science: submitted

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ABSTRACT

This study aimed to determine effects of diet density, fed until different ages, on broiler chicken growth performance and carcass composition using a 4 x 3 factorial design. Four levels of dietary fat (3.5, 8.2, 12.8, and 17.5%) were fed until different ages (d 7, 14, or 34). Broiler chickens fed different diet densities until d 7 or 14 received a 3.5% dietary fat diet for the remaining experimental period. An increase in dietary fat level was parallelled by an increase in amino acids, minerals, and all premix ingredients. Consequently, diets were not kept isocaloric nor isonitrogenous. Feed intake and BW of broiler chickens were determined at d 0, 7, 14, and 34. At d 35, carcass weight, breast meat yield, and weights of the abdominal fat pad, liver, and pancreas were determined. Regardless of feeding phase, feeding denser diets resulted in a higher ME intake, ADG, and gain to feed ratio during the period that the diet contrasts existed ($P < 0.001$). Feeding denser diets till d 7 did not affect overall growth performance and carcass characteristics. Feeding denser diets till d 14 resulted in a higher ADG and gain to feed ratio from d 0 to 34 and a lowered relative fat pad weight at slaughter ($P < 0.001$). Feeding denser diets till d 34 resulted in higher ME efficiencies, higher BW at slaughter, and lower relative breast meat yield ($P < 0.001$). ME efficiencies were neither affected by diet density from d 0 to 7 nor from d 14 to 34. However, from d 7 to 14 and d 0 to 34, the ME efficiency increased when feeding denser diets was continued ($P < 0.001$). It was determined that feeding increased diet densities to broiler chickens results in a higher ADG and gain to feed ratio but only during the period these diets are provided.

Key words: broiler chickens, dietary fat, diet density, carcass composition

INTRODUCTION

Increased dietary densities aim to increase nutrient intake of broiler chickens, resulting in higher gain and feed efficiency (Leeson et al., 1996). Diet density often refers to the dietary energy level. However, if one strives for a balanced increase in nutrient intake, an increase in dietary energy should be parallelled by an increase in protein (amino acids), minerals, and vitamins (Swennen et al., 2007). Elevated dietary energy levels are often achieved by increased inclusion of fats and oils in the diet at the expense of carbohydrates, as fats and oils deliver approximately twice as much energy than carbohydrates per gram included in the diet (Baião and Lara, 2005).

While increased diet densities (containing relatively high dietary fat levels) are commonly applied in older broiler chickens, it is quite uncommon to feed this type of diet during the first week of life. High inclusion levels of fats and oils in broiler diets during this age period are avoided due to the apparent lowered digestion and absorption of fats and oils at that age (Ravindran et al., 2016). Recent research has shown, however, that feeding broiler chickens high diet densities (containing up to 17.5% dietary fat; combined with increased mineral and amino acid levels) during the first week of life resulted in lower ADG and nutrient efficiency, whereas the post-absorptive protein and fat retention was not affected (**Chapter 4**). In the same study, broiler chickens adapted to high density diets by means of lowered liver and pancreas weights, whereas intestinal weights and lengths relative to body weight increased (**Chapter 5**). In other studies, broiler chickens were also found to physiologically adapt to increased diet densities by altered intestinal development (size, morphology), nutrient absorptive capacity (intestinal nutrient transporters), and other metabolic constituents (Scott, 2002; Wang et al., 2014; Wang et al., 2015; Li et al., 2016).

Even though broiler chickens seem capable of metabolizing high diet densities during the first week of life, it remains unknown whether there are any adaptive consequences when either switching to a low diet density, or when feeding broiler chickens high diet densities after the first week of life is continued during the starter and grower phase. Therefore, the objective of this study was to determine long-term effects of diet density, fed until different ages, on broiler chicken growth performance, ME efficiency, and carcass composition.

MATERIALS AND METHODS

All procedures in this study were approved by the Ethical Committee of the Animal Sciences group of Wageningen University & Research, the Netherlands.

Experimental design

The study was set up as a 4 x 3 factorial design. Four levels of diet density were combined with 3 feeding durations. The 4 levels of diet density (obtained through variable dietary fat levels: 3.5, 8.2, 12.8, and 17.5%) were fed to broiler chickens from d 0 till either d 7 (pre-starter phase), d 14 (pre-starter plus starter phase), or d 34 (pre-starter, starter, plus grower phase). An increase in dietary fat was parallelled by an increase in amino acids, minerals, and all premix ingredients. Treatments with different diet densities administered until d 7 or 14 were followed by a 3.5% dietary fat diet for the remaining experimental period (from d 7 till 34 or from d 14 till 34, respectively). Per treatment combination, 90 male broiler chickens were evenly distributed over 6 pens (n = 72 pens total). All chickens had *ad libitum* access to feed and water throughout the experimental period.

Birds and housing

A total of 1,080 Ross 308 male one-day old broiler chickens, derived from a 53 wk old broiler breeder flock, were purchased from a commercial hatchery (Morren BV, the Netherlands) and randomly allocated to 72 pens in a grow-out facility of Cargill (the Netherlands). Pens were divided over six blocks of 12 pens each, and each pen contained 15 broiler chickens that had similar total weights per pen within a block. Pens had a raised metal floor that was covered with a 2-cm layer of wood shavings. Artificial lighting was set for 23 h/d from d 0 to 2, 20 h/d from d 2 to 6, and 18 h/d from d 7 to 34. The temperature was set to decrease gradually by 0.5°C per day during the first 14 d, from 34°C at d 0. From d 14 onwards, the temperature was set to decrease gradually until a final temperature of 20°C was reached at d 34. At the start of the study, feed was supplied using a feeder placed within the pen. From d 14 onwards, the feed was supplied in a metal feeder trough placed in front of the pen. Each pen was equipped with four cup drinkers adjustable in height. The caretaking schedule consisted of two inspections per day, which also included a health check. Mortality was recorded daily.

Experimental diets

Diets were formulated based on digestibility and nutrient data provided by CVB (2007). As diet density increased, amino acids, minerals, and all premix ingredients increased at the same ratio as the dietary energy level. Consequently, diets neither remained isocaloric nor isonitrogenous. Soybean oil was used as the fat source to increase the dietary fat level among treatments. Diet compositions as well as calculated and analyzed nutrient values are shown in Table 1a-c. For each feeding phase (pre-starter, starter, and grower phase), basal diets were produced for the lowest (3.5% dietary fat) and highest (17.5% dietary fat) diet densities. Diet densities in between were obtained by blending the two basal diets for feeding each phase in different ratios. All diets were produced in mash form by Research Diet Services (the Netherlands).

Table 1a. Ingredient and nutritional composition of the experimental pre-starter (d 0 to 7) diets

Phase	**Pre-starter, d 0 to 7**			
Dietary fat level, %	**3.5**	**8.2**	**12.8**	**17.5**
Ingredient composition, g/kg				
Corn	655.3	565.4	475.4	385.6
Soybean meal HP, >48% CP	168.4	195.4	222.4	249.4
Potato protein , 77% CP	50.0	54.0	57.9	61.9
Soy protein isolate	50.0	54.0	57.9	61.9
Corn gluten meal	20.0	21.6	23.2	24.8
Soybean oil	8.5	57.7	107.0	156.2
Monocalciumphosphate	17.8	19.5	21.2	22.8
Limestone	14.3	15.3	16.3	17.3
Salt	1.1	1.4	1.7	2.0
Sodiumbicarbonate	1.1	1.0	0.9	0.8
DL-Methionine	1.9	2.4	2.8	3.3
L-Lysine HCl	1.6	1.6	1.7	1.7
Premix starter[1]	10.0	10.8	11.6	12.3
Calculated chemical composition, g/kg				
CP	235	248	261	274
Crude fat	35	82	128	175
Crude ash	57	61	64	68
DM	889	894	900	906
Carbohydrates[2]	561	504	447	389
AME_n, kcal/kg[3]	2,908	3,126	3,343	3,561
Dig. lys	12.0	12.9	13.9	14.8
Calcium	9.0	9.7	10.4	11.1
Phosphorous	7.6	8.0	8.3	8.7
Analyzed chemical composition, g/kg				
Crude fat	39	84	125	166
Crude ash	50	54	58	61
DM	892	898	903	913
Calcium	9.5	-	-	11.3
Phosphorous	7.6	-	-	8.6
Particle size, %				
>0.0 mm	58.8	56.3	18.5	12.3
>0.5 mm	25.6	27.9	60.3	44.8
>1.0 mm	9.1	9.6	12.9	23.1
>1.4 mm	3.6	3.3	4.4	10.6
>1.7mm	1.7	1.8	2.2	5.1
>2.0 mm	0.9	0.9	1.2	3.3
>2.5 mm	0.3	0.2	0.4	0.6
>2.8 mm	0.0	0.0	0.1	0.2

[1] Contributed per kilogram of diet: thiamine, 1.0 mg; riboflavin, 4.5 mg; niacinamide, 40 mg; D-pantothenic acid, 9 mg; pyridoxine-HCL, 2.7 mg; choline chloride, 500 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopherol), 33 IU; menadione, 2.3 mg; vitamin A (retinyl-acetate), 12,000 IU; cholecalciferol, 5,000 IU; biotin, 100 µg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 150 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSo_4 \cdot H_2O$, 145 mg; Na_2SeO_3, 0.56 mg; KI, 2.0 mg; antioxidant (oxytrap PXN), 125 mg. [2] Carbohydrate content calculated as: 1,000 - crude protein - crude fat - moisture - crude ash. [3] AME_n = calculated according to CVB (2007).

Table 1b. Ingredient and nutritional composition of the experimental starter (d 7 to 14) diets

Phase	**Starter, d 7 to 14**			
Dietary fat level, %	**3.5**	**8.2**	**12.8**	**17.5**
Ingredient composition, g/kg				
Corn	629.3	533.3	437.3	341.3
Soybean meal HP, >48% CP	315.6	357.9	400.2	442.6
Soybean oil	7.9	57.1	106.4	155.6
Monocalciumphosphate	13.9	15.3	16.7	18.1
Limestone	13.8	14.7	15.7	16.6
Salt	3.6	2.9	2.3	1.7
Sodiumbicarbonate	0.3	1.6	3.0	4.3
DL-Methionine	2.6	3.1	3.6	4.1
L-Lysine HCl	2.4	2.4	2.3	2.3
L-Threonine	0.7	0.8	1.0	1.1
Premix starter[1]	10.0	10.8	11.5	12.3
Calculated chemical composition, g/kg				
CP	210	223	235	247
Crude fat	35	82	128	175
Crude ash	59.9	64	69	73
DM	883	888	893	899
Carbohydrates[2]	578	520	462	404
AME_n, kcal/kg[3]	2,757	2,969	3,163	3,357
Dig. lys	11.1	12.0	12.8	13.7
Calcium	8.5	9.2	9.8	10.5
Phosphorous	7.0	7.3	7.6	8.0
Analyzed chemical composition, g/kg				
Crude fat	39	81	118	163
Crude ash	57	60	62	62
DM	888	893	899	903
Calcium	8.8	-	-	11.2
Phosphorous	7.1	-	-	8.1
Particle size, %				
>0.0 mm	27.9	38.6	28.9	1.3
>0.5 mm	48.3	39.3	44.4	5.6
>1.0 mm	15.1	13.6	16.6	37.3
>1.4 mm	4.8	4.7	5.6	26.2
>1.7mm	2.2	2.1	2.6	13.5
>2.0 mm	1.3	1.3	1.2	10.3
>2.5 mm	0.3	0.2	0.3	2.8
>2.8 mm	0.1	0.2	0.4	3.0

[1] Contributed per kilogram of diet: thiamine, 1.0 mg; riboflavin, 4.5 mg; niacinamide, 40 mg; D-pantothenic acid, 9 mg; pyridoxine-HCL, 2.7 mg; choline chloride, 500 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopherol), 33 IU; menadione, 2.3 mg; vitamin A (retinyl-acetate), 12,000 IU; cholecalciferol, 5,000 IU; biotin, 100 µg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 150 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSo_4 \cdot H_2O$, 145 mg; Na_2SeO_3, 0.56 mg; KI, 2.0 mg; antioxidant (oxytrap PXN), 125 mg. [2] Carbohydrate content calculated as: 1,000 - crude protein - crude fat - moisture - crude ash. [3] AME_n = calculated according to CVB (2007).

Table 1c. Ingredient and nutritional composition of the experimental grower (d 14 to 34) diets

Phase	**Grower, d 14 to 34**			
Dietary fat level, %	**3.5**	**8.2**	**12.8**	**17.5**
Ingredient composition, g/kg				
Corn	657.0	563.8	470.6	377.4
Soybean meal HP, >48% CP	291.1	331.5	371.9	412.3
Soybean oil	7.5	56.6	105.8	155.0
Monocalciumphosphate	12.5	13.7	14.9	16.2
Limestone	12.1	12.9	13.6	14.4
Sodiumbicarbonate	3.6	3.6	3.6	3.6
Salt	0.9	1.2	1.5	1.7
DL-Methionine	2.4	2.9	3.4	3.8
L-Lysine HCl	2.3	2.2	2.2	2.1
L-Threonine	0.7	0.9	1.0	1.2
Premix grower[1]	10.0	10.8	11.5	12.3
Calculated chemical composition, g/kg				
CP	201	212	224	236
Crude fat	35	82	128	175
Crude ash	56.4	60	63	67
DM	883	888	893	899
Carbohydrates[2]	591	534	478	421
AME_n, kcal/kg[3]	2,795	2,999	3,204	3,408
Dig. lys	10.4	11.2	12.0	12.8
Calcium	7.6	8.1	8.7	9.3
Phosphorous	6.6	6.9	7.1	7.4
Analyzed chemical composition, g/kg				
Crude fat	37	80	122	152
Crude ash	51	55	59	59
DM	886	894	900	904
Calcium	8.4	-	-	10.3
Phosphorous	6.7	-	-	7.6
Particle size. %				
>0.0 mm	30.9	36.9	27.5	1.1
>0.5 mm	44.4	39.9	46.3	5.3
>1.0 mm	16.4	14.6	16.6	32.1
>1.4 mm	4.8	5.0	5.7	26.1
>1.7mm	2.0	2.1	2.3	14.3
>2.0 mm	1.1	1.1	1.2	14
>2.5 mm	0.3	0.2	0.2	3.4
>2.8 mm	0.1	0.2	0.2	3.7

[1] Contributed per kilogram of diet: thiamine, 0.8 mg; riboflavin, 4.5 mg; niacinamide, 30 mg; D-pantothenic acid, 8 mg; pyridoxine-HCL, 1.9 mg; choline chloride, 400 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopherol), 22 IU; menadione, 2.3 mg; vitamin A (retinyl-acetate), 10,000 IU; cholecalciferol, 2,000 IU; biotin, 50 µg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 150 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSo_4 \cdot H_2O$, 145 mg; Na_2SeO_3, 0.50 mg; KI, 1.9 mg; antioxidant (oxytrap PXN), 125 mg. [2] Carbohydrate content calculated as: 1,000 - crude protein - crude fat - moisture - crude ash. [3] AME_n = calculated according to CVB (2007).

After production, all diets were analyzed for crude fat by acid hydrolysis (ISO 6492, 1999), DM (ISO 6496, 1999), ash (ISO 5984, 2002), and calcium and phosphorous content by ICP-AES (ISO 27085, 2009). Particle size was determined by dry sieving a 100 g feed sample for 3 min using a vibratory sieve shaker (Retsch AS 200; Retsch, Germany).

Data collection

Chickens were weighed on d 0, 7, 14, 21, 28, and 34. At d 0, only the group BW was determined, whereas at the other days individual BW were determined. Feed consumption was recorded per pen on the same days as the chickens were weighed. The gain to feed ratio (G:F; kg of weight gain / kg of feed consumed) was calculated based on calculated ADG and ADFI. ME efficiencies were calculated as g BW gain per MJ of ME intake. ME efficiencies were also calculated per MJ of ME available for growth (ME_{growth}), correcting for ME for maintenance (ME_m). The daily ME_m requirement was calculated using a ME_m value of 0.598 MJ/$kg^{0.75}$/d (Lopez and Leeson, 2005). ME_{growth} was calculated as the difference between total ME intake and ME_m. ADG, ADFI, G:F, and ME efficiencies (corrected and uncorrected for ME_m) were calculated from d 0 to 7, d 7 to 14, d 14 to 34, and d 0 to 34.

At d 35, after 8 h of starvation, five randomly chosen broiler chickens per pen were weighed, killed using carbon dioxide, and dissected to determine breast meat yield (pectoralis major, pectoralis minor, sternum, plus clavicle) and weights of the abdominal fat pad, liver, and pancreas. Breast meat yield, abdominal fat pad, liver, and pancreas weights were calculated as a percentage of carcass weight **(CW)**. Carcass weight included feathers, but excluded head, organs, intestines, blood, and legs distal to the hock. Mortality was checked on a daily basis and calculated per feeding phase.

Statistical analysis

Data were analyzed, using the pen as the experimental unit. Data were subjected to mixed model analysis, using the PROC GLIMMIX procedure in SAS (Version 9.3, 2011, SAS Institute Inc., Cary, NC, United States) according to the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha \times \beta_{ij} + c_k + \varepsilon_{ijk}$$

where Y_{ijk} = dependent variable, μ = overall mean, α_i = fixed effect of dietary fat level (i = 3.5, 8.2, 12.8, or 17.5%), β_j = fixed effect of feeding duration (j = d 0 to 7, d 0 to 14, or d 0 to 34), $\alpha \times \beta_{ij}$ = the interaction between dietary fat level and feeding duration, c_k = random block effect (k = 1, 2, ..., 6), and ε_{ijk} = residual error. Mortality data were analyzed as binomial distributed data, using the same statistical model. Data are expressed as least square **(LS)** means ± SEM. LSmeans were compared after being corrected with a Tukey test for multiple comparisons and effects were considered to be significant when $P \leq 0.05$. For age periods d 0 to 7 and d 7 to 14, only the biologically relevant main effects and interactions are reported.

RESULTS

Pre-starter phase (d 0 to 7)

During the pre-starter phase (d 0 to 7), feeding increased diet densities resulted in higher ADG (Δ = 22% or 3.7 g/d between 3.5 and 17.5% dietary fat) and G:F (Δ = 20% or 0.264 between 3.5 and 17.5% dietary fat; $P < 0.001$; Table 2). Feeding diets with 12.8 and 17.5% dietary fat resulted in higher relative (Figure 1a) and absolute (Figure 1) BW gain between d 0 and 7 than feeding diets with 3.5 and 8.2% dietary fat. Despite a lowered ADFI of broiler chickens as a result of feeding increased diet densities (Δ = 3% or 0.5 g/d between 3.5 and 17.5% dietary fat; $P < 0.001$; Table 2), the ME intake increased (Δ = 19% or 38 kJ/d between 3.5 and 17.5% dietary fat; $P < 0.001$; Table 3).

Starter phase (d 7 to 14)

During the starter phase (d 7 to 14), an interaction between diet density and feeding phase was found for ADG and G:F ($P < 0.001$; Table 2). ADG did not differ among diet densities when fed only from d 0 to 7, but increased with higher diet densities when fed from d 0 to 14 (Δ = 37% or 12.8 g/d between 3.5 and 17.5% dietary fat). G:F increased with higher diet densities when fed from d 0 to 7 (Δ = 3.7% or 0.028 between 3.5 and 17.5% dietary fat) and also from d 0 and 14 (Δ = 33% or 0.243 between 3.5 and 17.5% dietary fat). Continued feeding of increased diet densities resulted in a higher absolute BW difference from d 7 to 14 compared to the 3.5% dietary fat treatment, which was more pronounced for 17.5% compared with 8.2 and 12.8% dietary fat (Figure 1b). When switching from increased diet densitities to the 3.5% dietray fat control treatment after d 7, the relative BW difference decreased (especially after feeding 12.8 and 17.5% dietary fat from d 0 to 7; Figure 1a), whereas the absolute difference in BW was maintained between treatments (Figure 1b). ADFI from d 7 to 14 was not affected by diet density or feeding phase, but for ME intake an interaction between these two factors was found ($P < 0.001$; Table 3). ME intake did not differ among diet densities when fed from d 0 to 7, but increased with higher diet densities when fed from d 0 to 14 (Δ = 25% or 136 kJ/d between 3.5 and 17.5% dietary fat).

Interactions between diet density and feeding phase were found for ME efficiency between d 7 and 14 ($P < 0.001$; Table 4). The ME efficiency was not affected by diet density when fed from d 0 to 7, but it was lower (at 3.5% compared to 17.5% dietary fat) when fed from d 0 to 14, with the other dietary fat levels in between (Δ = 9.7% or 6.2 g gain/MJ ME between 3.5 and 17.5% dietary fat for ME efficiency). Continued feeding of increased diet densities resulted in a higher ME_{growth} to ME_m ratio compared to the 3.5% dietary fat treatment and the ratio lowered when feeding increased diet densities was discontinued (Figure 2a). Discontinued feeding increased diet densities (12.8 and 17.5% dietary fat) resulted in a higher ME efficiency corrected for ME_m compared to continued feeding of these density levels, whereas the opposite effect was found when feeding 8.2% dietary fat (Figure 2c).

Table 2. Effects of diet density and feeding phase on BW, ADG, ADFI, and gain to feed ratio (G:F) of broiler chickens, expressed as least squares means[1]

			BW, g			ADG, g/d				ADFI, g/d				G:F			
Item			**d 0**	**d 7**	**d 14**	**d 34**	**0-7**	**7-14**	**14-34**	**0-34**	**0-7**	**7-14**	**14-34**	**0-34**	**0-7**	**7-14**	**14-34**	**0-34**
Dietary Fat Level, %																		
3.5			47.9	168	418	1,995	17.0^{d}	35.6	78.2	56.3	16.6^{ab}	47.7	121.4^{b}	83.7^{b}	1.025^{d}	0.748	0.644	0.673
8.2			47.8	182	462	2,129	18.9^{c}	39.7	82.8	60.3	17.0^{a}	49.1	125.7^{a}	86.7^{a}	1.112^{c}	0.809	0.658	0.695
12.8			47.0	187	478	2,177	19.9^{b}	41.4	84.5	61.7	16.5^{ab}	47.9	125.6^{a}	86.2^{ab}	1.207^{b}	0.865	0.672	0.716
17.5			47.8	194	503	2,246	20.7^{a}	44.0	86.6	63.6	16.1^{b}	48.5	124.5^{ab}	85.5^{ab}	1.289^{a}	0.909	0.695	0.744
SEM (n = 18)			0.4	2	6	24	0.2	0.8	1.1	0.7	0.2	0.8	1.1	0.6	0.009	0.009	0.006	0.005
Feeding Phase																		
Pre-starter, d 0 to 7			-	-	440	2,053		36.6	80.0	58.0		48.5	124.2^{ab}	85.5		0.754	0.644	0.679
Pre-starter to starter, d 0 to 14			-	-	477	2,041		41.9	77.6	57.8		48.3	122.2^{b}	84.5		0.866	0.635	0.684
Starter to grower, d 0 to 34			-	-		2,315			91.4	65.6			126.5^{a}	86.5			0.722	0.758
SEM (n = 24)			-	-	5	22		0.7	1.0	0.6		0.7	0.6	0.6		0.008	0.006	0.005
Dietary Fat Level ×Feeding Phase[2]																		
PS	S	G																
3.5	-	-	-	-	429.6^{ab}	2,064^{cd}		36.5^{c}	81.0^{c}	58.1^{cd}		48.8	123.1	84.8		0.747^{de}	0.658^{d}	0.686^{de}
8.2	-	-	-	-	429.3^{ab}	2,009^{cd}		35.6^{c}	78.2^{c}	56.8^{cd}		48.3	122.0	84.4		0.737^{ed}	0.641^{de}	0.672^{e}
12.8	-	-	-	-	445.2^{abc}	2,054^{cd}		36.2^{c}	80.2^{c}	58.3^{cd}		47.9	126.0	86.6		0.756^{de}	0.636^{de}	0.674^{e}
17.5	-	-	-	-	457.1^{acd}	2,086^{cd}		37.9^{bc}	80.7^{c}	58.9^{cd}		48.9	125.8	86.3		0.775^{e}	0.642^{de}	0.682^{e}
3.5	3.5	-	-	-	411.7^{b}	1,946^{d}		35.0^{bc}	75.9^{c}	55.0^{d}		47.6	119.1	82.6		0.737^{d}	0.638^{de}	0.666^{e}
8.2	8.2	-	-	-	471.0^{cd}	2,056^{cd}		40.9^{ab}	78.5^{c}	57.9^{cd}		48.9	124.2	85.5		0.837^{c}	0.632^{de}	0.677^{e}
12.8	12.8	-	-	-	492.1^{d}	2,052^{cd}		43.7^{a}	77.1^{c}	58.0^{cd}		47.9	122.7	84.6		0.912^{b}	0.629^{e}	0.686^{de}
17.5	17.5	-	-	-	532.0^{e}	2,111^{c}		47.8^{a}	78.9^{c}	60.3^{c}		48.8	122.9	85.1		0.980^{a}	0.642^{de}	0.708^{d}
3.5	3.5	3.5	-	-		1,975^{cd}			77.7^{c}	55.8^{d}			122.1	83.7			0.637^{de}	0.666^{e}

Table 2. *Continued*

			BW, g				ADG, g/d				ADFI, g/d				G:F			
Item			**d 0**	**d 7**	**d 14**	**d 34**	**0-7**	**7-14**	**14-34**	**0-34**	**0-7**	**7-14**	**14-34**	**0-34**	**0-7**	**7-14**	**14-34**	**0-34**
Dietary Fat Level, %																		
8.2	8.2	8.2	-	-	-	2,322^{b}	-	-	91.8^{b}	66.2^{b}	-	-	131.0	90.0	-	-	0.701^{c}	0.735^{c}
12.8	12.8	12.8	-	-	-	2,425^{ab}	-	-	96.1^{ab}	68.9^{ab}	-	-	128.1	87.3	-	-	0.750^{b}	0.789^{b}
17.5	17.5	17.5	-	-	-	2,539^{a}	-	-	100.1^{a}	71.6^{a}	-	-	124.8	85.0	-	-	0.802^{a}	0.843^{a}
SEM (n=6)			-	-	8.6	36	-	1.1	1.6	1.0	-	1.0	0.3	1.1	-	0.011	0.008	0.007
***P*-value**																		
Dietary Fat Level			0.233	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.240	0.022	0.014	<0.001	<0.001	<0.001	<0.001
Feeding Phase			-	-	<0.001	<0.001	-	<0.001	<0.001	<0.001	-	0.740	0.008	0.051	-	<0.001	<0.001	<0.001
Dietary Fat Level × Feeding Phase			-	-	<0.001	<0.001	-	<0.001	<0.001	<0.001	-	0.500	0.136	0.062	-	<0.001	<0.001	<0.001

[1] Each treatment consisted of 6 pens with 15 males chickens per pen at the start of the study. [2] Dietary fat level expressed as percentage; feeding phases: pre-starter (d 0 to 7, **PS**), starter (d 7 to 14, **S**), grower (d 14 to 34, **G**) phase. [a-g] LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

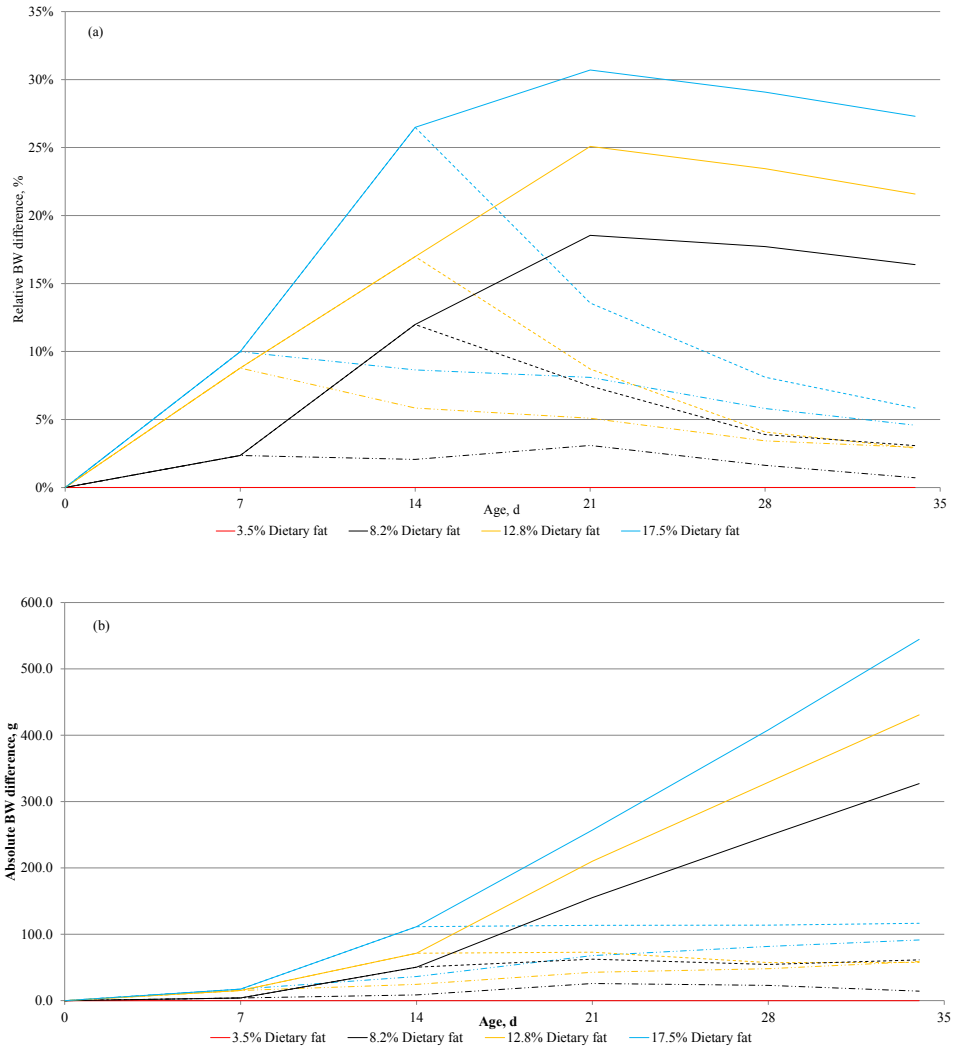


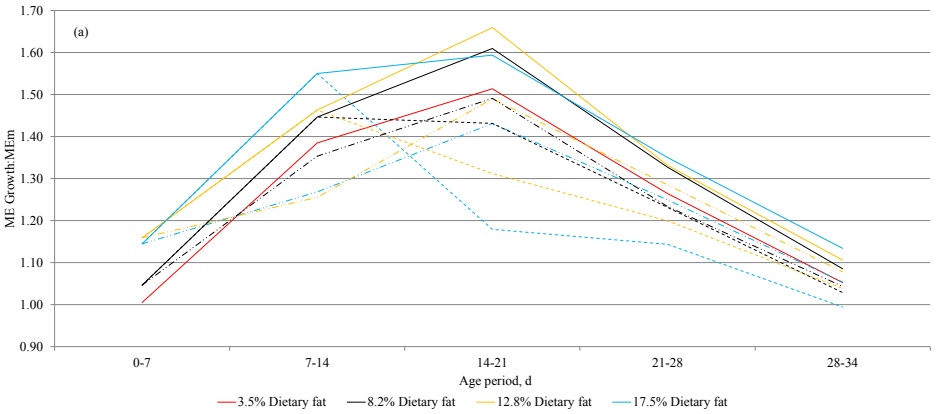
Figure 1. BW differences at d7, 14, 21, 28, and 34 when feeding different diet densities, expressed as relative difference **(A)** and absolute difference **(B)** to the lowest diet density (continuous feeding of 3.5% dietary fat). Continued (-), discontinued from d 7 (- · ·), and discontinued from d 14 (- -) feeding of increased diet densities shown.

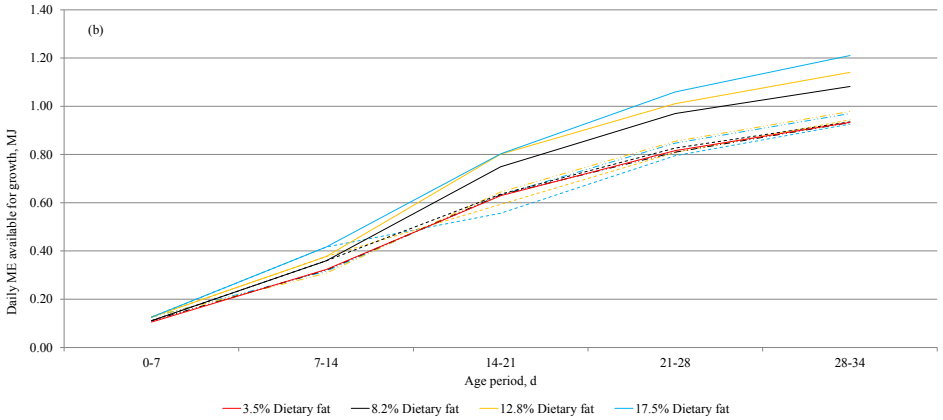
Grower phase (d 14 to 34)

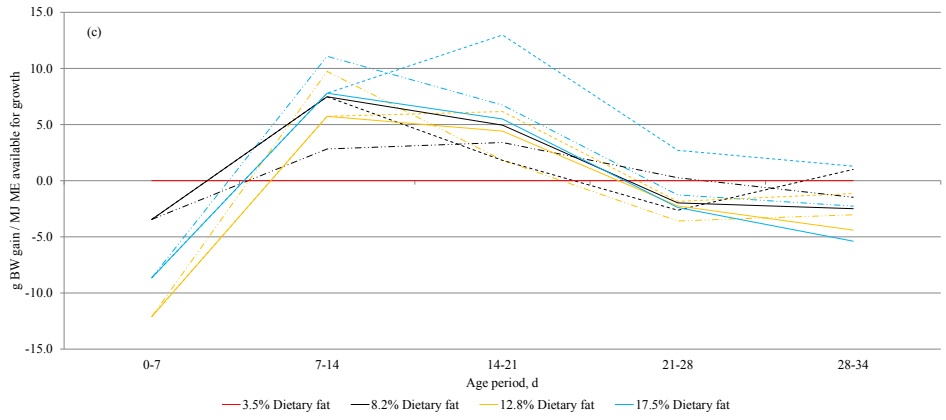
During the grower phase (d 14 to 34), an interaction between diet density and feeding phase was found for ADG and G:F ($P < 0.001$; Table 2). ADG and G:F did not differ among diet densities when fed from d 0 to 7 or d 0 to 14, but increased with higher diet densities when fed from d 0 to 34 (ADG Δ = 29% or 22.4 g/d between 3.5 and 17.5% dietary fat; G:F Δ = 26% or 0.165 between 3.5 and 17.5% dietary fat). In general, the relative BW difference as a result of continued feeding of increased diet densities increased until d 21, followed by a slow decrease from d 21 to 34 (Figure 1a). Continued feeding of increased diet densities resulted in a higher absolute BW difference from d 14 to 34 compared to discontinued treatments (Figure 1b). On the contrary, returning to 3.5% dietary fat after feeding higher diet density resulted in a lowered relative BW difference (that was steepest after feeding 17.5% dietary fat; Figure 1a), whereas the absolute difference in BW difference was maintained compared to continually feeding the 3.5% dietary fat level (Figure 1b). ADFI between d 14 and 34 was affected by diet density and feeding phase ($P = 0.022$ and $P = 0.008$, respectively; Table 2). Regardless of feeding phase, ADFI was lower at 3.5% dietary fat than at 8.2 and 12.8% dietary fat, with 17.5% in between (Δ = 3.5% or 4.3 g/d for 3.5 vs. 8.2 and 12.8% dietary fat, $P = 0.022$).

Furthermore, ADFI between d 14 and 34 was higher when increased diet densities were fed from d 0 to 34 compared to feeding them from d 0 to 14 only (Δ = 3.5% or 4.3 g/d). For ME intake an interaction between diet density and feeding phase was found ($P < 0.001$; Table 3). ME intake from d 14 to 34 did not differ among diet densities when fed only from d 0 to 7 or d 0 to 14, but increased with higher diet densities fed between d 0 to 34 (Δ = 25% or 352 kJ/d between 3.5 and 17.5% dietary fat).

ME efficiency between d 14 and 34 showed an interaction between diet density and feeding phase ($P < 0.015$; Table 4), but no clear pattern among treatments could be observed. The maximum difference among treatments was 2.5 g gain/MJ ME. Similar to the starter phase (d 7 to 14), continued feeding of increased diet densities resulted in a higher ME_{growth} to ME_m ratio compared to the 3.5% dietary fat treatment and lowered when feeding increased diet densities was discontinued (Figure 2a). Discontinued feeding of increased diet densities (17.5% dietary fat) at d 7 resulted in a higher ME efficiency corrected for ME_m from d 14 to 34 compared to continued feeding at this density level, whereas the opposite effect was found for the treatment with 12.8% dietary fat and no effect was found for the treatment with 8.2% dietary fat (Figure 2c). Discontinued feeding of increased diet densities (12.8 and 17.5% dietary fat) at d 14 resulted in higher ME efficiencies corrected for ME_m from d 14 to 34 compared to continued feeding of these density levels, whereas no effect was found when feeding 8.2% dietary fat (Figure 2c).


(a)
ME Growth:MEm
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
0.90
0-7
7-14
14-21
21-28
28-34
Age period, d
3.5% Dietary fat
8.2% Dietary fat
12.8% Dietary fat
17.5% Dietary fat


(b)
Daily ME available for growth, MJ
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0-7
7-14
14-21
21-28
28-34
Age period, d
3.5% Dietary fat
8.2% Dietary fat
12.8% Dietary fat
17.5% Dietary fat


(c)
g BW gain / MJ ME available for growth
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
0-7
7-14
14-21
21-28
28-34
Age period, d
3.5% Dietary fat
8.2% Dietary fat
12.8% Dietary fat
17.5% Dietary fat

Overall (d 0 to 34)

An interaction between diet density and feeding phase was found for ADG and G:F between d 0 and 34 ($P < 0.001$; Table 2). ADG was not affected by diet density when fed only from d 0 to 7, but increased with higher diet densities when fed from d 0 to 14 (Δ = 10% or 5.3 g/d between 3.5 and 17.5% dietary fat), with 8.2 and 12.8% dietary fat in between. The relative increase in ADG was even higher when increased diet densities were fed from d 0 to 34 (Δ = 28% or 15.8 g/d between 3.5 and 17.5% dietary fat). Similar to ADG, G:F did not differ among diet densities when fed only from d 0 to 7. However, G:F increased with higher diet densities when fed from d 0 to 14 (Δ = 6.3% or 0.042 between 3.5 and 17.5% dietary fat), with 12.8% dietary fat in between. The increase in G:F was even higher when increased diet densities were fed from d 0 to 34 (Δ = 27% or 0.177 between 3.5 and 17.5% dietary fat).

Between d 0 and 34, a diet containing 8.2% dietary fat resulted in higher ADFI than when feeding a diet containing 3.5% dietary fat (Δ = 3.6% or 3.0 g/d; $P = 0.014$; Table 2), with 12.8 and 17.5% dietary fat in between. ME intake did not differ among diet densities when fed only from d 0 to 7, but increased with higher diet densities when fed from d 0 to 14 (Δ = 13% or 122 kJ/d between 3.5 and 17.5% dietary fat). The relative increase in ME intake was even higher when increased diet densities were fed from d 0 to 34 (Δ = 24% or 235 kJ/d between 3.5 and 17.5% dietary fat).

An interaction between diet density and feeding phase was found for ME efficiency between d 0 and 34 ($P < 0.01$, Table 4). ME efficiency was not affected by diet density when fed from d 0 to 7 or from d 0 to 14, but increased with higher diet densities when fed from d 0 to 34 (Δ = 3.9% or 2.2 g BW gain/MJ ME between 3.5 and 17.5% dietary fat). Diet density and feeding phase did not affect mortality (average mortality between d 0 and 34: 6.43%; $P > 0.05$; data not shown).

◀ **Figure 2.** The ME for growth to ME for maintenance ratio (**A**), the daily ME available for growth (**B**), and grams of BW gain per MJ of ME available for growth (**C**) at different age periods; d 0 to 7, 7 to 14, 14 to 21, 21 to 28, and 28 to 34 when feeding different diet densities. Grams of BW gain expressed per MJ is expressed as absolute difference to the lowest diet density (continuous feeding of 3.5% dietary fat). Continued (-), discontinued from d 7 (- · ·), and discontinued from d 14 (- -) feeding of increased diet densities shown.

Table 3. The effect of diet density and feeding duration on AME intake (kJ/d) of broiler chickens, expressed as least squares means[1]

			AME intake, kJ/d			
Item			**0-7**	**7-14**	**14-34**	**0-34**
Dietary Fat Level, %						
3.5			202^{d}	550	1,420	985
8.2			223^{c}	596	1,508	1,061
12.8			231^{b}	607	1,543	1,095
17.5			240^{a}	640	1,563	1,126
SEM (n = 18)			2	10	13	8
Feeding Phase						
Pre-starter, d 0 to 7			-	560	1,453	1,030
Pre-starter to Starter, d 0 to 14			-	619	1,430	1,040
Pre-starter to Grower, d 0 to 34			-	-	1,643	1,130
SEM (n = 24)			-	9	11	7
Dietary Fat Level × Feeding Phase[2]						
PS	S	G				
3.5	-	-	-	564^{cd}	1,440^{c}	997^{efg}
8.2	-	-	-	558^{cd}	1,427^{c}	1,009^{efg}
12.8	-	-	-	553^{cd}	1,474^{c}	1,051^{def}
17.5	-	-	-	565^{cd}	1,472^{c}	1,064^{de}
3.5	3.5	-	-	549^{d}	1,393^{c}	972^{g}
8.2	8.2	-	-	607^{bc}	1,453^{c}	1,038^{defg}
12.8	12.8	-	-	634^{ab}	1,435^{c}	1,057^{de}
17.5	17.5	-	-	685^{a}	1,438^{c}	1,094^{cd}
3.5	3.5	3.5	-	-	1,428^{c}	985^{gf}
8.2	8.2	8.2	-	-	1,645^{b}	1,138^{bc}
12.8	12.8	12.8	-	-	1,718^{ab}	1,178^{ab}
17.5	17.5	17.5	-	-	1,780^{a}	1,220^{a}
SEM (n = 6)			-	14	22	14
***P*-value**						
Dietary Fat Level			<0.001	<0.001	<0.001	<0.001
Feeding Phase			-	<0.001	<0.001	<0.001
Dietary Fat Level × Feeding Phase			-	<0.001	<0.001	<0.001

[1] Each treatment consisted of 6 pens with 15 male chickens per pen at the start of the study. [2] Dietary fat level expressed as percentage; feeding phases: pre-starter (d 0 to 7, **PS**), starter (d 7 to 14, **S**), grower (d 14 to 34, **G**) phase. $^{a-g}$ LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

Table 4. Effects of diet density and feeding phase on ME efficiency (MEη), expressed as g gain per MJ consumed, expressed as least squares means[1,2]

			ME_η, g BW gain / MJ consumed			
Item			**0-7 d**	**7-14 d**	**14-34 d**	**0-34 d**
Dietary Fat Level, %						
3.5			85.1	64.8	55.3	57.7
8.2			85.8	67.1	55.1	57.8
12.8			86.3	68.6	54.8	57.8
17.5			86.8	68.9	55.5	58.5
SEM (n = 18)			0.6	0.7	0.5	0.5
Feeding Phase						
Pre-starter, d 0 to 7			-	65.6	55.3	57.9
Pre-starter to Starter, d 0 to 14			-	67.6	54.6	57.6
Pre-starter to Grower, d 0 to 34			-	-	55.7	58.4
SEM (n = 24)			-	0.7	0.5	0.4
Dietary Fat Level ×Feeding Phase[3]						
PS	S	G				
3.5	-	-	-	64.7^{cd}	56.6^{a}	58.8^{ab}
8.2	-	-	-	64.3^{cd}	55.1^{ab}	57.6^{ab}
12.8	-	-	-	66.1^{bcd}	54.5^{ab}	57.2^{ab}
17.5	-	-	-	67.1^{abd}	55.2^{ab}	58.0^{ab}
3.5	3.5	-	-	63.7^{b}	54.9^{ab}	57.2^{ab}
8.2	8.2	-	-	68.1^{ab}	54.4^{ab}	57.5^{ab}
12.8	12.8	-	-	68.9^{ab}	54.1^{b}	57.4^{ab}
17.5	17.5	-	-	69.9^{a}	54.8^{ab}	58.4^{ab}
3.5	3.5	3.5	-	-	54.5^{ab}	57.0^{c}
8.2	8.2	8.2	-	-	55.8^{ab}	58.5^{ab}
12.8	12.8	12.8	-	-	56.0^{ab}	58.8^{ab}
17.5	17.5	17.5	-	-	56.5^{a}	59.2^{a}
SEM (n = 6)			-	0.9	0.6	0.6
***P*-value**						
Dietary Fat Level			0.293	<0.001	0.389	0.066
Feeding Phase			-	<0.001	0.006	0.048
Dietary Fat Level ×Feeding Phase			-	0.006	0.015	0.006

[1] Calculated based on analyzed nutrient values, except for metabolizable energy (calculated). [2] Each treatment consisted of 6 pens with 15 male chickens per pen at the start of the study. [3] Dietary fat level expressed as percentage; feeding phases: pre-starter (d 0 to 7, **PS**), starter (d 7 to 14, **S**), grower (d 14 to 34, **G**) phase.
[a-i] LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

Carcass composition (d 35)

An interaction between diet density and feeding phase was found for BW, carcass yield, and breast meat yield at d 35 ($P \leq 0.015$; Table 5). BW and carcass yield did not differ among diet densities when fed only from d 0 to 7 or d 0 to 14, but increased with higher diet densities when fed from d 0 to 34 (Δ = 24% and 3.5% relatively or 464 g and 2.7% absolute between 3.5 and 17.5% dietary fat for BW at 35 d and carcass yield, respectively). Breast meat yield also did not differ among diet densities when fed only from d 0 to 7 or d 0 to 14, but was lowered with higher diet densities when fed from d 0 to 34 (Δ = 6.2% relatively or 2.0% absolute between 3.5 and 17.5% dietary fat). A tendency (P = 0.058) for an interaction between diet density and feeding phase was observed for relative fat pad weights (Table 5). Feeding increased diet densities from either d 0 to 7 or 0 to 14 resulted in lowered fat pad weights (average Δ = -13%), whereas this effect was the opposite when feeding increased diet densities from d 0 to 34 (Δ = 22%; Table 5). The relative fat pad size was higher when increased diet densities were fed from d 0 to 7 or d 0 to 34 compared to feeding increased diet densities from d 0 to 14 (Δ = 19% relatively or 0.14% absolute; $P < 0.001$; Table 5). The relative pancreas weight was lower when increased diet densities were fed from d 0 to 34 compared with feeding increased diet densities from d 0 to 7 and d 0 to 14 (Δ = 17% relatively or 0.045% absolute ; $P < 0.001$; Table 5).

DISCUSSION

The aim of the current study was to determine whether increased diet density, fed until different ages, affected broiler chicken growth performance and carcass composition. In general, increased diet density increased ADG, G:F, and ME intake during the periods the denser diets were fed. ME efficiency was influenced from d 7 onward by diet density. An increased diet density, fed during the entire grow-out period, resulted in increased BW at slaughter and lowered breast meat yield. In addition, feeding increased diet densities resulted in a lowered relative pancreas weight when fed between d 0 and 34, whereas a lower relative fat pad weight was found when increased diet densities were only fed between d 0 and 14.

Growth performance

In the current study, increased diet densities resulted in increased ME intake, ADG, and G:F during the first week of life, whereas ADFI was lowered and ME efficiency was not affected. Current results are in line with Brickett et al. (2007), where feeding mash diets with increased densities also increased ADG and G:F during the first week. However, in that study ADFI was not affected by diet density. The latter might be explained by differences in diet density level used, as in the current study, the increase in density level was about

Table 5. Effects of diet density and feeding phase on empty BW, relative carcass and organ size of broiler chickens at d 35, expressed as least squares means[1]

Item			BW,	Carcass[2]	Breast[3]	Fat pad[4]	Liver	Pancreas
			g	%	%	%	%	%
Dietary Fat Level, %								
3.5			1,923	77.7	34.3	0.83	2.93	0.30
8.2			2,058	78.6	34.4	0.85	2.94	0.30
12.8			2,075	78.5	33.8	0.80	2.94	0.27
17.5			2,153	79.1	33.9	0.81	3.03	0.29
SEM (n = 18)			24	0.3	0.3	0.04	0.04	0.01
Feeding Phase								
Pre-starter, d 0 to 7			1,981	78.5	34.5	0.85^{a}	2.91	0.31^{a}
Pre-starter to Starter, d 0 to 14			1,978	78.0	34.3	0.73^{b}	3.01	0.30^{a}
Pre-starter to Grower, d 0 to 34			2,199	78.9	33.6	0.89^{a}	2.97	0.26^{b}
SEM (n = 24)			20	0.3	0.2	0.03	0.04	0.01
Dietary Fat Level × Feeding Phase [5]								
PS	S	G						
3.5	-	-	1,937^{c}	78.5^{abcd}	34.5^{a}	0.91	2.90	0.31
8.2	-	-	1,966^{c}	78.4^{abcd}	34.3^{ab}	0.90	2.88	0.32
12.8	-	-	1,977^{c}	78.3^{abcd}	34.7^{a}	0.82	2.92	0.30
17.5	-	-	2,042^{bc}	79.0^{abc}	34.6^{a}	0.76	2.93	0.33
3.5	3.5	-	1,922^{c}	77.4^{cd}	34.1^{ab}	0.76	2.97	0.31
8.2	8.2	-	1,993^{c}	78.3^{abcd}	34.4^{ab}	0.76	2.98	0.30
12.8	12.8	-	1,954^{c}	78.0^{bcd}	33.9^{abc}	0.70	2.98	0.29
17.5	17.5	-	2,042^{bc}	78.3^{abcd}	34.8^{a}	0.69	3.10	0.30
3.5	3.5	3.5	1,911^{c}	77.2^{d}	34.4^{ab}	0.81	2.94	0.29
8.2	8.2	8.2	2,216^{ab}	79.0^{abc}	34.5^{a}	0.88	2.95	0.29
12.8	12.8	12.8	2,293^{a}	79.3^{ab}	32.9^{bc}	0.88	2.91	0.22
17.5	17.5	17.5	2,375^{a}	79.9^{a}	32.4^{c}	0.99	3.07	0.23
SEM (n = 6)			41	0.4	0.4	0.05	0.07	0.02
***P*-value**								
Dietary Fat Level			<0.001	<0.001	0.099	0.688	0.207	0.119
Feeding Phase			<0.001	0.004	<0.001	<0.001	0.106	<0.001
Dietary Fat Level × Feeding Phase			<0.001	0.015	<0.001	0.058	0.950	0.179

[1] Each treatment consisted of 6 pens with 5 males chickens dissected per pen. [2] Carcass: including feathers, but excluding head, organs, intestines, blood, and inferior to the hock. [3] Breast meat yield (pectoralis major, pectoralis minor, sternum, plus clavicle), expressed as percentage of carcass weight. [4] Abdominal fat pad. [5] Dietary fat level expressed as percentage; feeding phases: pre-starter (d 0 to 7, **PS**), starter (d 7 to 14, **S**), grower (d 14 to 34, **G**) phase. [a-d] LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

two times higher (600 vs. 300 kcal AME/kg) compared to the work of Brickett et al. (2007). Jackson et al. (1982a; b) already suggested that an increased diet density reduces ADFI with increasing ME intake, but only if the difference in diet density is large enough (1,000 kcal AME/kg).

Current results on growth performance during the first week of life partially contradict previous work (**Chapter 4**), where feeding of similar diet density levels during the first week of life resulted in linearly decreased BW, whereas ME intake was maintained. Differences in ME intake response between the two studies might be explained by differences in absolute feed intake. The average absolute feed intake level during the first week of life appears to be similar in the current and previous work (16.6 g/d in current study vs. 16.7 g/d from d 0 to 7 in **Chapter 4**). However, the difference in feed intake between low and high density diets appears to be smaller in the current work (0.5 g/d in the current study vs. 3.4 g/d in previous work, comparing 3.5 vs. 17.5% dietary fat).

Feeding increased diet densities resulted in increased ME intake, ADG, and G:F not only in the first week, but also during the entire experimental period (d0 to 34). The linear increase in ME intake due to feeding increased diet densities is contradictory to previous research, where broiler chickens were found to maintain a constant ME intake (Leeson et al., 1996; **Chapter 4**), but it is in agreement with other research where a similar effect of increased diet density on ME intake was observed (Jackson et al., 1982a; Saleh et al., 2004; Brickett et al., 2007).

Differences in feed intake and ME intake between the current study, results of **Chapter 4**, and literature may be explained by the physical form of the diet. Pelleted diets (as fed in **Chapter 4**) facilitate an easier uptake of nutrients compared to mash diets. The latter would enable chickens to easier fulfil their maximal nutritional requirements and thus subsequently reduce their feed intake with high density diets. This is confirmed by other work, where broiler chickens were better capable of regulating their nutrient intake with varying diet densities when provided pelleted rather than mash diets (Brickett et al., 2007). This suggests that in the current study, the lower diet densities may have caused suboptimal performance, because the physical feed intake capacity was limiting.

Although feeding increased diet densities until 7 d of age was found to have significant effects on ADG, ADFI, and G:F, these effects did not persist during the remainder of the study (d 7 to 34) when a high density diet was followed by a low diet density diet (Table 2). Feeding increased diet densities until d 14 resulted in higher ADG and G:F from d 0 to 34 when feeding a high (17.5% dietary fat) instead of low (3.5% dietary fat) density diet from d 0 to 14. Although it is known that broiler chickens are capable of physiological adaptation during the period that increased diet densities are provided (Scott, 2002; Wang et al., 2014; Wang et al., 2015; Li et al., 2016; **Chapter 5**), these physiological adaptations did not result in altered growth performance at a later age. This suggests that the induced physiological adaptions in early life, which were expected to occur in the current study, may have been

too small to cause long-term performance effects. Additionally, increasing the weight and length of the gastro-intestinal tract to increase the absorptive capacity, which may compensate for lacking hydrolyzing capacity, as seen in young broiler chickens as suggested in **Chapter 5**, may be less relevant for older broiler chickens that have a more mature intestinal tract with sufficient enzyme secretion.

The limited carry-over effects of feeding increased diet densities until either d 7 or 14 for the remainder of the experimental period are also shown by the relative and absolute BW differences (Figure 1a and b). Broiler chickens returned to the growth curve of the chickens continually fed the 3.5% fat diet once their diet was switched from an increased density diet to the low density diet. Whereas the relative BW difference after switching from feeding increased diet densities reduced and approached that of the treatment that was continually fed a 3.5% dietary fat diet, the absolute BW differences generated at the moment of diets change, were maintained until the end of the study (Figure 1b).

The increased G:F as a result of feeding increased diet densities is the logical consequence of feeding higher nutrient concentrations in the diet. This allows the broiler chicken to consume less or equal feed amounts, while maintaining or even increasing its nutrient intake levels. As such, the G:F provides only limited insight into the true efficiency of the broiler chicken, and we, therefore, suggest to rather consider ME efficiencies.

ME efficiency

In the current study, the calculated ME efficiencies are expressed as BW gain per MJ of ME consumed. The calculated efficiencies should be interpreted with care, as BW gain is actually the sum of protein and fat accretion, metabolic water, and gut fill. Thus, the calculated efficiencies do not represent energy retention. Furthermore, nutrient excretion in the feces was not determined, which would allow to correct for any energy inefficiencies. Nevertheless, the calculated energy efficiencies can be interpreted as alternative values for feed efficiency.

Calculated ME efficiencies were not affected by diet density from d 0 to 7 nor from d 14 to 34. During the starter phase (d 7 to 14), continued feeding of a diet density (3.5% dietary fat) resulted in a lower ME efficiency compared to the other diet densities (≥ 8.2% dietary fat). A similar effect was found for the overall (d 0 to 34) period when feeding of increased diet densities was continued during the grower phase (d 14 to 34). In previous research, an increase in diet density also resulted in increased energy utilization (Jackson et al., 1982a; Saleh et al., 2004). The difference in ME efficiency response between diet densities within different age periods showed that the response for energy efficiency is age-dependent (Table 4). Current results also suggest that continued feeding of increased diet densities resulted in higher efficiency, whereas this effect disappears when changing to a low diet density (3.5% dietary fat). However, a disadvantage of the calculated ME efficiencies in the current study is that no distinction is made between ME intake used for maintenance (ME_m) or

growth (ME_{growth}). This will impact the calculated ME efficiencies, as broiler chickens fed high density diets are expected to have more ME_{growth} compared to maintenance. On the contrary, the chickens fed high diet densities are likely to have a higher BW, which results in a higher ME_m.

The ME_{growth} to ME_m ratio, daily ME_{growth}, and ME efficiencies corrected for ME_m are presented in Figure 2a-c. These calculations show that feeding increased diet densities resulted indeed in more energy available for growth, reflected by an increased ME_{growth} to ME_m ratio (Figure 2a). Furthermore, this ratio decreased after switching from high (≥ 8.2% dietary fat) density diets to a low (3.5% dietary fat) density diet, an effect that was more pronounced when broiler chickens were fed increased diet densities for a prolonged period of time (7 vs. 14 d). Broiler chickens that change from a high (≥ 8.2% dietary fat) to a low (3.5% dietary fat) density diet did not only have a lower overall ME intake after they changed diet density level (Table 3), but also their BW at the moment of change was relatively high compared to their new feed and energy intake level, thus spending relatively more of the total ME intake on maintenance than on growth.

Based on the calculated ME efficiencies in Table 4, it was concluded that continued feeding of increased diet densities resulted in higher efficiency and that this effect disappeared when changing to a low diet density (3.5% dietary fat). However, when corrected for ME_m, the ME efficiencies depend not only on age and diet density, but also on the duration the diet is provided. Changing from diets with either 12.8 or 17.5% dietary fat to a 3.5% dietary fat diet resulted in a higher ME efficiency corrected for ME_m in the period directly after the change in diet density (Figure 2c). The latter may be explained through physiological adaptations towards increased diet densities that are expected to occur in young broiler chickens, such as repartitioning of visceral organ development to increase the size and length of the intestinal tract (**Chapter 5**).

Carcass composition

In the current study, the empty BW and relative carcass yield at d 35 were not affected by different diet densities fed until either d 7 or 14, whereas the relative carcass weight increased as a result of continued feeding of increased diet densities (≥ 8.2% dietary fat) until d 34. It is generally accepted that higher BW results in higher relative carcass weights (Brake et al., 1993), thus explaining current treatment differences in cases of continued feeding of increased diet densities. Similar to carcass yield, breast meat yield at d 35 was also not affected when feeding increased diet densities until either d 7 or 14. This is contradictory to previous research, where an increased amino acid density in the diet of broiler chickens was associated with increased satellite cell proliferation and muscle development, thus resulting in increased breast meat yield at slaughter (Dozier et al., 2008). However, in contrast to our study, Dozier et al. did not increase energy density along with amino acid density. Continued feeding of increased diet densities from d 0 to 34 resulted

in a lower breast meat yield (>8.2% dietary fat). This is in contrast with Saleh et al. (2004), who found that relative breast meat yield and fat pad size remained rather constant when feeding increased diet densities, ranging from 3,023 to 3,383 AME kcal/kg, while maintaining a constant energy to protein ratio. Differences between studies might be caused by the limited increase in diet density as used by Saleh et al. (2004) compared to the current study.

In the current study, the increased energy density was combined with an increased dietary amino acid density. Dietary fat that is ingested but not metabolized to yield energy to facilitate protein synthesis may be stored as body fat. As found by Jackson et al. (1982a), continued feeding of high density, thus high energy, diets resulted in an increased relative fat pad size at slaughter. Interestingly, in the current study, the relative fat pad size was lower for broiler chickens fed increased diet densities until d 14, which subsequently were switched to a low density diet. This effect may be explained by a physiological response of the chicken that is similar to temporary feed restriction, sometimes used to induce and study compensatory growth (Zubair and Leeson, 1996). Feed restriction at relatively young ages (between 2 and 3 wk of age) is hypothesized to impair proliferation of adipocytes, associated with a lower fat deposition at a later age (Zubair and Leeson, 1996). As discussed earlier, broiler chickens may have difficulties regulating their feed (and thus energy) intake, especially when fed low density mash diets (Brickett et al., 2007). Therefore, the switch from high density diets to a low density diet as in the current study may also be perceived as a kind of feed restriction, resulting in the suggested impairment of adipocyte proliferation. The lowered relative fat pad weight may also be indirectly linked to the previously discussed higher ME efficiency corrected for ME_m, when switching from a high (12.8 or 17.5% dietary fat) to a low (3.5% dietary fat) density diet at 7 or 14 d of age (Figure 2c). The higher ME efficiency corrected for ME_m is hypothesized to be the result of increased protein deposition, at the expense of fat deposition, in order for the broiler chicken to maintain its growth. Protein deposition is, in contrast to fat, accompanied with water deposition in a ratio of 3 to 1 (Leenstra, 1986), hence the higher ME efficiency. Increased protein deposition results in lowered fat deposition, explaining the subsequent lowered relative fat pad weight at slaughter.

In conclusion, feeding increased diet densities containing up to 17.5% dietary fat resulted in increased ADG and G:F but mainly for the duration these diets were provided. ME efficiency depended on both age and diet density level.

CONFLICT OF INTEREST

D.M. Lamot and P.J.A. Wijtten are employed at the Cargill Animal Nutrition Innovation Center Velddriel, the Netherlands, but do not have any conflict of interest regarding the topic described in this article. The other authors also do not have any conflict of interest.

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CHAPTER 7

General Discussion

INTRODUCTION

First week nutrition should support the broiler chicken during a very dynamic period in life. Various physiological developments occur in the first week of life, such as growth and maturation of the intestinal tract, development of the immune system, and development of the thermoregulatory system (Christensen, 2009). While these physiological developments have to be supported through nutrition, the effect of nutrition may depend on other factors, such as breed, breeder age, incubation conditions, housing hygiene, housing temperature, and moment of feed access after hatch (Figure 1). These factors may strongly affect physiological development of broiler chickens during the first week of life; therefore, the effect of nutrition on physiological development of the chicken may also interact with these factors.

Limited research has been conducted to study these factors of influence in relation to nutrition during the first week of life, although some examples exist. For example, the effect of breeder age (Noy and Pinchasov, 1993; Vargas et al., 2009) and housing conditions (Simon et al., 2015) have been examined in relation to the moment of first feed intake and how this affects growth performance and immune status. Another example is the modeling of lysine requirements for slow vs. fast growing broiler chickens (breed effect) during the first week of life (Gous, 2010). Finally, Van den Brand et al. (2010) studied the effects of first week nutrition, focusing on individual dietary components (dextrose, albumen) and more complex pre-starter diets in relation to cold stress during the first days after hatch.

The above examples demonstrate the interdependency of nutrition and other factors in relation to (physiological) development during the first week of life; therefore, these factors should not be ignored. This thesis focused in particular on 1) the moment of first feed intake after hatch and 2) feed composition during the first week of life.

1. Moment of first feed intake after hatch

A factor that has been studied more extensively, either by itself or in relation to other environmental conditions, is the moment of first feed access after hatch. Delayed access to feed during the first days after hatch impairs growth performance of the broiler chicken, e.g., by impaired development of the intestinal tract (Corless and Sell, 1999), despite that chickens have a residual yolk that supplies nutrients for initial survival and development after hatch (Gonzales et al., 2003). Despite the abundant availability of studies focusing on the effect of first feed access after hatch, there is large variation in the used experimental designs that may affect the results as well. This causes ambiguities about the exact duration of delayed feed access after hatch, ignoring potential interactions between the moment of first feed intake after hatch and the actual moment of hatch (Careghi et al., 2005; Van de Ven et al., 2011). In many studies that addressed the moment of feed access in relation to hatch moment, the hatch window was often longer than 30 h, thereby raising the question

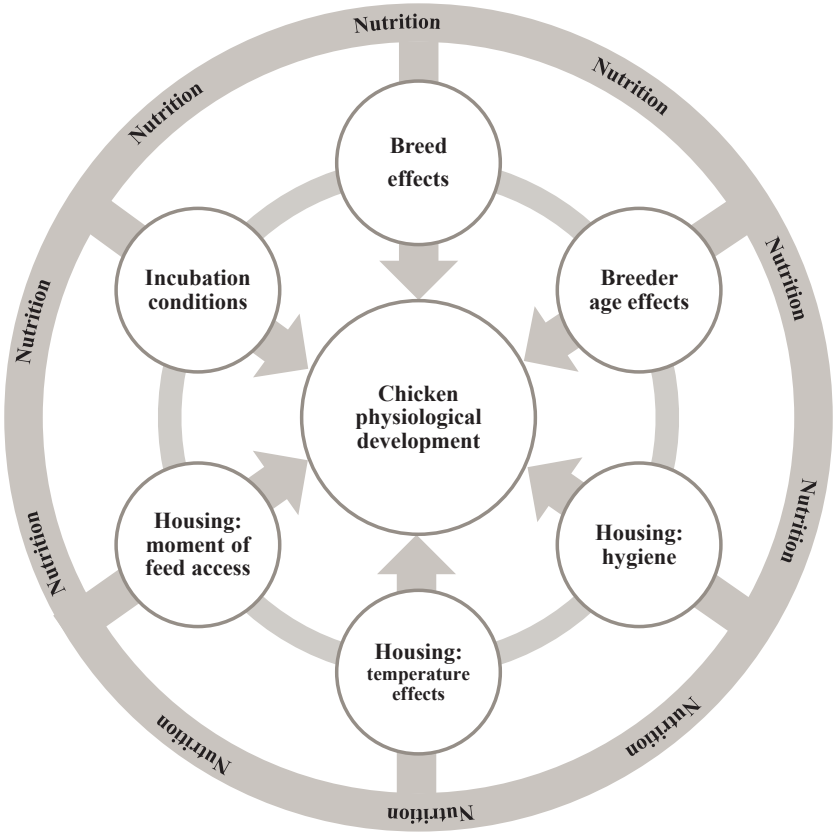

Figure 1. Nutrition provided during the first week of life is intended to support the broiler chicken in its (physiological) development. However, other factors may also influence development of the chicken during this age period. Therefore, the supportive role of nutrition on development during the first week of life may interact with these factors too.

whether similar effects would be observed in a short hatch-window. Furthermore, studies that tried to quantify the physiological and metabolic effects of hatch moment and feed access mainly focused on the first week after hatch. Consequently, potential carry-over effects of hatch moment and moment of feed access on organ growth or carcass composition at a later age are rarely considered.

2. Feed composition during the first week of life

Effects of feed composition during the first week of life on physiological development of the broiler chicken have been examined before and covered specific ingredients, such as albumen and dextrose (Van den Brand et al., 2010), protein levels and sources (Noy and Sklan, 2002; Longo et al., 2007; Everaert et al., 2010; Wijtten et al., 2012), minerals (Maiorka

et al., 2004; Garcia et al., 2006), cellulose (Noy and Sklan, 2002), and macro nutrient ratios (Swennen et al., 2009). Although some studies have looked into the inclusion of increased dietary fat levels in the diet (Noy and Sklan, 2002; Van den Brand et al., 2010), in general the inclusion of high levels of fats and oils in a pre-starter diet is considered undesirable due to their assumed low digestibility in young broiler chickens compared to starch (Batal and Parsons, 2002b). Consequently, over recent years, limited research has been conducted related to the role of dietary fats and oils in diets for broiler chickens during the first week of life.

The aim of this thesis was to determine the effects of the moment of first feed intake after hatch and the effect of feed composition (with special attention to dietary fat inclusion) during the first week of life on short-term physiological development as well as long-term effects on growth, organ development, metabolic status and carcass composition.
The objective of this discussion is to combine data from different chapters and literature and to discuss the obtained results on feed access after hatch in relation to the experimental setup chosen to study these effects, as well as underlying physiological mechanisms for the effects observed. Next, the role of fats and oils in high density pre-starter diets will be discussed, reflecting on the underlying physiological mechanisms for the effects observed (pre vs. post-absorption) and diet physical form. Finally, the potential implications of the current results for practice will be discussed.

FEED ACCESS AFTER HATCH

Experimental designs to examine effects of feed access after hatch

A relatively short (Chapter 2) as well as long (Chapter 3) delay in feed access after hatch resulted in lowered growth and altered development, partially compensated for at a later age. In Chapter 2, with a delayed feed access of 13 to 26 h, the difference in BW gain after hatch compared to immediate fed chickens only lasted until 7 d of age, whereas in Chapter 3, with a delayed feed access of 48 h, the difference in BW gain lasted up to 25 d of age. The long-term consequences of delayed feed access on BW gain should be carefully considered in relation to the methodology used to induce delayed feed access.
In Chapter 2, early, midterm, and late hatched chickens were pulled from the incubator at different time intervals (475 to 481, 483 to 487 or 489 to 493 h after onset of incubation) and either directly or delayed fed (26, 19, and 13h after pulling, depending on hatch moment). The moment of feed access coincided with the regular first feed intake in practice (21.5 d after the onset of incubation).
In Chapter 3, chickens were pulled from the incubator all at once and only after all chickens had hatched (normal pulling moment), not differentiating for the moment of hatch. From

the moment of placement in the grow-out facility onwards, half of the chickens received delayed feed access for 48 h. In both experimental designs, broiler chickens received variable durations of delayed feed access. In Chapter 2, the variation in duration was caused by variable hatch time between treatments, whereas in Chapter 3, the variation was within treatments as we could not distinguish when chickens had hatched. Furthermore, in Chapter 3, the 48 h delayed feed access was on top of the time spent within the incubator, plus processing time within the hatchery and transportation time from the hatchery to the grow-out facility.

In general, three types of experimental designs can be distinguished when conducting research on effects of direct vs. delayed feed access after hatch (Figure 7.2). Careghi et al. (2005) and Willemsen et al. (2010) already emphasized the importance of distinguishing between the biological and chronological age of broiler chickens. According to this principle, the chronological age is defined as a fixed moment in time and is the same for one batch of chickens, whereas the biological age is different for early, midterm, and late hatched chickens (Figure 2). Based on the chronological age, delayed feed access can be applied, e.g., from the arrival in the grow-out unit onward for a fixed time period (Design I; Chapter 3), thereby ignoring variation in hatching time (biological age). Based on the biological age, delayed feed access can be applied from the moment of hatch onward until pulling or placement in a grow-out facility, resulting in differences in delay of feed access depending on the moment of hatch (Design II; Chapter 2). Lastly, delayed feed access can be applied from the moment of hatch onward for a fixed time duration (Design III). The appropriate selection of Design I, II, or III depends on the research question to be answered.

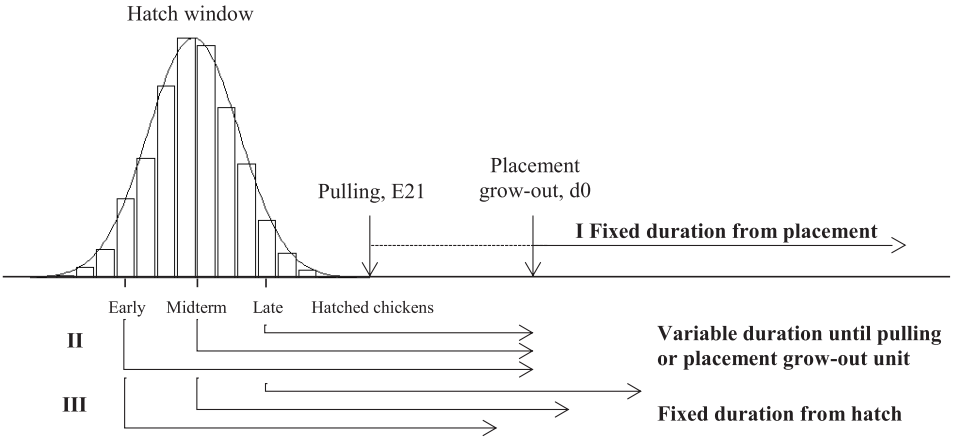

Figure 2. Experimental designs that are in general applied related to research concerning moment of first feed access: (I) fixed duration from pulling or placement in a grow-out facility onwards, (II) variable duration until pulling or placement in grow-out unit, or (III) fixed duration from hatch onward (modified from Willemsen et al., 2010).

Design I is suitable to create a larger contrast between treatments and to 'model' the effect of feed access on subsequent growth or health status (Chapter 3). However, a drawback of this design is that the potential differences in biological age between chickens due to their spread of hatch are ignored.
Design II accounts for differences in biological age (Chapter 2), while the contrasts of direct or delayed feed access are applied between hatch and pulling or placement in a grow-out unit. This results in varying delayed feed access durations based on biological age. Therefore, this design may inevitably result in confounding effects between the moment of hatch and feed access. Related to this, in Chapter 2, it was also found that especially early hatched chickens benefit from direct feed access compared to midterm and late hatched chickens, which was likely due to the longer exposure time to feed.
Design III accounts for differences in biological age, but delayed feed access is applied from the moment of hatch onward for a fixed duration (e.g., 48 h) for all chickens hatched at different moments. Compared to the other designs, Design III is considered the most elegant if one truly wants to study the effects of delayed feed access on physiological development of early, midterm, or late hatched chickens within the hatch window.
Although all three types of experimental designs are applied in the literature (Table 1), studies often remain inconclusive or unclear about the exact used design.

Table 1. Examples of studies with different experimental designs that can be applied related to moment of feed access: (I) fixed duration from pulling or placement in a grow-out facility onwards, (II) variable duration until pulling or placement in grow-out unit, or (III) fixed duration from hatch onward.

Design	Reference
I	Moran, 1990; Corless and Sell, 1999; Batal and Parsons, 2002a; Gonzales et al., 2003; Franco et al., 2006; Halevy et al., 2000; Maiorka et al., 2003; Biloni et al., 2013; **Chapter 3**
II	Noy et al., 2001; Careghi et al., 2005; Van de Ven et al., 2011; Van de Ven et al., 2013; **Chapter 2**
III	Bigot et al., 2003; Careghi et al., 2005; Debonne et al., 2010; Geyra et al., 2002; Juul-Madsen et al., 2004; Simon et al., 2014; Simon et al., 2015; Wang et al., 2014

Unclear aspects about the experimental design used often arise from the wording used in the Materials and Methods section of papers that is either inconclusive or not specific. For example, whereas in Designs II and III the moment of hatch is defined as the moment of hatch within the hatch-window (having monitored the spread of hatch), in some studies the moment of hatch is referred to as the moment of pulling from the incubator (Gonzales et al., 2003). Also, terminology is inconsistent between studies. An example of this is the use of wording like *"day old chickens"*, *"newly hatched chickens"*, or *"freshly hatched chickens"*, or chickens that were placed *"immediately after hatch"*, therewith probably all meaning to say *"chickens at pulling"*. However, it remains unclear when these chickens were pulled from the incubator and how old they are from a biological or chronological age perspective.

Improved reporting and the use of more standardized terminology will make it easier to compare different studies in the same research area, and it will improve the overall quality of the research that is being conducted.

The effect of delayed feed access after hatch may also be confounded with other factors, such as housing and climate conditions. In some studies, chickens with delayed feed access up to 48 h remained in shipping boxes until they received feed access (Moran, 1990; Corless and Sell, 1999; Design I). These kinds of experimental designs make it impossible to conclude whether differences in, e.g., body weight losses, are either caused by climate conditions after hatch, as it is known that a lowered environmental temperature during the first days after hatch results in a lowered feed intake (van der Pol et al., 2013), or the effect of delayed feed access after hatch. Other examples of factors that may potentially be confounded with the effects of delayed feed access after hatch (and that are often not reported) include breeder age, incubation conditions, spread of hatch (hatch window), and time between pulling and arrival in the grow-out facility.

It can be concluded that the chosen approach with respect to the effect of direct or delayed feed access after hatch is dependent on the research question to be answered. More importantly, future efforts should be put towards agreeing on standardized terminology related to this kind of research describing the experimental design and methodology. Lastly, care should be taken on how the results are interpreted based on the different designs available.

Physiological consequences of delayed feed access after hatch

Intestinal development

Especially during the first days after hatch, delayed access to feed impairs intestinal development, potentially affecting the chicken's development (see Chapter 1, General Introduction). In Chapter 2, delayed feed access after hatch resulted in a reduced weight to length ratio of the jejunum and ileum at 4 d of age, and for the jejunum this effect remained until 18 d of age. The altered weight to length ratio may be associated with altered villi development, but this was not confirmed by histological analysis. On the contrary, delayed feed access resulted in wider villi at 4 d of age. The delay in feed access in Chapter 2 (13 to 26 h) is relatively short compared to previous studies with delayed feed access for 12 to 48 h that show more profound effects on impaired villi development due to delayed feed access (Noy et al., 2001; Franco et al., 2006; Design II and I). However, it could be that a short duration of delayed feed access, as applied in Chapter 2, might affect intestinal development differently and consequently might have an effect on digestibility and absorption of nutrients. It is known that the duodenum facilitates most of the hydrolyzation of the ingested feed, whereas the jejunum facilitates most of the nutrient absorption. Based on the results in Chapter 2, an increased villi width in the duodenum in delayed fed chickens may be an adaptive reaction of the intestinal tract that tries to increase its hydrolyzation capacity, trying

to anticipate future changes in substrate availability, whereas the jejunum and ileum develop less as there is no stimulus (yet) to increase the absorptive capacity. The phenomenon of villi growth that is stimulated by the availability of nutrients is supported by Moran (1985) and Bohórquez et al. (2011), who suggested that during incubation, it is the amniotic fluid that stimulates villi development, whereas from hatch onwards its exogenous feed.
Limited research has focused on effects of delayed feed access on the digestive capacity of broiler chickens. However, Batal and Parsons (2002a) have shown that delayed feed access for 48h after placement in a grow-out facility (Design I) resulted in impaired amino acid digestion up to 7 d of age (Table 2).

Table 2. Summarized amino acid digestibility coefficients for direct or delayed (48 h; Design I) feed access, derived from Batal and Parsons (2002a).

	Age, d				
Digestibility Coefficient	1-2	4	7	14	21
Average for all AA[1]	0.78	0.77	0.82	0.86	0.88
Average for all AA, 48 h fasting	0.76	0.75	0.79	0.86	0.90

[1]Arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine.

If the digestive capacity of broiler chickens is truly impaired due to delayed feed access after hatch, this could be anticipated by providing dietary ingredients that are more easily digestible at this age. This can be done by, e.g., providing high quality soy products, such as soy protein concentrate or soy protein isolate (Batal and Parsons, 2003). Chapter 3 indicates that intestinal development may also be supported through provision of a fat source that is relatively easy to digest and absorb, such as medium chain fatty acids (MCFA). Regardless of the moment of feed access, the addition of 3% MCFA in the diet during the first week of life resulted in higher BW gain and feed efficiency. However, there was also a tendency for an interaction where especially chickens with delayed feed access (48 h after placement) appeared to benefit from feeding an MCFA blend. The exact underlying mechanism that causes this potential benefit for delayed hatched chickens remains unclear. It may be hypothesized that MCFA affect tight junction permeability of mucosal tissue (Lindmark et al., 1998) or function as an easily accessible energy source due to their high digestibility (Noy and Sklan, 1995).
In conclusion, it appears that short durations of delayed feed access (as applied in Chapter 2) impact intestinal development of young broiler chickens. MCFA can be added to the diet to increase growth performance during the first week of life, especially when fed to broiler chickens with delayed feed access.

Infobox 1. Moment of hatch: more than just a timing difference

In Chapter 2, breast meat yield of broiler chickens at d 18 was higher for early hatched chickens compared with midterm and late hatched chickens (Figure 3). Thus far, differences in carcass development, such as breast meat yield were mainly linked with the effect of feed access in relation to satellite cell proliferation at a young age (Halevy et al., 2000). However, it appears that the moment of hatch is also important.
Various studies have looked into the underlying physiological differences between early, midterm, and late hatched chickens within the hatch-window, by determining blood metabolites, such as glucose, lactate, uric acid, as well as thyroid hormones triiodothyronine (T3) and thyroxine (T4) (Van de Ven et al., 2011; Van de Ven et al., 2013; Wang et al., 2014b). None of these studies looked into potential effects on breast meat yield, except for Wang et al. (2014), who found no effect of hatch moment nor feed access on the proportional breast muscle weight at biological age d 2 and 5.

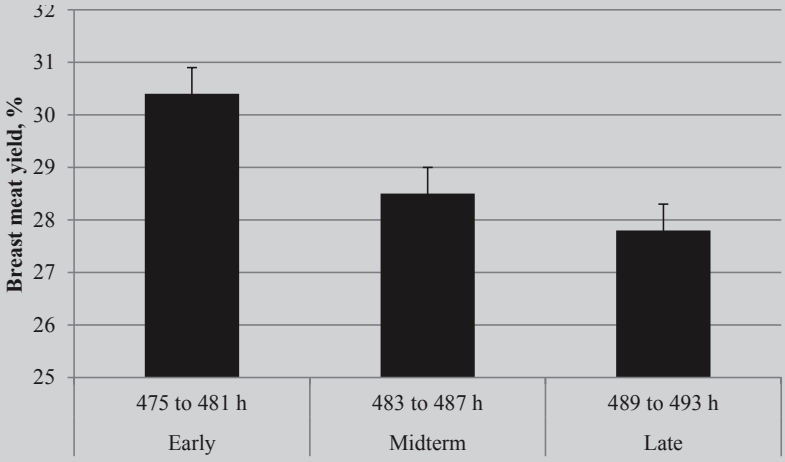

Figure 3. Breast meat yield at 18 d of age of broiler chickens hatched early (475 to 481h), midterm (483 to 487h), or late (489 to 493h after the onset of incubation) within the hatch-window (Chapter 2).

However, Powell et al. (2016a,b) recently showed an effect of moment of hatch on breast meat yield and intramuscular adipose tissue formation at a later age. Early and midterm hatched chickens had a higher relative breast weight compared to late hatched chickens, regardless of having feed access.

Infobox 1. *Continued*

Powell et al. (2016a) hypothesized that satellite cell proliferation is impaired through reduced T3 levels in late compared to early hatched chickens, having a critical role in myoblast cell differentiation.
Powell's hypothesis is in line with observed differences in T3 and T4 expression between early, midterm, and late hatched chickens in previous work (Van de Ven et al., 2011) and requires further validation.

Immune status

In contrast to growth performance and intestinal development, relatively little attention is given to effects of the moment of feed access after hatch and feed composition during the first week of life in relationship to immune status. In Chapter 3, it was examined whether a delayed feed access (48 h; Design I) and the addition of fats to the diet provided during the first week of life would alter the innate and specific humoral immune response of chickens from 0 to 28 d of age. Fish oil, expected to reduce the inflammatory response (Korver and Klasing, 1996), and MCFA, expected to induce an inflammatory response (Lee et al., 2001), were compared to a control diet containing only soybean oil. At different ages (2, 7, 14, 21, 25 and 28 d of age), blood serum was analyzed for Nab IgM and IgY titers against Keyhole Limpet Hemocyanin (KLH), interferon gamma (IFN- γ), and both the classic and alternative complement activity (not at 2 d of age). Total serum antibody titers against sheep red blood cells (SRCB) were only analyzed from immunization at 21 d of age onwards. Neither feed access nor inclusion of different fats affected any of the immune parameters measured from 2 to 21 d of age. Only during the immunization period (d 21 to 28), chickens with direct feed access showed higher IFN-γ levels compared to chickens with delayed feed. It was hypothesized that delayed colonization of the cecum and colon by T cells as a result of delayed feed access, resulted in a lower production of IFN-γ in the gastro-intestinal tract of direct fed chickens at a later age. Based on the results of Chapter 3, the inclusion of fish oil and MCFA at the selected inclusion levels had limited effect on the innate and acquired immune response. However, delayed feed access did have some effects on the immune response, an effect that is also found in the literature.
For example, Bar-Shira et al. (2005) found that the gut associated lymphoid tissue (GALT) activity of broiler chickens with delayed feed access for 72 h (after placement; Design I) was lower in the hindgut up to 14 d of age as a result of rectal immunization with hemocyanin (Hem), whereas GALT activity in the small intestine was not affected after

immunization using bovine serum albumin (BSA). Additionally, delayed feeding resulted in a lowered T lymphocyte distribution in the colon up to 8 d of age and reduced colonization of T and B lymphocytes in the bursa up to 14 d of age. Although it can be questioned whether a 72 h delay in feed access is too long and therefore does not represent current practices in broiler farming, less severe lengths of delayed feed access also affected immune status. Simon et al. (2015) demonstrated that chickens with delayed feed access from hatch onwards (72 h; Design III) had increased antibody titers after immunization with human serum albumin (HuSA) at 28 d of age compared to direct fed chickens. This suggests that delayed fed chickens may potentially be more susceptible to disease pressure at a later age. Juul-Madsen et al. (2004) demonstrated that delayed feeding for 24 or 48 h after hatch (Design III) resulted in higher expression of MHC-II molecules (involved in the presentation of antigens) on the surface of lymphocytes up to 42 d of age compared to direct fed chickens. Furthermore, direct feeding resulted in an increased number of cytotoxic T cells ($CD8^+$) versus T helper cells ($CD4^+$) compared to delayed fed chickens (24 and 48 h). Lastly, and in agreement with Chapter 3, shorter periods of delayed feed access neither affected the levels of infectious bursal disease virus (IBDV) antibodies nor IgM and IgY antibodies in the blood, which represented the innate humoral immune system (Juul-Madsen et al., 2004).

To date, the majority of studies that have assessed the immune response of broiler chickens towards delayed and direct feed access after hatch have used model antigens' such as E. coli lipopolysaccharides, HuSA, SRBC, IBDV, Hem, BSA, and KLH. Although these studies demonstrated that the immune system response is different for broiler chickens with delayed or direct feed access after hatch, no definite conclusions can be drawn if the observed responses are actually positive or negative for the general health status of the chicken. To date, no studies have assessed the effects of feed access after hatch and feed composition using disease models, such as necrotic enteritis or an E. coli infection. The use of disease models may enable us to get better insight if alterations in the immune response due to treatment effects are advantageous or disadvantageous for the chicken.

In conclusion, delayed feed access after hatch results in an altered immune response compared to chickens with direct feed access after hatch. However, for future research it is strongly advised to use disease models, rather than model antigens, to study the effects of moment of feed access after hatch and feed composition in relation to health status of the broiler chicken.

Extended delays in feed access: how long before growth capacity is impaired?

The effects of delayed feed access after hatch on growth and development of broiler chickens are often reported and discussed compared to a control treatment, in which chickens had received direct feed access. Delayed feed access is often reported to result in lower growth and less development of, e.g., the intestinal tract compared to chickens with direct feed access. Although this conclusion is basically correct, it may also be considered inconclusive. It can be hypothesized that delayed feed access only results in a later onset of growth and that these chickens follow a similar growth and development curve compared to direct fed chickens. So, delayed feed intake may delay the onset of growth, but the capacity for growth may not be impaired. However, this may also be dependent on the duration of delayed feed intake.

Generally, the effect of delayed feed access is strongest during the first week of life, and only small delays in feed access after hatch have a significant impact on BW gain. In Chapter 2 (Design III), delayed feed access for 13-16 h resulted in a lowered BW gain until 7 d of age, but this effect disappeared thereafter. Delayed feed access for 24 h (Design III) was only found to result in lowered BW gain until 5 d of age when compared to direct feed access, but not thereafter (Juul-Madsen et al., 2004). In the same study, delayed feed access for 48 h resulted in lowered BW gain until 42 d of age compared to direct feed access, whereas the difference in BW gain due to a delay of 24 h remained only until 28 d of age. Careghi et al. (2005) already reported that a 48 h period of delayed feed access (Design III) resulted in lower absolute and relative growth at d 7 compared to direct fed chickens, but unfortunately these broiler chickens were not followed to a later age. In Chapter 3, it was shown that delayed feed intake (48 h; Design I) resulted in a lowered BW gain of broiler chickens up to 28 d of age, compared to those that received direct feed access.

The above results suggest that the age until such differences in BW gain between direct and delayed fed chickens remain, is highly correlated with the duration of delayed feed access after hatch. A longer delay in feed access results in a prolonged difference in BW between direct and delayed fed broiler chickens. The question that remains is whether the growth capacity of these delayed fed chickens is impaired or just delayed. If the growth capacity is not impaired, the growth curve for delayed and direct fed chickens should be similar. Although the inability for full compensatory growth till 28 d of age (Chapter 3) or 42 d of age (Juul-Madsen et al., 2004) may suggest a more permanent inhibitory effect on growth competence of the chicken, extrapolation of BW data obtained in Chapter 3 suggests otherwise. A polynomial curve was fitted to the growth data of direct fed chickens (Figure 4) and used to extrapolate growth data for delayed fed chickens up to 30 days of age. Compensating for the initial 48 h delay in feed and water access, the predicted BW at 30 days of delayed fed chickens matches the BW of direct fed chickens after hatch at 28 days (1761 vs. 1751 g; Figure 4). As such, the growth curve for direct and 48 h delayed fed

chickens is almost identical. It can be concluded that delayed feed access for 48 h does not impair the chickens' growth competence but only delays onset of growth. The latter is supported by earlier work, where delayed feeding ranging from 15 to 49 h decreased BW at slaughter age but disappeared when chickens were provided the additional time for feed intake that was previously lost due to the delayed feed and water access after hatch (Van de Ven, 2012).

Based on the conclusion that delayed feed access for 48 h after placement only results in a delayed onset for growth, it is of interest to examine if delayed feed access also results in a delayed onset of (digestive) organ development. A study where weights of the jejunum were compared after 2 d of feeding (4 days old delayed fed chickens vs. 2 days old direct fed chickens; Design III) showed that the relative intestinal weights were similar (Bigot et al., 2003), suggesting a similar delay in intestinal development as suggested for growth. However, a less consistent response was found when comparing villi development at 7, 21, and 42 d of age in the duodenum and jejunum when comparing direct with delayed feed access for 12, 24, 34, and 48 h, respectively (Design I; Franco et al., 2006).

Lastly, although it may be concluded that growth capacity is not impaired by delayed feed access up to 48 h, the effect of delayed feed access on health status of the broiler chicken should also be considered (see *Feed access after hatch, Immune status*).

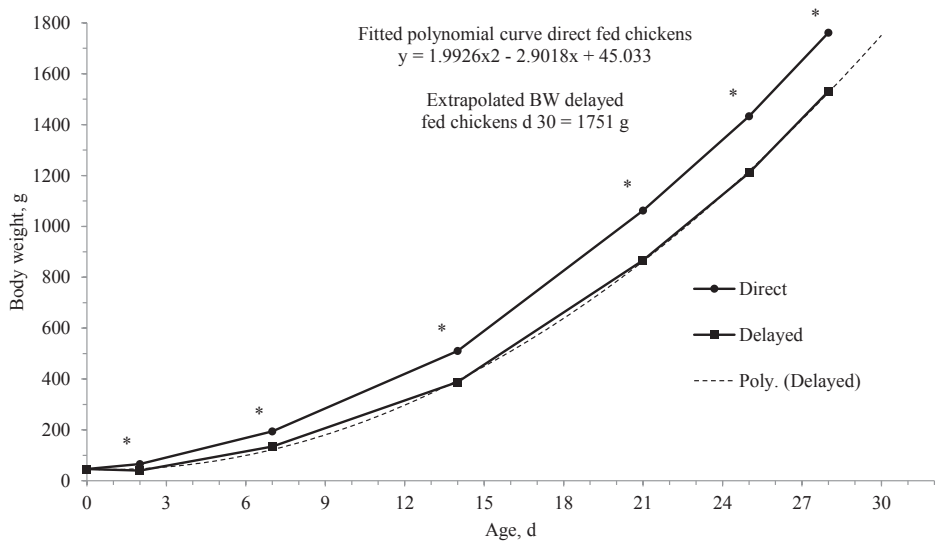

Figure 4 Body weight development from 0 to 28 d of broiler chickens based on moment of feed and water access. Direct vs. delayed (48 h; Design I) fed chickens; data obtained from Chapter 3. Asterisks indicate a significant difference for direct vs. delayed fed chickens for that age ($P \leq 0.05$). A polynomial curve was fitted to the growth data of direct fed chickens and used to extrapolate growth data for delayed fed chickens up to 30 days. The predicted BW at 30 d of delayed fed chickens is 1751 g, whereas BW d 28 of early fed chickens was 1761 g.

In conclusion, a prolonged delay in feed access after hatch results in a lowered growth rate, compared to chickens with direct feed access. Depending on the duration of delayed feed access after hatch, differences in BW between direct and delayed fed chickens persist up to 4-6 weeks of age. A delayed feed intake up to 48 h does not result in impaired growth capacity but rather a delayed onset of growth. However, more work is required to validate whether the growth capacity of these chickens is truly affected and to examine whether similar growth observations can be made for individual organ development.

Conclusions Feed access after hatch

The effect of nutrition was discussed in relation to the moment of first feed intake after hatch. Data from Chapters 2 and 3 were combined with literature and discussed in relation to the experimental setup chosen to study these effects, as well as underlying physiological mechanisms for the effects observed.

The following can be concluded:

- Efforts should be made to standardized terminology related to research around the first week of life.
- Short durations of delayed feed access impact intestinal development of young broiler chickens.
- MCFA can be added to the diet to increase growth performance during the first week of life, especially when fed to broiler chickens with delayed feed access.
- Dietary composition, by means of fish oil and MCFA inclusion in the diet during the first week of life, have limited effect on the innate and acquired immune response.
- The effects of delayed feed access on health remain unclear. It is recommended to select relevant disease models when studying the effects of first week nutrition on the health status of broiler chickens.
- A delay in feed access up to 48 h does not affect the growth capacity of chickens, reflected by a growth curve that is identical to direct fed chickens.
- More work is required to examine if delayed feed access also results in a delayed onset of (digestive) organ development.

DIET DENSITY

In Chapters 4 and 5, effects of diet density during the first week of life on growth performance, organ development, nutrient digestion, and metabolism were examined. Broiler chickens appeared to adapt themselves towards increased diet densities by increasing their intestinal weight and length, while lowering liver and pancreas size to cope with reduced feed intake, but increased nutrient concentration (Chapter 5). Intestinal length

(duodenum, jejunum, ileum, and cecum) increased, coinciding with a higher duodenum weight and a higher weight to length ratio for the ileum and cecum when feeding increased diet densities (Chapter 5). Furthermore, although the gross (GE) and metabolizable energy (ME) intake was not affected, the ME to GE ratio decreased with increased diet densities (Chapter 4). Moreover, heat production and the respiratory exchange ratio decreased as a result of increased diet density, whereas protein and fat retention were not affected by diet density, despite a shift in dietary energy supply from carbohydrates to fat (Chapter 4).
Based on the combined results of Chapters 4 and 5, high density diets, containing increased dietary fat levels at the expense of carbohydrates, did not limit metabolism of broiler chickens during the first week of life. Adaptation by broiler chickens towards these diets can be discussed by differentiating in pre- and post-absorptive nutrient utilization, an approach used by De Lange et al. (2013) as well, to better understand and model nutrient utilization in farm animals.

Pre- and post-absorptive nutrient utilization

Fat oxidation during incubation vs. fat digestion post-hatch

The low fat digestibility during the first week after hatch (see Chapter 1, General Introduction) appears to contradict the metabolic status of the broiler chicken during embryonic development. During embryonic development, the chicken embryo primarily relies on fat oxidation as an energy source for growth and development (De Oliveira et al., 2008). The chick embryo appears to be already capable of breaking down lipids into glycerol and free fatty acids, using lipoproteins (Sunny, 2007), a group of proteins involved in transportation of hydrophobic lipids. The process of lipid uptake through the yolk sac membrane during late incubation is significantly different compared to digestive and absorptive processes in the intestine at a later age (Speake et al., 1998). However, it can be hypothesized that broiler chickens are capable of metabolizing fat during the first week of life once absorbed by the intestine, as they are also capable of doing so during embryonic development.

Pre-absorption adaptation: hydrolyzation vs. absorption

Digestibility is the sum of hydrolyzation and absorptive processes, resulting in a net uptake of nutrients. As discussed in Chapter 1, the General Introduction, hydrolyzation of nutrients depends on enzyme and bile salt secretion. Enzyme secretion in the duodenum (amylase, trypsin and lipase) increases significantly as the chicken ages (Noy and Sklan, 1995), supported by age-dependent activity of amylase, trypsin, lipase, and maltase in the pancreas (Sell et al., 1991). The age-dependent enzyme activity suggests that in particular the hydrolyzation capacity is limiting in young broiler chickens.

In Chapter 5, nitrogen metabolizability and fat digestibility were not affected by diet density. To explain these results, it is hypothesized that broiler chickens during the first week of life either 1) have low but still sufficient enzyme secretion (hydrolyzation capacity) for fat and nitrogen digestion or 2) have adapted their digestive system for increased diet densities using another strategy. Although based on the results of this thesis no inferences can be made about the hydrolyzation capacity, the results suggest that broiler chickens repartition visceral organ development in response to being fed more concentrated diets, at least during the first week of life. Broiler chickens experience increased intestinal size (length and weight) in response to more concentrated diets, which is likely to increase the absorptive capacity of the intestinal tract (Chapter 5).
Larger intestines facilitate an increased villi surface area for absorption of nutrients, as nutrients are absorbed either actively (protein, carbohydrates) or passively (fats and oils) by transport via the enterocytes located in these villi (Denbow, 2015). Furthermore, an extended intestinal tract may affect the transit time of digesta through the gut, allowing the intestine more time to absorb nutrients. Lastly, the uptake of nutrients in the intestinal tract may also be enlarged by increasing the absorptive capacity per defined surface area. In broiler chickens, feeding an increased diet density (+200 kcal ME/kg) from 21 to 42 d of age was found to upregulate various genes involved in phosphate transport in the duodenum at 42 d of age (Li et al., 2016). However, additional research is required to validate similar effects of feeding more concentrated diets on protein and carbohydrate related transporters in broiler chickens.

Post-absorption adaptation
It was already hypothesized that broiler chickens are very well capable of metabolizing more concentrated diets with high fat inclusion levels during the first week of life once absorbed by the intestine, as they are also capable of doing so during embryonic development. This hypothesis is confirmed by the results of Chapter 4, where feeding increased diet densities did not affect protein and fat retention, despite a shift in dietary energy supply from carbohydrates to fat. In Chapter 5, it was found that feeding of increased diet densities resulted in a lowered relative liver size that might be explained by changes in post-absorptive metabolism.
Protein and fat retention in young broilers was not affected by feeding increased diet densities, which can be explained by the maintained energy to amino acid ratio as diet density increased. Young (<14 d of age) broiler chickens have a high capacity for protein deposition, higher than older broiler chickens (Marcato et al., 2008). Feeding increased diet densities, where an increase in dietary fat (energy) level is parallelled by an increase in amino acids, fulfills not only the high demand for protein deposition, it also provides the necessary energy to facilitate this process. It is also hypothesized that as long as the maximum capacity for protein deposition is not reached yet, the dietary energy is primarly

used as an energy source and not stored as body fat reserves. This is confirmed in Chapter 6, where feeding increased diet densities until slaughter (35 d of age) resulted in a very limited increase in relative fat pad weight. Especially in young broiler chickens, it may be the energy supply to support this protein accretion that is limiting rather than that a maximum protein accretion is reached. Finally, as hypothesized in Chapter 5, the reduced liver size as a result of feeding high density diets may be explained by a reduced availability of glucose for storage of glycogen in the liver due to the high energy demand for growth and protein accretion; whereas the kidneys facilitate the process of gluconeogenesis for endogenous glucose.

In conclusion, broiler chickens repartition visceral organ development in response to being fed more concentrated diets during the first week of life, probably to increase the absorptive capacity of the intestinal tract. Feeding more concentrated diets to broiler chickens facilitates the high demand for protein deposition and does not result in chickens with increased fat deposition, as long as the increase in dietary fat (energy) level is parallelled by an increase in amino acids and the maximum protein deposition rate is not reached.

Long-term effects of feeding increased diet densities at young ages

Even though it was shown in Chapters 4 and 5 that broiler chickens are capable of metabolizing high diet densities during the first week of life, it remained unknown to what extent adaptations towards these diets at this age (repartitioning of visceral organ development, see *Pre- and post-absorptive nutrient utilization*), would affect performance at a later age when either continuing to feed increased diet densities or switching from high density diets to a low density diet.

Depending on the duration that high density diets were provided (until 7 or 14 d of age), broiler chickens that switched from a high to a low density diet showed reduced BW compared to broilers that were fed the high density diets till the end of the grower phase (Chapter 6; Figure 1a). Although absolute BW differences remained after switching to a low density diet, the relative growth decreased compared to chickens fed a low density diet during the entire grow-out period. Based on the observed effects on BW and taking the diet physical form into account (see *Diet density, Diet physical form*), it appears logic that the physical feed intake capacity is limiting to allow broiler chickens to compensate for the lower nutrient levels in the low density diets.

In Chapter 6, it was suggested that based on the observed effects in growth performance, the induced physiological adaptations in early life with increased diet density, as observed in Chapter 5, were too small to result in altered growth performance at a later age. Also, these adaptations are also probably less relevant for older broiler chickens that have a more matured intestinal tract with sufficient hydrolyzation capacity. Additionally, based on the calculated ME efficiencies in Chapter 6, without differentiating for maintenance and growth requirements, limited carry-over effects were observed when switching from a high to a

low density diet. However, when calculating the amount of ME available for growth (ME_{growth}) by correcting the total ME intake for the maintenance requirement (ME_m), it was shown that switching from a high to low density diet resulted in a lowered ME_{growth} to ME_m ratio, indicating that the ME_m requirement relatively increased at the expense of ME available for growth. Also, it was shown that the ME efficiency, corrected for ME_m, did not only depend on age and diet density, but also depended on the duration these increased diet densities were provided. The latter is also illustrated in Figure 5, where changing from either a 12.8 or 17.5% dietary fat diet to a 3.5% dietary fat diet at 14 d of age resulted in the highest BW gain per MJ ME_{growth} for the overall experimental period (d 0 to 34) compared to changing on either 7 d of age or when continuing feeding of increased diet densities until 34 d of age.

Data in this thesis suggests that feeding of increased diet densities (≥12.8% dietary fat) until especially 14 d of age did result in higher ME efficiency corrected for ME_m. This may be explained by physiological adaptations towards increased diet densities when fed during early life as observed in Chapter 5. In addition, other physiological adaptations as described in literature, such as altered intestinal development (size, morphology), nutrient absorptive capacity (intestinal nutrient transporters), and other metabolic constituents (Scott, 2002; Wang et al., 2014a; Wang et al., 2015; Li et al., 2016) may occur.

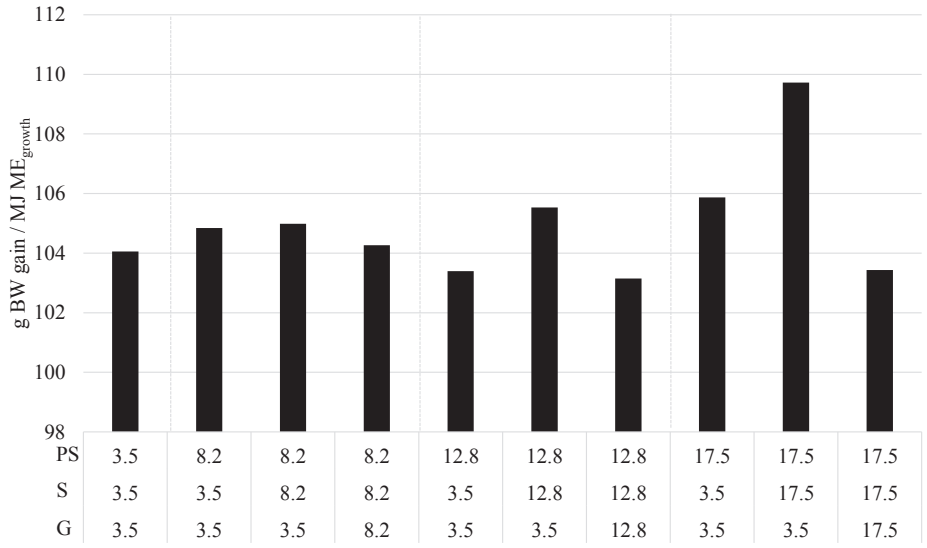

Figure 5. ME efficiency for growth, expressed as grams of BW gain expressed per MJ of ME available for growth (ME_{growth}) from 0 to 34 d of age and when feeding different diet densities. Feeding phases: pre-starter (d 0 to 7, PS), starter (d 7 to 14, S), and grower (d 14 to 34, G) phase. Based on data from Chapter 6.

The improved ME efficiency corrected for ME_m may also be explained by increased protein over fat deposition, as also discussed in Chapter 6. In short, the easiest way for the broiler chicken to maintain its growth despite a reduction in total energy intake is through protein deposition. Based on deposition efficiencies for protein (kp) and fat (kf) of 0.66 and 0.86 (Boekholt et al., 1994) and energy values of 23.6 and 38.2 kJ/g for protein and fat (Larbier and Leclercq, 1994), it can be calculated that 35.8 kJ (1/0.66*23.6) are required to deposit 1g of protein, whereas 44.4 kJ (1/0.86*38.2) are required to deposit 1g of fat, respectively. Although it may appear to be more efficient to deposit fat rather than protein (relatively lower costs for deposition compared to protein), protein deposition is, in contrast to fat, accompanied with water deposition in a ratio of 3 to 1 (Leenstra, 1986). Thus, to maintain growth, it is more efficient to deposit protein. Higher protein deposition likely occurs at the expense of fat deposition, explaining the subsequent lowered relative fat pad weight at slaughter for broiler chickens fed increased diet densities up to 14 d of age.

In conclusion, long-term effects of feeding high density diets up to two weeks of age are limited with respect to growth performance. However, there appears to be a more prolonged effect of feeding increased diet densities on ME efficiency corrected for ME_m, which increased when feeding increased diet densities (≥ 12.8% dietary fat) until 14 d of age.

Diet physical form

In Chapters 4, 5, and 6, the effect of feed composition (with special attention to dietary fat inclusion) during the first week of life was studied with respect to short and long-term effects on growth, organ development, metabolic status, and carcass composition. In Chapters 4 and 5, feeding increased diet densities to broiler chickens during the first week of life resulted in lowered BW gain and feed intake, whereas feeding similar diet densities in Chapter 6 resulted in higher BW gain and maintained or even higher feed intake during this age period. In addition, in Chapters 4 and 5, the ME intake was maintained irrespectively of diet density, whereas in Chapter 6, the feeding of increased diet densities resulted in an increased ME intake, regardless of age.

Differences in growth performance, as well as ME intake may be explained by diet physical form. In Chapters 4 and 5 the diets were pelleted, whereas a mash diet was provided in Chapter 6. In a study by Brickett et al. (2007), broiler chickens were fed low, medium, or high diet densities (+150 kcal per density level; ME levels of low density starter, grower, finisher diet: 2800, 2850, 2900 kcal/kg) either as mash or pelleted diets until 35 d of age. Feeding pelleted diets resulted in similar increases in ME intake when comparing low to medium and medium to high density diets, and feeding mash diets resulted in a much larger increase of ME intake between low and medium density diets compared to medium and high density diets (Figure 6a).

The work of Brickett et al. (2007) clearly demonstrates that broiler chickens are limited by their maximum feed intake capacity, especially when feeding low density mash diets.

A similar response can be observed in Chapter 6 (Figure 6b), where broiler chickens that were continually fed a low density diet (3.5% dietary fat) showed the largest relative difference in ME intake compared to more concentrated diets, most likely because their physical intake capacity is limited. If the study in Chapter 6 were repeated with feeding pelleted diets, differences in BW gain and ME efficiency between diet densities would probably be reduced. Interestingly, feed intake with mash diets was neither affected in Chapter 6, nor in the work of Brickett et al. (2007). However, it has already been suggested that an increased diet density of mash diets only reduces ADFI if the difference in diet density is large (>1,000 kcal AME/kg) enough (Jackson et al., 1982a; b). Another reason why feed intake in Chapter 6 was not decreased, despite an increase in diet density, might be related to the dietary fat level that resulted in increased 'stickiness' of the diet, which might have improved the palatability of the feed.

In conclusion, the diet physical form appears to have a large impact on the effect of feeding increased diet densities, especially with respect to ME intake. Feeding lower density mash diets may limit the physical intake capacity, resulting in suboptimal growth performance compared to higher density diets.

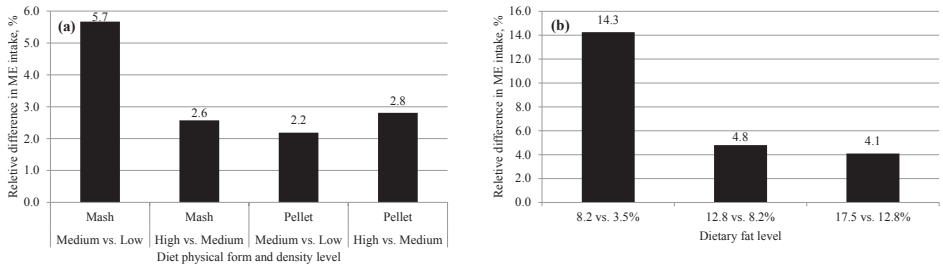

Figure 6. Relative differences in ME intake between treatments **(A)** of broiler chickens from 0 to 35 d of age, fed different diets density levels (+150 kcal per density level; ME levels low density starter, grower, finisher diet: 2,800, 2,850, 2,900 kcal/kg) provided as either mash or pellet (based on data of Brickett et al., 2007). Relative differences in ME intake between treatments **(B)** of broiler chickens from 0 to 34 d of age that were continuously fed 3.5, 8.2, 12.8, or 17.5% dietary fat (+150 kcal per density level; ME levels low density pre-starter, starter, grower diet: 2,908, 2,757, 2,795 kcal/kg; **Chapter 6**).

Conclusions diet density

The effect feed composition (with special attention to dietary fat inclusion) during the first week of life was discussed in relation to short-term physiological development as well as on long-term effects on growth, organ development, metabolic status, and carcass composition. Data from Chapters 4, 5, and 6 were combined with the literature and discussed in relation to the underlying physiological mechanisms for the effects observed (pre- vs. post- absorption) and diet physical form.

The following can be concluded:

- Young broiler chickens appear to repartition visceral organ development in response to more concentrated diets during the first week of life.
- Feeding concentrated diets does not result in increased fat deposition, as long as the increase in dietary fat (energy) level is parallelled by an increase in amino acids.
- Diet physical form appears to influence the effect of feeding increased diet densities, especially with respect to nutrient intake and consequently BW gain.
- Physical feed intake limitations are hypothesized to be the main driver for altered performance when feeding lower diet densities in mash form.
- Feeding high density diets up to two weeks of age results in the following:
  - Limited effects with respect to long-term growth performance (driven by subsequent physical feed intake capacity).
  - Increased ME efficiency for growth, which is hypothesized to be facilitated by a relative higher protein and lower fat deposition.

PRACTICAL RECOMMENDATIONS

Based on results of the current thesis, the following practical recommendations can be made:

- Direct feed access after hatch may be used to initiate BW gain of broiler chickens directly after hatch, resulting in faster onset of growth compared to chickens in traditional incubation and housing systems. An earlier onset of growth, especially when chickens are kept at a different location than the grow-out facility during this period, may shorten the production cycle of broiler farmers, save costs by spending less on climate control (temperature, ventilation) around hatch, and thus result in increased savings.
- Feeding of concentrated mash diets to young broilers will result in higher growth performance. Although feeding increased diet densities during the entire grow-out results in overall best performance, an economical evaluation on the return on investment of feeding denser (and more expensive) diets on broiler performance is required to determine the optimal feeding strategy.

Infobox 2. Respiratory Exchange Ratio: age and diet dependent substrate oxidation.

In Chapter 4, the effects of feeding increased diet densities during the first week of life, coinciding with increased dietary fat levels, on growth performance and metabolism were examined. Although not part of the original study aim, the collected gas flow data (O_2 and CO_2) allows one to observe age-related development of the respiratory exchange ratio (RER) and examine the primary substrate that is being metabolized at a specific age. The respiratory quotient (RQ) for glucose is 1.0, whereas it is approximately 0.7 for fat and 0.8 for protein oxidation (Ferrannini, 1988).

As an average over treatments, the RER at d 0 was 0.75 to 0.80 (Chapter 4). This is somewhat higher than the RER value of 0.71 to 0.72 determined for incubated eggs at embryonic day 18 (Nangsuay et al., 2015), an incubation stage during which the chick primarily relies on lipids in the yolk as an energy source. However, during the final stage of incubation, the RER slightly increases due to glycogenolysis, which will deplete glycogen reserves at the actual moment of hatch (De Oliveira et al., 2008; Van de Ven et al., 2013).

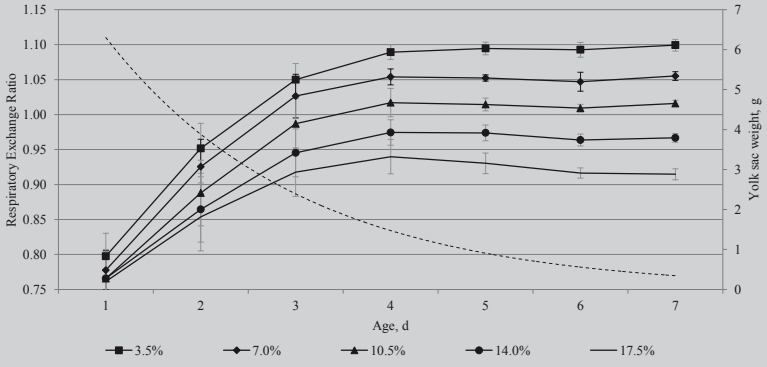

Figure 7. Effect of increased diet density through dietary fat level (3.5, 7.0, 10.5, 14.0, and 17.5%) on the respiratory exchange ratio (ratio of CO_2 produced to O_2 consumed) of broiler chickens of 0 to 7 d (Chapter 4). Exponential curve (---) for weight of the residual yolk based on data from Bigot et al. (2003).

Infobox 2. *Continued*

The residual yolk at hatch, still consisting mainly of fatty acids (Yadgary et al., 2010), continues to be metabolized during the first days after hatch and gradually lowers in weight as the chicken ages (Bigot et al., 2003).
This results in a gradual shift from fat metabolization to a mix of fat, protein, and glucose metabolization due to the ingestion of an exogenous nutrient source.
The RER is also highly affected by dietary composition, as in Chapter 4 it was observed that diets with relatively high fat content (at the expense of carbohydrates) resulted in a lower RER (Figure 7).

CONCLUSIONS

The duration of delayed feed access after hatch is strongly related with the duration that the broiler chicken requires to "catch up" on growth, compared to chickens with direct feed access. However, delayed feed access up to 48 h after placement does not seem to result in an impaired growth capacity of broiler chickens, but rather a delayed onset of growth. MCFA can be added to the diet to increase growth performance during the first week of life, especially when fed to broiler chickens with delayed feed access.
However, dietary composition, by means of fish oil and MCFA inclusion in the diet during the first week of life, was found to have limited effect on the innate and acquired immune response. A prolonged delayed feed access requires a longer grower period and will result in a negative economic value for the farmer.
With respect to the effect of dietary fat in pre-starter diets, it can be concluded that young broiler chickens adapt to high density diets with high fat inclusion levels in the first week of life, enabling them to digest and metabolize these diet types despite a suboptimal capacity for fat digestion. Feeding concentrated diets does not result in increased fat deposition, as long as the increase in dietary fat (energy) level is parallelled by an increase in amino acids. Diet physical form appears to influence the effect of feeding increased diet densities on growth performance and in particular feeding these diets in mash form to young broiler chickens results in improved growth performance.

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Summary

Broiler chickens start their life inside an incubator without any exogenous feed and water access, before being sorted and transported to a grow-out facility. During incubation, important organs, such as the gastro-intestinal tract and the immune system start to develop, a process that continues for several weeks after hatching. Hence, broiler chickens undergo a number of changes during the perinatal period, including the first week of life, both physiological and environmental related. Although broiler chickens have a residual yolk that may provide nutritional support during the first week of life, the period between hatch and first (exogenous) feed intake might have an impact on subsequent growth performance. Furthermore, the physiological status of the chicken at hatch determines the nutritional requirements of the chicken for this age period. Interestingly, that same physiological status also sets boundaries with respect to feed ingredients and nutrients constraints within the diet that the chicken can digest and metabolize.

Immature development of the gastro-intestinal tract is associated with a suboptimal digestive capacity for fat. To anticipate this, young broiler chickens are fed (pre-) starter diets that are primarily based on starch and protein sources that are perceived to be easily digestible at this age. Immature development of the immune system may expose the broiler chicken to a higher risk for diseases and mortality during the first week of life. Limited knowledge is available about how physiological development during the first week of life, reflected by digestive organ and immune status, is affected by feed availability directly after hatch, and macro nutrient composition in complex pre-starter diets. Furthermore, it is not well known how nutrition during the first week of life affects long-term physiological development, growth performance, and health status. Based on these knowledge gaps, the aim of this thesis was to determine the impact of feed availability and feed composition provided during the first week of life on short-term physiological development, as well as potential long-term effects on growth performance of broiler chickens.

Chapter 2 focused on the effect of the spread of hatch and moment of first feed intake on subsequent broiler chicken growth performance till day 18 of age. Additionally, Chapter 3 focused on the effect of the moment of first feed intake and the inclusion of dietary fats on broiler chicken growth and humoral immune status. Chapters 4 and 5 focused on effects of increased diet density on nutrient digestion and metabolism of broiler chickens during the first week of life. This was followed by a grow-out study in Chapter 6, to examine long-term effects of feeding increased diet densities, fed until different ages, on growth performance and carcass composition at slaughter age of broiler chickens.

In traditional hatcheries, the time between hatch and first feed intake is variable (up to 48 or even 72 h) due to differences in the spread of hatch, processing and transportation time. Various studies have looked at the effect of time between hatch and first feed intake on development and growth later in life, but often focused on delayed feed intake in relation to large time intervals (48 to 72 h). Therefore **Chapter 2** examined whether the moment of hatch within a relatively short (24 h) hatch-window and the moment of first feed and

water intake after hatch affected growth, organ development, and carcass composition of broiler chickens until 18 d of age. Newly hatched chickens were pulled from an incubator at three time intervals (475 to 481, 483 to 487, and 489 to 493 h after onset of incubation) and were provided feed and water either directly or delayed (504 h after onset of incubation). The results of this study suggested that especially early hatched chickens benefit from direct feed access compared to midterm and late hatched chickens, as they tended to have a higher body weight gain from 0 to 18 d of age. Furthermore, irrespective of the moment of first feed intake, early hatched chickens were found to be physiologically different compared to midterm and late hatched chickens by means of a higher breast meat yield at 18 d of age. A short (13 to 26 h) delay in feed access after hatching resulted in lowered body weight gain and feed intake compared to direct feed access during the first week of life. In addition, delayed feed access resulted in a lower weight to length ratio of the jejunum and ileum at 4 d of age compared with chickens with direct feed access.

Chapter 2 focused on the moment of hatch and first feed intake on growth performance, organ development, and carcass composition. Therefore, it remained unknown whether diet composition immediately after hatching would affect broiler chicken growth and development during the first week of life. To address this question, **Chapter 3** focused on the effect of the moment of first feed and water intake after hatch, as well as the inclusion of different dietary fat sources in the pre-starter diet (fed from 0 to 7 d of age) on the growth rate and immune function of broiler chickens until 28 d of age. Broiler chickens received either direct or delayed (48 h) feed access after placement in the grow-out facility. Pre-starter treatments consisted of a control diet (with soybean oil), a diet with fish oil (5 g/kg) or a diet with a blend of medium chain fatty acids (MCFA; C10:0 and C12:0; 30 g/kg), followed by a standard starter and grower diet. Immune function was measured through innate and specific humoral immunity, represented by immunoglobulins, interferon gamma, complement activity, and agglutination titres, of which agglutination titres were determined after a challenge with sheep red blood cells at 21 d of age. Results showed that chickens with direct feed access after placement that were fed the control pre-starter diet had a higher risk for mortality than chickens with delayed feed access and fed the control pre-starter diet, with the other treatment groups in between. Although the inclusion of MCFA resulted in higher body weight gain and lowered feed efficiency during the first week of life, this effect was not maintained for the remainder of the grow-out period. For direct fed chickens, the body weight at 28 d of age was highest for the control diet and lowest for the diet with MCFA, whereas this was the opposite in delayed fed chickens. The inclusion of fish oil and MCFA had minor effects on humoral immune function. Until 25 d of age, delayed feed access resulted in lowered body weight gain and feed intake compared to direct feed access, whereas gain to feed ratio was higher. Although results of Chapters 2 and 3 suggested that a delayed feed access after hatch resulted in lower body weight gain during the first week after

hatch and thereafter, it can be disputed whether this is truly an impairment of long-term growth or just a delayed onset of growth.

Chapters 4 and **5** investigated effects of feeding increased diet densities during the first week of life. Despite that embryonic development of broiler chickens largely depends on fat oxidation, it is generally assumed that fats and oils are not well digested and metabolized by young broiler chickens. To validate this, broiler chickens were fed increased diet densities in pelleted form from 0 to 7 d of age. Increased diet densities were obtained by formulating diets with different dietary fat levels (3.5, 7.0, 10.5, 14.0, and 17.5% dietary fat). With an increase in dietary fat level, amino acid levels, mineral levels, and the premix inclusion level were increased as well to preserve a similar ratio between energy, amino acids, minerals, and premix level in the diet. In Chapter 4, the effects of diet density on broiler chicken growth performance as well as energy and nitrogen metabolism were studied. Increased diet densities resulted in decreased body weight gain and feed intake, but increased gain to feed ratio. On the contrary, fat, nitrogen, and gross energy (GE) efficiencies (gain per gram or MJ of nutrient consumed) decreased. Furthermore, the GE and ME intake were not affected, but the ME to GE ratio decreased with increased diet densities, in line with the decreased nutrient efficiencies. Heat production and the respiratory exchange ratio decreased as a result of increased diet density, whereas protein and fat retention were not affected, despite a shift in dietary energy supply from carbohydrates to fat. These results suggested that even though digestibility of fats and oils may be suboptimal in young broiler chickens, protein and fat accretion are not affected by increased diet densities.

The effects of increased diet density on nutrient digestion and digestive organ sizes were studied in Chapter 5. Nitrogen metabolizability and fat digestibility were not affected by diet density, but dry matter metabolizability was decreased as a consequence of increased diet densities. Furthermore, the crop, liver, and pancreas weights (as a percentage of body weight) decreased due to increased diet densities. On the contrary, the length of the entire intestinal tract (duodenum, jejunum, ileum, and cecum) increased, coinciding with an increased duodenum weight and an increased weight to length ratio for the ileum and cecum. These results suggest that increasing diet densities with increased dietary fat levels do not have to result in lowered performance per se, as the broiler chicken appears to repartition visceral organ development in response to more concentrated diets during the first week of life. The results of Chapters 4 and 5 suggest that young broiler chickens can metabolize high density diets with a relatively high dietary fat content and that feeding more concentrated diets to broiler chickens facilitates a high demand for protein deposition. In Chapters 4 and 5, the broiler chickens were grown until 7 d of age. However, consequences of different diet densities on later life performance and development were not investigated. Therefore, in **Chapter 6,** the long-term effects of feeding increased diet densities on broiler chicken growth performance and carcass composition were studied by feeding mash diets until different ages. Diet density levels similar to Chapters 4 and 5 (3.5, 8.2, 12.8, and 17.5%

dietary fat) were fed until different ages (d 7, 14, or 34). Treatments fed different diet densities until d 7 or 14 received a 3.5% dietary fat diet for the remaining period. Regardless of feeding phase, feeding increased diet densities resulted in a higher ME intake, ADG, and gain to feed ratio during the period these diets were provided. Feeding increased diet densities until 7 d of age had no effect on overall (0 to 34 d of age) growth performance, whereas feeding increased diet densities until 14 d of age resulted in increased body weight gain and feed efficiency from 0 to 34 d of age. The relative decrease in body weight gain as a result of switching from a high to a low density diet was higher when feeding increased diet densities until 14 d of age compared to 7 d of age. Metabolizable energy efficiency was only affected from the first week of life onwards. Continued feeding of increased diet densities resulted in higher energy efficiencies, but this effect disappeared when changing to a low density diet. When the metabolizable energy efficiency was corrected for the energy requirement for maintenance, the efficiency increased when high diet density levels (12.8 and 17.5% dietary fat) were followed by a low density diet at both 7 and 14 d of age. Continued feeding of increased diet densities resulted in a higher body weight at slaughter, but a lower breast meat yield, whereas feeding increased diet densities from 0 to 14 d of age resulted in a lower fat pad weight at 34 d of age. Results suggest that feeding increasing diet densities resulted in higher BW gain, G:F ratio and metabolizable energy intake, but mainly during the periods that these diets were provided.

CONCLUSIONS

It can be concluded that short durations of delayed feed access impact the intestinal development of young broiler chickens. Furthermore, MCFA can be added to the diet to increase growth performance during the first week of life, especially when fed to broiler chickens with delayed feed access.

Young broiler chickens are capable of metabolizing and utilizing high density diets through repartitioning the development of visceral organs. High density diets result in higher growth performance, but only for the period these diets are provided.

Acknowledgements

About the author

Training and supervision plan

Colophon

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I would like to thank Cargill for providing me the opportunity that allowed me to work on this PhD. Dave, I am very happy that you recognized the mutual benefit of conducting a PhD for both Cargill and myself. It requires trust and confidence as a company to make such a long-term investment, hoping that things will ultimately work out as intended for all parties involved.

Natuurlijk mogen ook Bas, Henry en Peter niet ontbreken in dit dankwoord. Bas, als hoofd van de leerstoelgroep heb je dagelijks te maken met veel verschillende onderzoeksonderwerpen. Ik heb veel bewondering voor de manier hoe je dit allemaal combineert (naast het aansturen van de groep) en hoe je tijdens onze besprekingen in staat bent geweest om in heel korte tijd de hoofd- en bijzaken te onderscheiden van een onderwerp waar je niet dagelijks mee bezig bent. Al snel heb je door of ergens 'muziek in zit' en klinkt alles ineens heel logisch. Bedankt voor je fijne begeleiding!

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While working on my PhD, I also continued to work as a researcher in the poultry R&D team. More than once this has resulted in a balancing act for me with a thin line between daily work and PhD related work. In any balancing act, there is the risk of loosing balance. And so this has also happened to me. It has forced me to frequently rethink my priorities and it forced me to become more selfish than I sometimes wanted to be. Hereby I like to thank all my colleagues at Cargill, but especially at the Cargill Innovation Center Velddriel for their understanding and patience during this period. In particular (former) colleagues in Velddriel and Elk River from the poultry team have experienced the direct effects of me trying to combine all the work, so thank you Evelien, Irene, Peter, Syrena, Matt, Elham, Lieske, Ahmed, Marijn, Henk, Jeroen, Janet, Twan, Roland, and Rafael.

Het onderzoek dat wordt beschreven in dit proefschrift is uitgevoerd op twee verschillende locaties, namelijk op het proefbedrijf van Cargill in Velddriel en dat van de Wageningen Universiteit in Wageningen. Zoals dat gaat met onderzoek, had ik dit natuurlijk nooit alleen kunnen doen en de inzet van vele personen is onmisbaar gebleken. Het team van dierverzorgers in Velddriel en Wageningen, maar ook iedereen van kantoor en de leerstoelgroep die is bijgesprongen voor het verzamelen van alle data (en soms op de meest onmogelijke tijden!).

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Mijn antwoord?

"A broiler chicken is physiologically more flexible than the fantasy of many nutritionists"

ABOUT THE AUTHOR

David Matthijs Lamot was born in Amstelveen, the Netherlands, on 18 October 1983. He grew up in Amstelveen and moved to Curaçao (Dutch Antilles) at the age of 16. There he graduated from the Radulphus College in 2003. When returning to the Netherlands, David obtained his BSc in Animal Sciences at the Wageningen University. In 2006 he started his MSc Animal Sciences at the same university, with a specialization in 'Animal Health and Behavior'. During his MSc, David conducted his minor thesis at the chair of Quantitative Veterinary Epidemiology, trying to quantify transmission rates of bovine tuberculosis between cattle herds in Ireland based on clustering of outbreaks. This was followed by an overseas internship at the Tokyo University of Agriculture in Japan, where he examined cryopreservation of poultry semen while maintaining optimal fertility rates. For his major thesis at the chair of Adaptation Physiology, David worked on the development of a technique that allowed continuous digital monitoring of angiogenesis in hatching eggs.

After his graduation in 2008, David started working at Provimi as a research nutritionist for pigs and poultry. At the beginning of his working life, a lot of focus was put on the development of nutritional solutions for piglets directly after weaning. As such, young animal nutrition already took a central role at the start of his career. After about two and half years, he started to fully focus on poultry with an emphasis on nutrition for broiler chickens during the first week of life. After the merger of Provimi and Cargill in 2011, David continued to work as a research scientist for poultry at the global R&D department of Cargill Animal Nutrition. In 2012 he got the opportunity to start his PhD on first week nutrition for broiler chickens at the chair of Adaptation Physiology at the Wageningen University, of which the results are presented in the current thesis.
After finalizing his PhD, David will continue to work at the global R&D department of Cargill Animal Nutrition with a strong focus on poultry. Although young animal nutrition remains one of his passions, he continues to expand his nutritional knowledge by working on a wide variety of nutrition related research topics.

PUBLICATIONS

Journal Papers

Lamot D.M., Wijtten P.J.A., van de Linde, I.B., Kemp B., and H. van den Brand. 2016. Diet density provided during different feeding phases: Effects on growth performance and carcass composition of broiler chickens. *Submitted to Poultry Science*

Lamot D.M., Sapkota D., Wijtten P.J.A., van den Anker I., Heetkamp M.J.W., Kemp B., and H. van den Brand. 2016. Diet density during the first week of life: Effects on growth performance, digestive organ weight, and nutrient digestion of broiler chickens. *Submitted to Poultry Science*

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Conference Papers / Presentations

Lamot D.M., Sapkota D., Wijtten P.J.A., van den Anker I., Heetkamp M.J.W., Kemp B., and H. van den Brand. 2016. Effects of increased diet density through increased dietary fat level on growth performance, organ size, and dietary fat metabolizability of broilers during the first week of life. Proceedings of the XXV World's Poultry Congress, Beijing, China. *Accepted for oral presentation.*

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the 5th EAAP International Symposium on Energy and Protein Metabolism and Nutrition, 12-15 September 2016, Krakow, Poland. *Accepted for oral presentation.*

Koedijk R.M., van de Linde I.B., **Lamot D.M.**, Hilbert M., and Enting H. 2015. Antioxidants in broiler breeder diets can affect offspring performance. Proc. 26th Annual Australian Poultry Science Symposium, Sydney, New South Wales, Australia. *Accepted for oral presentation.*

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Lamot D.M., Van der Klein S.A.S., Wijtten P.J.A., Kemp B., Van den brand H., and A. Lammers. 2014. Does nutritional immunomodulation during the first week after hatch affect the immune status of broiler chickens in later life. Proc. XIII Avian Immunology Research Group Meeting, Guelph, ON, Canada, Guelph, ON, Canada. *Accepted for oral presentation.*

Molenaar R., Gooding T., **Lamot D.M.**, Wijtten P.J.A., Van der Pol C.W., Maatjens C.M., and I.A.M. van Roovert-Reijrink. 2014. Incubation and brooding conditions essential for the optimisation of neonatal nutrition. Proc. 25th Annual Australian Poultry Science Symposium, Sydney, New South Wales, Australia. *Accepted for oral presentation.*

Lamot D.M., van de Linde I.B., Molenaar R., van der Pol C.W., Maatjens C.M., Wijtten P.J.A., Kemp B. and H. van den Brand. 2013. The effect of hatch moment and moment of feed access after hatch on broiler development. Proc. 38th Animal Nutrition Research Forum, Roeselare, Belgium. *Accepted for oral presentation.*

Lamot D.M., Van de Linde I.B., Molenaar R., Van der Pol C.W., Maatjens C.M. and P.J.A. Wijtten. 2012. The effect of hatch window and early feed access on broiler development. Proc. World Poultry Congress 2012, Salvador de Bahia, Brazil. *Accepted for poster presentation.*

Van de Linde I.B., **Lamot D.M**., Molenaar R., Van der Pol C.W., and P.J.A. Wijtten. 2012. The effect of hatch window on broiler quality and yolk utilization with and without feed access. Proc. World Poultry Congress 2012, Salvador de Bahia, Brazil. *Accepted for poster presentation.*

Wijtten P.J.A., **Lamot D.M.**, Hangoor E., Greijmans J., Koedijk R.M., and J.K.W.M. Sparla. 2012. Differential effects of dietary protein quality and ideal protein level from 0 to 3 days versus 4 to 14 days of age on performance of male broilers. Proc. 3rd Mediterranean Poultry Summit. 6th International Poultry Conference of the Egyptian Poultry Science Association, Alexandria, Egypt. *Accepted for oral presentation.*

Prüst, H.G., Hangoor E., Brock F., Pearce M. and **D.M. Lamot**. 2010. A comparison of carcass characteristics of entire boars, boars vaccinated with Improvac™, barrows and gilts fattened on low and high energy diets. 21st International Pig Veterinary Society Congress, Vancouver, Canada.

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Others

Lamot D.M., Van de Linde I.B., Molenaar R., Van der Pol C.W., Maatjens C.M., and P.J.A. Wijtten. 2012. Investigación - ficha nº 1.028 - efectos de la ventana de nacimientos y el acceso precoz al pienso sobre el desarrollo de los broilers. Pages 21-21 in Selecciones avícolas.

Koedijk R.M., van de Linde I.B., **Lamot D.M.**, Hilbert M., and H. Enting. 2016. Antioxidants in broiler breeder diets can affect offspring performance. Pages 50-52 in AFMA MATRIX October 2016, magazine of the Animal Feed Manufacturers Association of South Africa.

TRAINING AND SUPERVISION PLAN OF GRADUATE SCHOOL WIAS

DESCRIPTION	YEAR
Education And Training	
The Basic Package (3.00 ECTS)	
WIAS Introduction course	2012
Course on philosophy of science and/or ethics	2012
SCIENTIFIC EXPOSURE	
International conferences (5.10 ECTS)	
24th World Poultry Congress, Salvador Bahia, Brazil	2012
XIII Avian Immunology Research Group Meeting, Guelph, Canada	2014
26th Australian Poultry Science Symposium, Sydney, Australia	2015
5th EAAP ISEP, Krakow, Poland	2016
Seminars and workshops (2.55 ECTS)	
5th Workshop on Fundamental Physiology and Perinatal Development in Poultry (PDP), Wageningen, the Netherlands	2011
Developments in Phosphorus Nutrition in Pigs and Poultry, Wageningen, the Netherlands	2012
38th Animal Nutrition and Research Forum, Roeselare, Belgium	2013
Symposium "Innate immunity and infection", Wageningen, the Netherlands	2013
39th Animal Nutrition and Research Forum, Utrecht, the Netherlands	2014
Immunoforce Symposium, Veenendaal, the Netherlands	2016
Presentations (5.00 ECTS)	
The effect of hatch window and early feed access on broiler development. Poster. *Presented at the 24th World's Poultry Congress, Salvador, Brazil.*	2012
The effect of hatch moment and moment of feed access after hatch on broiler development. *Presented at the 38th Animal Nutrition and Resarch Forum, Roeselare, Belgium.*	2013
Does nutritional immunomodulation during the first week after hatch affect the immune status of broiler chickens in later life? *Presented at the XIII Avian Immunology Research Group Meeting, Guelph, Canada.*	2014
The effect of direct feed access after hatch and diet type on broiler chicken growth performance. *Presented at the 26th Annual Australian Poultry Science Symposium, Sydney, Australia.*	2015

Effects of increased diet density through increased dietary fat level on energy balance characteristics of broilers during the first week of life. *Presented at the 5th EAAP ISEP, Krakow, Poland.*	2016

In-Depth Studies

Disciplinary and interdisciplinary courses (2.90 ECTS)

Epigenesis & Epigenetics (VLAG course), Wageningen, the Netherlands.	2014
Gut health in pigs and poultry, the influence of nutrition and immunology (WBS course), Wageningen, the Netherlands.	2014
Energy metabolism and body composition in nutrition and health research (VLAG course), Wageningen, the Netherlands.	2016
Indirect calorimetry (WUR / INRA), Krakow, Poland.	2016

Advanced statistics courses (3.50 ECTS)

Applied statistics (WBS course), Wageningen, the Netherlands	2012
Cargill in-house course Mixed Models, Velddriel, the Netherlands	2012-2013

Statutory Courses (0.00 ECTS)

Use of Laboratory Animals *(conducted during MSc Animal Sciences)*	2007

Professional Skills Support Courses (6.60 ECTS)

Writing for academic publication, Wageningen, the Netherlands	2012
Provimi Potential Managers Training, various locations across Europe	2012-2013

Research Skills Training (6.00 ECTS)

Preparing own PhD research proposal	2012

Didactic Skills Training

Supervising Theses (9.00 ECTS)

Supervising 4 MSc theses (4 x 2.0 ECTS)	2012-2016
Supervising 1 BSc thesis (1 x 1.0 ECTS)	2013

Education and Training Total	**43.65 ECTS**[1]

[1]1 ECTS credit equals a study load of approximately 28 hours

Colophon

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