MULTIPHASIC GROWTH IN THE LAYER PULLET

- EFFECTS OF NUTRIENT RESTRICTIONS DURING REARING -

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MULTIPHASIC GROWTH IN THE LAYER PULLET

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René P. Kwakkel

Proefschrift

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Traditionally, layer pullets are reared on a least-cost basis. Feeding regimens, that allow pullets to consume a restricted amount of nutrients, have been designed to control body growth towards a 'target' weight and age. It was questioned whether the pattern of body growth in relation to the rate of development of particular organs during rearing interferes with the productive potential of the young hen. For the present study, it was hypothesized that the supply of nutrients for some organs may be critical at certain ages, as a result of their individual growth patterns. In this thesis, growth and development of the pullet body and its constituents has been studied by means of multiphasic growth functions. It was found that body weight at end of rearing ('target weight') is less important than type (which nutrient?) and phase (what age?) of restriction in determining egg performance. Furthermore, a pronounced growth spurt in the body growth curve was distinguished at around 19 wk of age ('the maturity growth spurt'), that appeared to be related to both the development of the reproductive organs and the onset of lay. The assessment of this growth spurt in a flock may help the producer in taking nutritional decisions. A certain amount of fat-free tissue in the body is suggested to be critical for the initiation of sexual growth. Fat growth at early rearing seemed to be functionally related to growth of the fat-free body: pullets on a low-lysine diet did not increase their fat-to-protein ratio at that stage of development. Fat growth at late rearing is stored as an energy buffer (abdominal fat pad). The composition of the fat-free body was not affected by dietary treatment. It was concluded that the fat-free body of pullets is a better measure of physiological age than body weight. Effects of nutrient restrictions on growth of body constituents should be presented relative to the fat-free body. Multiphasic analyses of pullet growth quantified some growth relationships between body components which had not been revealed if a simple monophasic growth approach had been used.
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NN08201, 1773

STELLINGEN

1. Het lichaamsgewicht aan het einde van de opfokperiode als maat voor de kwaliteit van de jonge leghen is van geringe betekenis.

   Dit proefschrift

2. Het bepalen van de zogenaamde puberteitsgroeispurt bij jonge hennen kan voor de pluimveehouder een waardevol hulpmiddel zijn bij het nemen van een juiste beslissing met betrekking tot de te volgen voerstrategie.

   Dit proefschrift

3. De ontwikkeling van het vetvrije kuikenlichaam bepaalt in belangrijke mate het op gang komen van biologische processen die leiden tot legrijpheid.

   Dit proefschrift

4. Vetgroei tijdens de vroege jeugdfase van opfokkuikens is biologisch gerelateerd aan de groei van het vetvrije lichaam.

   Dit proefschrift

5. De samenstelling van het vetvrije kuikenlichaam wordt door de leeftijd van het kuiken beïnvloed en niet door de voerstrategie.

   Dit proefschrift

6. De door Katanbaf *et al.* (1989) en Yu *et al.* (1992) gevonden effecten van voederbeperking op het relatieve gewicht (t.o.v. lichaamsgewicht) van enkele organen verdwijnen grotendeels wanneer deze gewichten worden uitgedrukt ten opzichte van het vetvrije lichaam.

   Katanbaf, M.N., E.A. Dunnington, and P.B. Siegel, 1989. Restricted feeding in early and late-feathering chickens. 3. Organ size and carcass composition. Poultry Sci. 68:359-368.

   Yu, M.W., F.E. Robinson, and A.R. Robblee, 1992. Effect of feed allowance during rearing and breeding on female broiler breeders. 1. Growth and carcass characteristics. Poultry Sci. 71:1739-1749.

   Dit proefschrift

40951

7. De zowel in de humane als zoötechnische literatuur veelvuldig beschreven relatie tussen vetmassa en puberteit berust volgens Bronson and Manning (1991) op een reeks waarnemingen die een *gevolg* in plaats van een *oorzakelijk* verband weergeven, hetgeen door het onderhavige onderzoek lijkt te worden bevestigd.

   Bronson, F.H., and J.M. Manning, 1991. The energetic regulation of ovulation: a realistic role for body fat (minireview). Biol Reprod 44:945-950.

   Dit proefschrift

8. De Gompertz curve verhult een tweefasisch groeipatroon.

9. De armen zitten niet te wachten op wat wij over hebben, maar op wat zij missen.

   'Andersom', 16e jrg. no. 4, 1993

10. In het streven naar een duurzame samenleving wordt een gebrek aan persoonlijke verantwoordelijkheid nogal eens verward met politieke besluiteloosheid.

11. Het verstrekken van volwaardige, plantaardige voeders aan pluimvee voor de produktie van het 'viergranen-ei' of het 'maiskuiken' (de 'light'-trend) gaat volledig voorbij aan het feit dat deze dieren uitstekend geschikt zijn om hoogwaardige voedingsmiddelen te produceren uit laagwaardige voor de menselijke consumptie ongeschikte grondstoffen.

12. Wie zijn strop niet kan strikken, doet zichzelf de das om.

13. Een beetje geloof doet wonderen.

René P. Kwakkel
Multiphasic growth in the layer pullet. Effects of nutrient restrictions during rearing
Wageningen, 13 mei 1994

Voor Emmy, Hedi en Sanna

VOORWOORD

Het schrijven van een proefschrift, en zeker een die gaat over meerfase groei, is te vergelijken met het maken van een bergtocht: 't is uiterst vermoeiend en kent zowel hoogte- (hè, hè, eindelijk gesubmit...) als dieptepunten (oei, 'major revisions'...). Gelukkig trek je er meestal niet alleen op uit. Een aantal van mijn 'tochtgenoten' wil ik aan u voorstellen.

Allereerst mijn promotor, prof.dr.ir. M.W.A. Verstegen. Martin, ik weet dat ik in bepaalde opzichten een beetje een 'Einzelgänger' ben geweest, maar op de 'kritieke momenten' wist ik van jouw diensten dankbaar gebruik te maken. Dankzij jou vond ik steeds weer de juiste route door soms 'onherbergzaam' gebied. Dr.ir. G. Hof, mijn copromotor, heeft zijn taak van dagelijks begeleider uiterst serieus genomen. Ik kon eigenlijk elk gewenst moment bij je binnen lopen, en deed dit dan ook. 't Is plezierig te weten dat iemand tijd voor je maakt en constructief meedenkt. Ondanks het feit dat ik niet de weelde van een 'echte' begeleidingscommissie heb gekend, was dr.ing. W.J. Koops bereid dit duo, met name in de tweede fase van het onderzoek, aan te vullen. Wiebe, de gesprekken met jou waren vaak zeer verhelderend, maar brachten ook vaak 'nieuw werk' met zich mee. Ik ben deze 'denktank' veel dank verschuldigd.

Een belangrijke schakel in een promotie-onderzoek vormt het team dat de resultaten 'op de werkvloer' verzamelt. De bedrijfsleider van De Haar - Pluimvee, Roel Terluin, heeft samen met Aad Rodenburg en Arie van den Dool een groot deel van de proeven uitstekend begeleid. Aad, het moet gezegd: wat duren die legproeven toch verschrikkelijk lang! Heren, alle drie heel hartelijk bedankt.

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Drie omvangrijke opfok-legproeven betekenen veel werk. Een groot aantal doctoraalstudenten en stagiairs heeft een bijdrage geleverd door in de experimentele fase van het onderzoek te participeren. Het feest der herkenning kan beginnen: Han Laumen, Ron Eek, Dolf de Rouw, Mariëtte de Bruyn, Hans Koole, Bart van den Assum, Dagmar van Rijnberk, Lieuwe Appel, Fred de Koning, Margret Kok, Madelaine van Kraay, Wilfried Zijlmans, Peter Paul Jansen, Herbert Wisselink, Gea Jalving, Jan van Esch, Nancy Nieuwenboom en Nic Langeveld (in min of meer chronologische volgorde).

Geen enkele doctoraalstudent kan ook maar iets aanvangen zonder de bezielende leiding van onze 'supporting supervisor non-ruminants' Tamme Zandstra. Tamme, ik heb me met name het laatste jaar wel eens schuldig gevoeld. Experimenten gaan door en ik had mijn hoofd alleen nog maar bij 'Chapter zus of zo'. Tamme zorgde ervoor dat alles op rolletjes bleef lopen. Maar je bent geen supervisor als je niet wordt bijgestaan door (in de voltooid verleden tijd) Piet Linders en (in de tegenwoordige en toekomende tijd)

Peter van der Togt. Tamme, Piet en Peter: heel erg bedankt.

Ik ben in het rijke bezit van twee paranimfen: Barbara Williams en Bart Ducro. Barbara (kortweg 'Bar') heeft een groot aantal hoofdstukken op de engelse taal gecorrigeerd. For the next month we agreed to have our daily conversations in English, to prepare for 'nasty' questions from external examiners during the defence in May.
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Barbara en Bart, alvast bedankt voor jullie hulp voor, tijdens en na de promotie.

Zonder de collegialiteit van de 'Veevoedingsmensen' lukt zo'n proefschrift nooit. Een aantal collega's heeft mij schromelijk verwend. Bijvoorbeeld Marlou Bosch, m'n buurvrouw, die er meestal voor zorgt dat bij mijn (late) binnenkomst de koffie al ingeschonken staat. Of Josien Bos en Maria Lippelt: "Ik heb nog wat koffie over.... heb je zin....?". Truus mogen we ook niet vergeten. Tussen alle werkzaamheden door vond zij tijd om te 'werken' aan onze vis-relatie (laat ze maar kletsen hoor, Truus). Zonder dat 'lekkerbekkie' was dit proefschrift nooit......, toch?

Niet te verwaarlozen is de invloed van mijn ouders geweest. Zij hebben mij altijd gestimuleerd in datgene wat ik graag wilde bereiken (dierenarts worden). Tijdens de middelbare-schooltijd waren zij zeer belangrijk voor mij en dat is eigenlijk nog steeds zo. Mede aan hen draag ik dit proefschrift op.

Lieve Em, jij bent degene die mij door dik en dun hebt bijgestaan. Naast het dagelijks 'runnen' van ons gezin in deze vrij hectische maanden (jaren...), heb je in de avonduren nog tijd weten te vinden om je secretariële vaardigheden te gebruiken ten behoeve van de lay-out van dit proefschrift.

Hedi kende de regels als ik zat te 'computeren'. Maar als ik weer eens om kwart voor zes opbelde met de mededeling 'dat het wel wat later kon worden', viel dat bij haar niet altijd in goede aarde. Zij beantwoordde het telefoongerinkel rond die tijd dan ook steevast met de opmerking: " Nee hè, daar heb je 'em weer.....". Sanna heeft van dit alles nog weinig gemerkt. Zij bleef optimistisch en toverde dus immer een brede lach op haar gezichtje wanneer ik thuiskwam. Voor m'n drie meiden krijg ik gelukkig weer wat meer tijd.

Het moet gezegd: zoveel hulp en belangstelling bij het vervaardigen van een proefschrift doet een mens goed. Ik heb lang niet iedereen genoemd, ik weet het, maar toch: een ieder bedankt!

En nu snel naar de drukker. Nog even een aantal extra exemplaren van het proefschrift bestellen: de vakgroep verscheurt er immers minstens één tijdens het 'Veevoedings-stukje'.....

René

"An animal is not solely composed of protein, fat, ash, and water;
it is formed by the functional completeness of organs which contain them".

CONTENTS

GENERAL INTRODUCTION

GENERAL INTRODUCTION[1]

Traditionally, egg-type pullets are reared under nutrient restrictive conditions (Scott *et al.*, 1982; Leeson and Summers, 1991). The main reason for this type of feeding management, is the discrepancy between the objectives of the pullet rearer and those of the egg producer (Belyavin, 1993). For any particular flock, the egg producer is generally not the one who has previously reared the birds. This means that the producer usually has little or no information concerning the ecomomic potential of the birds on the rearing farm, and therefore aims for the lowest possible price per bird. This type of organization within the layer industry consequently forces the rearer to produce a young hen on as low a budget as possible. This separation of a hen's lifespan into two unrelated phases, which is based on purely economic motives, is biologically irrelevant: both phases are parts of a continous whole; egg production is the ultimate goal for rearing a layer pullet (Leeson, 1986; Belyavin, 1993).

It is well known that a certain feeding strategy during rearing affects laying performance, particularly through physiological alterations in the growing bird (McCance, 1977; Leeson and Summers, 1980; Wells, 1980). These physiological alterations may involve distinctive and possibly irreversible changes in body growth characteristics during the pre-lay period, as well as alter processes which relate to organ and tissue development (Table 1). It is, however, still unclear in what way body composition, body size and/or rate of development of the reproductive tract during rearing exactly determine the potential for optimal adult performance (Leeson, 1986).

Table 1. Possible mechanisms, through which pullet feeding strategies (input) may affect laying performance (output).

Input	- Restricted energy or protein (amino acids) - Period, Severity and Moment of cessation of restriction
Mechanism	- Critical periods in organ and tissue growth - Alterations in nutrient partitioning - Changes in multiphasic growth patterns and Adult organ weights - Endocrine control of growth processes
Output	- Onset of lay and Rate of lay - Egg size and Egg quality - Feed intake and Adult body growth

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[1] Parts of the 'General Introduction' have been published in *Recent Advances in Animal Nutrition - 1993:* Proceedings of the 27th University of Nottingham Feed Manufacturers Conference, pages 109-129, Sutton Bonington, UK.

PULLET FEEDING STRATEGIES: A MATTER OF CONCERN

A better understanding of the growth processes in pullets is needed as part of the effort to improve the quality (i.e., the potential ability to perform successfully during the laying period) of the young layer and to reduce the proportion of nutritional resources used for its rearing. Pullet growth can be defined in terms of multiphasic growth (Koops, 1989). In this thesis, growth and development of laying-type pullets has been studied by means of two multiphasic growth functions. These mathematical functions were used to describe absolute and relative growth, of the body and its constituents. The interpretation of the results by use of the multiphasic growth approach, may help to explain differences in laying performance as a result of specific nutritional conditions during rearing.

In this thesis, the terms 'feeding strategy' or 'feeding regime' refer to a set of feeding measures (*ad libitum* or restricted feeding level as well as variable dietary compositions) applied to a group of birds of defined chronological age.

During the last 30 years, the modern layer has been characterized by a yearly improvement of egg production (Leeson, 1986). This has been, amongst others, due to a genetic selection for optimum performance: today's young hen matures several weeks earlier than the hen of two decades ago. Onset of lay is now at around 19 wk of age, and the hen reaches peak production a few weeks later (Summers and Leeson, 1983; Summers *et al.*, 1987). Moreover, a high rate of lay and a low adult body weight of the modern hen have improved net performance (Luiting, 1992). The consequence of this, is that pullet feeding strategies have to be reconsidered and evaluated every few years, in order to match feeding strategy to the physiological requirements of the growing pullet (Summers, 1983; Leeson, 1986).

In the next section follows a brief review of twenty years of research on pullet feeding strategies.

RESEARCH ON PULLET FEEDING STRATEGIES: 1970 - 1992

For 30 years, the optimization of nutritional conditions for rearing hens in order to maximize adult laying performance has been a key subject for research (for reviews see Lee *et al.*, 1971; Balnave, 1973; Karunajeewa, 1987).

Lee *et al.* (1971) presented a comprehensive review of the effects of pullet feeding strategies during the sixties. They concluded that, although restricted fed pullets had a delayed onset of lay, egg output had increased, due to a better utilization of nutrients during lay. Moreover, saving of feed costs during rearing was an obvious advantage of restricted feeding (Balnave, 1973).

Target weights

Breeding companies emphasized the importance of achieving a so-called 'target' body weight for a 'ready-to-lay' pullet (Balnave, 1984). The main goal was to achieve these target weights with a minimum of nutritional input. All rearing methods were based on this least-cost principle (Leeson, 1986). Body weight served as a convenient tool for evaluating the rearing period under practical conditions. The rearing period was 'non-profitable' and so nutritional requirements were therefore not properly defined (Leeson and Summers, 1980). Physiological relationships between target weights and egg production features were not clear. Most of the experiments described in the literature were conducted to evaluate each feeding strategy in relation to economic goals (a.o. Mbugua and Cunningham, 1983).

Growth patterns and feeding regimens

Wells (1980) criticized the philosophy of feeding to a particular target weight and stated that "body weight at 18 wk of age is not a reliable indicator of subsequent laying performance, when considered in isolation from the pattern of growth leading to that weight". Undoubtedly, a minimum pre-lay body weight is necessary for onset of lay (Dunnington *et al.*, 1983; Dunnington and Siegel, 1984), but the shape of the body growth curve may give additional information on subsequent performance ability (Leeson and Summers, 1980). From the early 1980's, research on feeding strategies for layer pullets became more focussed on the growth pattern during development, rather than on target weights (Leeson and Summers, 1980; Wells, 1980; Summers, 1986).

Pullet feeding strategies were usually 'step-down protein' programmes. This meant that the protein content of the diet was decreased with age. The programmes were characterized by high protein starter diets which resulted in high growth rates. In order not to reach weights above the target weights, pullets were then restricted in feed intake during the grower period. As a consequence, the growth curve followed pattern A (Figure 1; Summers, 1983). With these regimens, the young hen did not build up any body reserve for the first few weeks of lay when feed intake had not yet matched nutrient requirements. As a result, post-peak production dips and small eggs were a common phenomenon (Summers, 1983; Leeson, 1986).

Leeson and Summers (1979; 1980) stated that on the basis of choice-feeding experiments, the requirements for protein increased with age, and that pullets were fed 'back to front' up to that time. They postulated that the birds would be better fed with a low protein starter and high protein grower diets, a 'step-up' regime, rather than with the conventional step-down diets. Their theory was based on a rather slow rate of muscle growth during the starter period and an increased ovarian and oviduct growth during the grower period, which would stimulate appetite for high protein diets during that period (Leeson and Summers, 1980). The step-up method resulted in a linear growth curve B (Figure 1). This new method saved feed costs during early rearing and delayed sexual maturity. However, it also led to decreased egg sizes and increased mortality during the rearing and laying period. These problems were eradicated by increased protein levels during the first few weeks of rearing (the 'modified' step-up regimens: a.o. Robinson *et al.*, 1986). These modified regimens resulted in normal egg sizes and average mortalities.

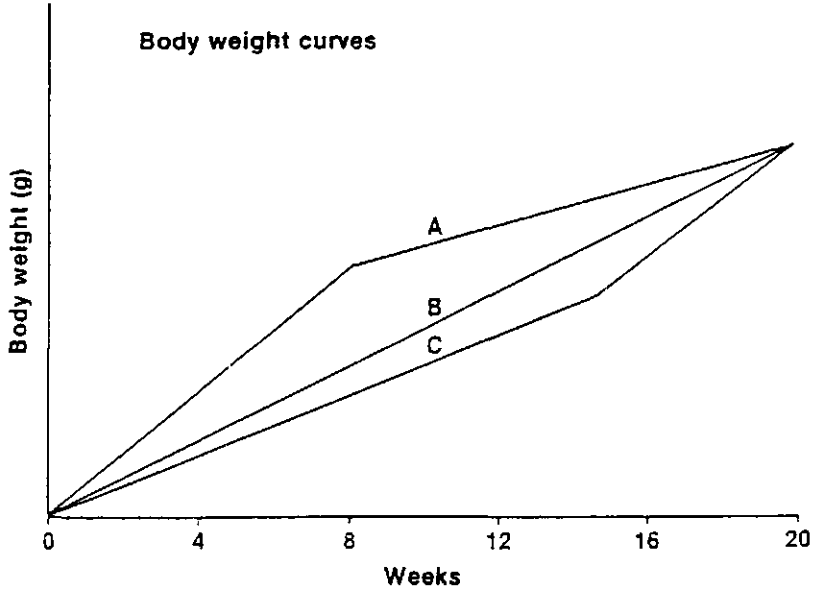

Figure 1. Different growth patterns for pullets as proposed in literature (After Summers, 1983).

Wells (1980), suggested a so-called 'mid-term' feed restriction, from about 7 to 15 wk of age, to be suitable for the layer pullet. This method was based on the idea that one has to restrict the bird's diet during periods of overconsumption and so prevent excessive fat deposition. By allowing the young hen free access to feed three weeks before onset of lay, she presumably could compensate for the previous growth retardation and to adjust appetite to the increased demands (Wells, 1980).

Summers (1983) postulated that the aim of a good rearing programme should be to produce large pullets carrying sufficient reserves of body protein and more especially fat, at the onset of lay. Energy intake had to be stimulated as being the promotor of body weight gain during the grower period (Summers, 1986; Summers *et al.*, 1987). The bird should gain in body weight at a high rate during late rearing (energy as body reserve). A growth reduction during mid-term rearing followed by high density diets during late rearing seemed to be the most appropriate method of promoting feed consumption just before lay (curve C; Figure 1).

An early maturation of the skeletal body (frame size) was considered beneficial by Leeson (1986) and Summers (1986). The basis of their advice was the frequent occurrence of prolapse in young hens at start of lay (Leeson, personal communication). They suggested that feeding regimens should be based on diet/body weight changes instead of diet/age changes. This was a step forward in pullet rearing: dietary changes should take place only if a certain physiological age, in their view defined as body weight, was achieved, rather than if a certain chronological age had been reached.

Basically, this type of 'trial and error' research did not elucidate any of the physiological mechanisms involved in pullet growth and maturation conclusively.

Body composition

Differences in the growth pattern may influence body composition during and at the end of rearing. Experiments have been designed to elucidate the role of body composition in order to explain differences in the onset of lay and overall performance (Brody *et al.*, 1984; Soller et *al.*, 1984; Chi, 1985; Johnson *et al.*, 1985; Leeson, 1986; Zelenka et *al.*, 1986; Summers et *al.*,1987). It was generally agreed that body composition during rearing and at onset of lay could have an important role in the maturation of the young hen. However, the exact causal pathways are not yet understood (Dunnington and Siegel, 1984). Timing of the onset of lay may be determined by a number of interrelated factors such as age, body weight, body fat and/or fat free tissue (both in relative and absolute amounts), body size, and genetic strain (Brody *et al.*, 1980; 1984; Dunnington *et al.*, 1983; Dunnington and Siegel, 1984; Soller *et al.*, 1984; Summers *et al.*, 1987; Zelenka *et al.*, 1986). Age and body weight, in particular, were thought to be important as threshold factors for onset of lay (Dunnington *et al.*, 1983; Dunnington and Siegel, 1984). Work on broiler breeders showed that a specific percentage of body fat seemed to be required for onset of lay

(Brody *et al.*, 1980, 1984; Zelenka *et al.*, 1986). Soller *et al.* (1984) confirmed these results, but they also concluded that a certain lean body mass was required.

In agreement with Summers et *al.* (1987), most researchers concluded from their experiments that "a profitable young hen must probably attain a minimum body weight in combination with a 'particular' body composition in order to initiate egg production" (Brody et *al.*, 1984; Soller et *al.*, 1984; Zelenka et *al.*, 1986).

Other proposed mechanisms

Hollands and Gowe (1961) suggested that a mild stress, as a consequence of a feed restriction during rearing, could have stimulated the development of endocrine glands, and so result in higher rates of lay.

According to this theory, an altered gonadotrophin output or an increased sensitivity of the ovary and oviduct to gonadotrophins should lead to larger oviducts, faster rates of follicular growth, and improved production by restricted birds (Frankham and Doornenbal, 1970; Watson, 1975).

Johnson *et al.* (1984) concluded that the increased feed or energy intake after cessation of the restriction could play an important role in obtaining increased egg size.

Hocking (1987) and Hocking *et al.* (1989) studied the physiology of reproduction in the young layer. They examined the hierarchical structure of follicular development in the ovaries of rearing hens fed different feeding levels. They found a high incidence of shell-less and broken eggs in birds that had been fed *ad libitum* during the pre-lay period. This lead to an irregular egg production. On the basis of these results, they postulated that this unsatisfactory performance could be explained by the large amount of 'ready' yellow follicles, inducing multiple and internal ovulations. Hocking and colleagues therefore suggested a feeding programme with a moderate restriction until the onset of lay.

Discrepancies in the literature

Results in the literature which describe egg performance as a result of particular feeding strategies, are hard to compare and sometimes even conflicting (Lee, 1987). The variation in conclusions are the result of differences in body weight at the onset of lay, the method of restriction (Wells, 1980), the severity of the restriction (Balnave, 1984; Lee, 1987), the start, period and cessation of restriction (Johnson et *al.*, 1984), the levels of essential amino acids in low protein diets (Balnave, 1973), management (uniformity, feeding space allowance: Robinson and Sheridan (1982)), and genetic strain (Abu-Serewa, 1979; Lee, 1987).

A NEW APPROACH: MULTIPHASIC GROWTH ANALYSES

Three basic questions concerning the effects of pullet feeding strategies remain:

1. At what physiological stage(s) of development do organs and tissues receive signals which result in irreversible preparations, a kind of "setting", for subsequent egg production ?
2. Which system (e.g. endocrine or nervous) controls this 'setting' of the body ?
3. What is the relation between a certain feeding strategy and the physiological alterations related to this setting for egg production ?
   In other words: in what way, to what extent, and during which period are nutrient restrictions appropriate ?

The answers to these questions need a biological approach.

Critical periods

If nutrient supply is not limited, each body organ or tissue will follow its own distinctive maturation curve (Ricklefs, 1975). As a consequence, there will be a variation in the nutritional demand of every organ or tissue in the course of time, related to the development of that specific body structure (Ricklefs, 1985). This means that the supply of nutrients for certain organs may be critical at particular stages of immature growth (McCance, 1977). Ignoring such critical periods may negatively influence subsequent performance. An overall moderate feed restriction for layer pullets during the entire rearing period is common practice in the Netherlands. However, it does not take into account the existence of such critical periods.

Nutritional programmes for pullets should be adjusted to take into account the stages of development of important body structures (e.g., the ovary and oviduct for the layer: Hurwitz and Plavnik, 1989). For example, results of multiple regression analysis indicated that the magnitude of the influence of oviduct weight on egg weight is almost three times larger than the influence of body weight on egg weight (Elahi and Horst, 1985). Critical periods need to be identified, and, as a consequence, nutrient restrictions at these stages avoided.

Monophasic versus multiphasic growth

Growth can be considered biologically as being a discontinuous process. The Count de Montbeillard was one of the first who perceived the multiphasic nature of growth. He described the adolescent growth spurt of his son by collecting his son's height data from birth in 1759 till the age of 18 years in 1777 on a yearly basis (Short, 1980; Koops, 1989).

The assumption of the existence of several distinguishable growth 'waves' (Hammond, 1932), which is the basis of the multiphasic growth theory, is widely accepted and has been incorporated into human medical research for a long time.

In contrast, growth in farm animals (e.g. in poultry) has usually been described by the well-known monophasic sigmoidal (S-) curve, assuming only one inflection point, in which growth rate is at a maximum. The main reason that the multiphasic approach has not been used widely by animal researchers is a lack of detailed observations over time, a prerequisite for the assessment of multiphasic growth (Koops, 1989). An example of a diphasic growth function is presented in Figure 2.

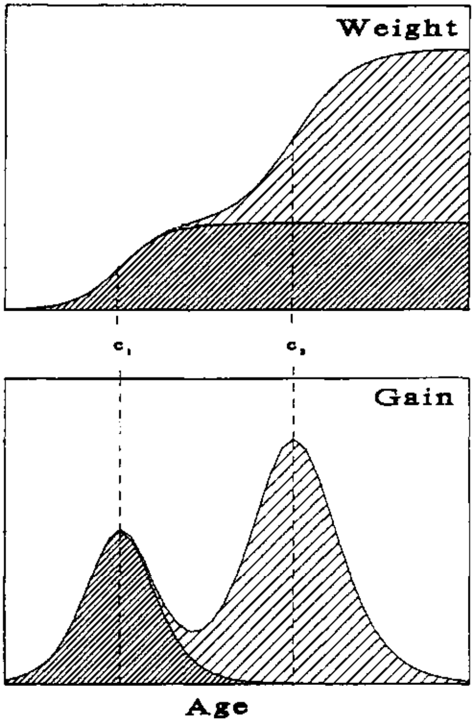

Figure 2.
An example of a diphasic weight-age (upper) and gain-age (lower) curve. C_1 and C_2 are *points of inflection* (ages at maximum gain) for the first and the second phase, respectively (After Koops and Grossman, 1991).

Multiphasic growth: two mathematical functions

The mathematical functions that are presented in this thesis are used to distinguish stages of growth and development of the total body or particular body constituents (organs and tissues). Both functions were developed at the Wageningen Agricultural University by Koops (1989) and Koops and Grossman (1993).

The Multiphasic Growth Function (MGF) is based on a summation of 'n' different and partly overlapping sigmoidal curves. Each curve or growth cycle is described by a logistic function. The curve represents growth data as a function of age. The MGF equation defines the number of phases, growth within phases, age at maximum gain in each growth phase, and the duration of each phase.

The second model, the Multiphasic Allometric Function (MAF), was applied in this study to compare relative growth of body constituents in pullets which had been reared using different feeding strategies. The MAF equation is an extension of the simple allometric model of Huxley (1932). The Huxley model ($y=\alpha . x^{\beta}$) is often used in studies on relative growth (Ricklefs, 1975; Lilja *et al.*, 1985). It incorporates the *allometric growth coefficient ß*, a parameter which is easy to compare between treatments. The description of changes in the relationship between two body structures with advancing age or stage of development is not possible with the simple model. However, this can be done with the multiphasic growth functions. Moreover, the MAF function allows a smooth transition from one allometric level to another.

SCOPE OF THE STUDY

Pullet growth and development has previously been studied mainly according to the pattern of body growth in combination with body weight at a particular age as the 'setpoint' for the onset of lay. It is hypothesized here, that changes in body composition during rearing, which are due to the provision of insufficient nutrients during certain critical periods in the growth of particular organs, may irreversibly affect adult layer performance.

The objectives of the present study were:

1. To investigate the effects of type, period and severity of nutrient restrictions on pullet growth and subsequent laying performance.
2. To validate the presumed key role of body composition (tissues and organs) as being more relevant than body weight in determining the onset of lay and adult egg-producing abilities.
3. To define the empirical relationships between body components and organ systems that quantify biological principles of pullet growth, thus providing a mechanistic framework.

These aspects were studied by means of applying multiphasic growth analyses. The answers may serve as a step forward in elucidating the causal relationships between immature growth and mature performance in the layer.

The aim of this study was to give starting-points for a feeding strategy in which a pullet's nutritional status throughout rearing is attuned its physiological requirements for subsequent egg production. The focus for the nutritionists of today is clear. It must be to adjust the feeding programmes for rearing hens to the processes of multiphasic growth in order to find a balance between the competing demands for nutrient resources and an undisturbed biological development of the fowl.

OUTLINE OF THE THESIS

This thesis describes the results of three large-scale experiments in which young layer pullets were subjected to different feeding regimens. These regimens were mainly based on quantitative (daily amount of feed restricted) and qualitative (restricted amount of lysine in the diet) restriction methods. All experiments were carried out with a commercial type of the White Leghorn breed.

Chapter 1 describes a pilot experiment. In this experiment, three regular methods of nutrient restriction were compared to investigate changes in body weight gain and/or feed intake during rearing as a consequence of the method of restriction. Moreover, the possibilities of the pullet to compensate for body weight losses before the start of lay were studied. The birds were not examined during the laying period.

In the second experiment, different levels of severity of a lysine and feed restriction were investigated to verify critical levels with respect to compensatory growth and subsequent productive performance (Chapter 2).

Based on the results of the second experiment, a third factorial experiment was designed to study method and phase of restriction as independent effects. Pullets were reared on two different methods of restriction during early or late rearing. Both groups were pair-gained, i.e. a similar weekly body weight gain in each group was pursued. Effects of body weight *per se*, one of the major problems in evaluating results in the literature, were excluded in this way. The results on body weight gain, feed intake and egg performance are presented and discussed in Chapter 3.

In Chapter 4, the multiphasic growth approach is introduced. Two multiphasic functions are presented. The multiphasic growth function describes the relationship between growth and age, whereas the multiphasic allometric function describes the relative growth relationships between body constituents.

Throughout the rearing and early laying period, birds of the third experiment were killed for dissection purposes. Chemical body compositions and organ weights were determined (Chapters 5, 6, and 7).

Factors which determine for the onset of lay are a matter of debate in the literature. Some critical body factors for initiating maturation processes to induce subsequent egg production are proposed and discussed in Chapter 5.

Relative changes in body composition within the whole and the fat-free body are related to processes of aging and the effects of feeding strategies (Chapter 6). Functional pullet growth is defined.

In Chapter 7, changes in the relative growth, and moments of individual maturation were investigated in some skeletal bones, the organs associated with feed processing and digestion and some organs related to reproductive processes. Comparisons between treatments were made.

In the General Discussion the results reported in the Chapters 1-7 are discussed and evaluated with respect to theories on post-hatch growth and nutrition. Prospects for further research are suggested. Practical implications to define a pullet feeding strategy based on this study are presented.

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Chapter 1

EFFECT OF LYSINE INTAKE AND FEEDING LEVEL DURING REARING ON GROWTH PERFORMANCE OF LAYING-TYPE PULLETS

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ABSTRACT

Effects of a conventional step-down lysine regime (0-18 wk of age), a step-up lysine regime (0-18 wk of age) and a quantitative 'mid-term' restriction (7-15 wk of age) on weight development and feed consumption in 640 laying-type pullets were studied. Digestible lysine levels of the diet varied according to the experimental regime between .57 and .85% during the rearing phases. Applied 'mid-term' restriction was 85% of *ad libitum*. Whereas no differences in body weight at 18 wk of age could be demonstrated between the feeding regimens, some regimens decreased feed consumption during rearing to a maximum of 7% due to the changed pattern of weight development.

Keywords: lysine, body weight development, step-up, restricted feeding, growth, laying-type pullets.

INTRODUCTION

Over the last few years, the laying performance of leghorn strains used for egg production has been changed genetically (Leeson, 1986). Therefore it is necessary to revaluate the feeding programmes during rearing on their suitability for the modern hen.

The weight of a hen at 18 wk of age is thought to be a reliable parameter to predict subsequent laying performance (Wells, 1980). Achieving this weight through a conventional step-down feeding programme (Scott *et al.*, 1982) is a good insurance to obtain a profitable hen. Summers and Leeson (1978) assumed from their experiments on diet self-selection that the nutritional demands of a young pullet reflect its physiological requirements. They promoted the use of a 'reserve protein' feeding strategy during rearing; this is referred to as the step-up method.With this method a more 'optimum' development of the hen's body size, in addition to its target weight, can be achieved (Leeson and Summers, 1979). On the other hand, Wells (1980) claimed that a 'mid-term' feed restriction is an 'economic' method for rearing the modern pullet, that still achieves the desired production goals.

These considerations led to this study of the effect of step-down, step-up or 'mid-term' restricted feeding strategies on body weight development, feed consumption and feed conversation ratio (FCR) of growing pullets. According to data of Scott *et al.* (1982), lysine was the first limiting amino acid used in the experimental diets. Therefore lysine was used as 'growth'-controlling factor in the step-wise rearing treatments.

MATERIAL AND METHODS

A total of 640 one-day-old White Leghorn pullets were housed during rearing in battery cages (16 pullets per cage) and submitted randomly to 8 treatments (Table 1). Pullets of Treatments 1 and 2 were subjected to a step-down lysine programme; those of Treatments 3 and 4 were reared on a step-up lysine programme. As stated in Table 1, pullets of Treatments 1-4 were fed *ad libitum* (AL). Pullets of Treatments 5-8 were fed a .85% digestible lysine starter diet from 0-6 wk of age, followed by a 'mid-term' restricted feed intake of a normal grower diet (R: 85% of AL) from 7-15 wk of age.

Table 1. Digestible lysine content of the experimental diets.

Treatment[1]	*Age (wk)* 0-6	 7-15	 16-18
Step-down (AL)[2]			
1	.85	.67	.57
2	.85	.67	.67
Step-up (AL)			
3	.65	.67	.77
4	.65	.67	.67
Mid-term restriction	*AL*	*R*	*AL*
5	.85	.79	.77
6	.85	.79	.67
7	.85	.67	.77
8	.85	.67	.67

[1] Each treatment consisted of 5 replicates.

[2] AL = ad libitum feeding level; R = restricted: 85% of AL.

Table 1 shows the digestible lysine contents of the experimental diets. Lysine levels of the starter (0-6 wk: 19.6% crude protein (CP), 11.7 MJ ME kg^{-1}) and grower (7-18 wk: 17.3% CP, 11.7 MJ ME kg^{-1}) diets were increased by addition of synthetic lysine to a basal corn-soybean meal diet. The amount of lysine added was dependent on the respective treatment. Half of the grower diets of the pulllets fed on a restricted feed intake were upgraded (15%) with digestible lysine. The body weight and feed consumption of the pullets were recorded weekly per cage. From these data FCRs were calculated.

RESULTS AND DISCUSSION

The results of the experiment are presented in Table 2. Pullets (0-6 wk of age) fed a diet with .65% digestible lysine (Treatments 3 and 4) had, on average, a 20 g lower body weight at 6 wk of age than pullets fed a diet with .85% digestible lysine (Treatments 1 and 2). However this difference was not significant ($P>.05$). Feed intake within this period did not significantly differ among treatments, although the feed conversion ratio (FCR) was higher for pullets fed on the low-lysine diet. No difference in feed intake was observed between pullets fed on step-down and step-up regimens in the period from 7-15 wk of age. Pullets subjected to the step-up regimens obviously converted their feed more efficiently during this period than the pullets fed on step-down regimens (Treatments 3 and 4 versus Treatments 1 and 2). A slight change of the lysine content in the diet after 15 wk of age had no effect on growth and feed consumption.

Pullets on restricted feed intake from 7-15 wk of age (Treatments 5-8) showed a significantly lower body weight at 15 wk of age than the *ad libitum* fed pullets. This was mainly due to the restricted allowance of feed. At 18 wk of age, no significant differences in body weight were found between pullets of all treatments. 'Mid-term' restricted pullets (Treatments 5-8) consumed a significantly lower amount of feed over the whole rearing period than the qualitative restricted pullets. This is illustrated by the FCR values in Table 2. Mortality rate during rearing was on average 3.0% and did not differ among treatments.

The main advantage of a 'mid-term' feed restriction seems to be the ability to control growth during rearing (thus saving feed costs), while allowing pullets to compensate 'growth-loss' during the last part of the rearing period. The overall lower FCR values (0-18 wk; Table 2) of Treatments 5-8 can be explained partly by the possibility of a changed protein-fat deposition during the restriction period.

This is in agreement with the results of Wells (1980), who promoted this method for rearing pullets.

Leeson and Summers (1979) reported a significantly lower body weight at 20 wk of age for step-up-reared pullets as compared to conventional step-down-reared pullets.

Table 2. *Means of least squares.*

	Treatment step-down 1	2	step-up 3	4	mid-term restriction 5	6	7	8	Signifi-cance[1]	Coefficient of variation (%)
Body weight (g)										
6 wk	0.38	0.38	0.36	0.35	0.38	0.38	0.38	0.37	n.s.	4.89
15 wk	1.10^{ab}	1.10^{ab}	1.10^{abd}	1.11^{a}	1.05^{c}	1.05^{c}	1.07^{bc}	1.06^{dc}		2.32
18 wk	1.17	1.17	1.17	1.17	1.14	1.15	1.15	1.16	n.s.	2.46
Cumulative feed consumption (g/hen housed)										
0 - 6 wk	0.87	0.87	0.85	0.84	0.86	0.86	0.87	0.85	n.s.	4.64
7 - 15 wk	3.66^{a}	3.70^{a}	3.64^{a}	3.62a	3.15^{b}	3.16^{b}	3.16^{b}	3.16^{b}		1.97
16 - 18 wk	1.29^{a}	1.30^{a}	1.28^{a}	1.39^{acd}	1.46^{bc}	1.55^{b}	1.51^{bd}	1.59^{b}		7.36
0 - 18 wk	5.83^{a}	5.87^{a}	5.77^{a}	5.86^{a}	5.46^{b}	5.57^{b}	5.55^{b}	5.60^{b}		2.30
Feed conversion ratio (FCR; g feed/g gain)										
0 - 6 wk	2.55^{ab}	2.55^{ab}	2.62^{bc}	2.68^{c}	2.52^{ab}	2.50^{a}	2.53^{ab}	2.53^{ab}		3.30
7 - 15 wk	5.12^{a}	5.12^{a}	4.97^{ad}	4.82^{cd}	4.66^{bc}	4.75^{bc}	4.60^{b}	4.60^{b}		2.99
16 - 18 wk	17.90	18.61	19.10	21.10	17.64	15.98	19.93	15.77	n.s.	18.32
0 - 18 wk	5.16a	5.16^{a}	5.13^{acd}	5.17^{a}	4.96^{b}	5.02^{ab}	5.00^{bd}	4.97^{bc}		2.32

[1] a, b, c, d Means in a row with the same superscript are not significantly different ($P > .05$).

The low digestble lysine level in our starter diet (.67%) was insufficient to cause a definite growth retardation and an accompanying decreased feed intake in the step-up groups.

From our experiments, it can be concluded that the feeding strategies used achieve equal body weights at 18 wk of age, but result in different patterns of body weight development (growth curves). After a thorough 'mapping' of the interactions between physiological and nutritional factors involving the maturing pullet, decisions about the use of specific feeding strategies during rearing can be made. However more research on the relation between growth curve and laying performance is desirable.

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Chapter 2

EFFECTS OF A LOW-LYSINE STARTER DIET AND A RESTRICTED FED GROWER DIET ON GROWTH AND PRODUCTIVE PERFORMANCE OF LAYING-TYPE PULLETS AND HENS

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ABSTRACT

A study was made of growth and laying performance of pullets and hens from a commercial stock (White Leghorn) that had been subjected to different feeding regimens during rearing (0 to 18 wk of age). Eight feeding regimens were imposed: four groups were each fed *ad libitum* one of four starter diets that differed in lysine content (RL: .40, .55, .70, and .85% digestible lysine) from 0 to 6 wk of age. From 7 to 18 wk of age, the pullets received a grower diet *ad libitum* with .65% digestible lysine. Moreover, in each of the two starter groups with .55 and .85% digestible lysine, pullets were subsequently restricted during the grower period (7 to 18 wk of age), whereby a restricted daily amount of feed (RF: 80% of *ad libitum*) was provided from 7 to 15 wk of age (16 to 18 wk of age *ad libitum*) or from 7 to 18 wk of age. Hens were fed a commercial layer feed *ad libitum* from 19 to 62 wk of age.

Body weight (BW) at 18 wk of age was about 100 g lower if the lysine content in the starter diet was decreased from .85 to .40% digestible lysine. A feed restriction during the grower phase reduced BW at 18 wk of age by about 60 (7 to 15 wk of age) to 220 g (7 to 18 wk of age), if compared to the *ad libitum* fed group. RL-pullets required more feed than RF-pullets to reach similar BW at 18 wk of age. The onset of lay (50% rate of lay) was delayed in all restricted treatments. Hence, RL-pullets commenced egg production at equal BW, whereas RF-pullets had lower BW at the onset of lay. RF-pullets maintained a lower BW throughout the entire laying period (19-62 wk of age). Only minor decreases in egg production were evident in both the restricted (RL and RF) feeding regimens, except for pullets of the .40% digestible lysine group and the up to 18 wk feed restricted pullets: these two groups had a significant lower egg output than all other groups. No differences in rearing and laying performance were found between pullets that received the .70% and .85% (control) digestible lysine starter diet.

Keywords: feed restriction, lysine, pullets, growth, onset of lay, egg performance.

INTRODUCTION

Breeder companies publish target weights for their layer stock: a particular body weight (BW), that has to be 'targeted' before egg production will start, and which should guarantee a 'good-quality' hen for the egg producer. Wells (1980) discussed the relevance of such target weights if there was no concomitant advice concerning the growth curve that should be followed. He posed the question of how to obtain a profitable ready-to-lay hen in terms of how, when, and to what extent the pullet should be fed restrictedly.

Various types of feed restriction programmes for egg-type pullets have been reported in the literature. Some of these have been incorporated into the modern poultry industry, despite public concerns on welfare (Karunajeewa, 1987). Some of those mentioned by Lee *et al.* (1971) are in common use today. Pullets may be restricted either quantitatively, by means of a physical feed reduction, or qualitatively, in which protein or one of the essential amino acids in the diet is decreased (Lee *et al.*, 1971; Karunajeewa, 1987). Quantitative feed restrictions have been applied mainly during the grower phase of rearing (after 8 wk of age; Lee, 1987). For example, the 'mid-term' feed restriction programme, a moderate reduction of feed supply during 'mid' rearing (7-15 wk of age), as promoted by Wells (1980), has been adopted widely by breeders and incorporated in their flock management programmes.

On the other hand, feeding programmes based on qualitative restrictive measures are often used during the starter phase of rearing (before 6 or 8 wk of age). For example, Leeson and Summers (1979) developed a feeding strategy, referred to as the 'step-up' method, in which high protein diets during the grower phase were preceded by low protein diets during the starter phase. They assumed that this method precisely reflected the pullets' nutritional demands (Leeson and Summers, 1980). It is a common belief that a certain level of "protein" is required only to provide sufficient amounts of the first limiting essential amino acids (Scott *et al.*, 1982). That is why that much research on qualitative restriction methods for pullets has focussed on the use of lysine instead of protein as growth manipulator.

The purpose of the present study was to investigate the consequences for pullet growth and subsequent performance during the laying period, of a nutrient restriction during rearing (carry-over effects). Low-lysine diets were used during the starter phase and/or a daily restricted feed allowance applied during two age-intervals during the grower phase.

MATERIALS AND METHODS

Birds and housing

864 day-old Single Comb White Leghorn pullets (Lohmann Selected Leghorn, Lohmann Breeding Company, Cuxhaven, FRG) were weighed and wingbanded. Pullets were randomly assigned to one of eight treatments (Table 1). Each treatment was represented by 8 or 10 replicate battery cages (1.00 x .45 m) with 12 pullets per cage. During rearing, light was maintained at 23 hours a day during the first wk, followed by

a gradual reduction to 8 hours a day in wk 5. This photoperiod was continued until 18 wk of age. Temperature was set to 35°C in the first wk and reduced by 2.5°C per wk to 20°C in wk 7 and maintained at this level during rearing. Pullets were given regular vaccines at proper intervals and were not debeaked.

After 19 wk of age, 8 hens of each cage were transferred to the laying house. Hens were placed in a three-deck battery with 2 birds per cage (.45 x .45 m). Hence, 4 cages with a joint feed trough represented one experimental unit as during the rearing period. Each experimental unit of 8 birds was allocated within the laying house using a randomized block design. The daily photoperiod was increased from 8 hours in wk 18 to 12 hours in wk 19 and 13 hours in wk 20. Subsequently, photoperiod was increased by .5 hour per wk to 16 hours by wk 26. From 26 to 62 wk of age, the daily photoperiod was kept constant at 16 hours, supplemented with artificial light if necessary.

Feeding regimens

Eight feeding regimens were evaluated in this trial (Table 1). Four groups of day-old pullets were each fed one of four experimental diets *ad libitum* that differed in digestible lysine content (RL-groups) from 0-6 wk of age. The diets contained .40, .55, .70, or .85% digestible lysine (.85% was assumed to be adequate). These experimental diets were based on corn and soybean meal, and formulated to provide .40% digestible lysine and adequate levels of the other essential amino acids in a basal experimental diet (Table 2). Thus, the first limiting amino acid in the diets was lysine (Edmonds *et al.*, 1985). The diets with .55, .70, and .85% digestible lysine were achieved by adding synthetic lysine.

Subsequently, from 7 to 18 wk of age, all pullets of the .40 and .70, and one third of the pullets of the .55 and .85% digestible lysine groups, received a grower diet with .65% digestible lysine *ad libitum*. Two third of the pullets in each of the .55 and .85% groups were feed restricted (RF-groups) during the grower period (7 to 18 wk of age). A restricted amount of feed (80% of *ad libitum*) was offered to one third from 7 to 15 wk of age (16 to 18 wk of age *ad libitum*) and to another one third from 7 to 18 wk of age (Table 1). The daily feed restriction was based on the amount of feed consumed by the respective *ad libitum* group during the previous wk and extrapolated over the actual wk, adjusted for differences in BW between both groups. The grower diet of the restricted fed pullets was upgraded by 20% synthetic lysine. During the first wk after transportation to the layer house, pullets were fed the grower diet *ad libitum* to minimize stress. All birds were fed a commercial layer feed *ad libitum* from 19 to 62 wk of age. The compositions of the diets, all provided in mash form, are shown in Table 2.

Table 2. Composition (g/kg) of the experimental diets, subdivided after digestible lysine content.

Ingredients	Experimental diets Starter[1] .40	.55	.70	.85	Grower .65	Layer .81	.66
Maize	372.0	365.0	357.0	350.0	321.0	313.0	512.0
Maize gluten feed (190 g/kg)	150.0	150.0	150.0	150.0	200.0	200.0	139.0
Maize gluten meal (640 g/kg)	102.0	102.0	102.0	102.0	30.0	30.0	...
Tapioca root meal (680 g/kg)	200.0	200.0	200.0	200.0	250.0	250.0	...
Wheat middlings	37.0	37.0	37.0	37.0	...	...	...
Soyabeans (heat treated)	...	...	...	...	...	...	100.0
Soyabean meal (490 g/kg)	29.0	29.0	29.0	29.0	128.0	128.0	35.0
Lucerne meal	...	...	...	...	...	...	30.0
Meat meal, high fat (580 g/kg)	7.0	7.0	7.0	7.0	...	...	36.0
Feather meal, hydrolysed	20.0	20.0	20.0	20.0	...	...	...
Herring meal	...	...	...	...	...	...	20.0
Sunflower meal hulls (350 g/kg)	50.0	50.0	50.0	50.0	15.0	15.0	...
Animal fats	...	...	...	...	16.0	16.0	30.0
Vitamin/mineral premix[2]	20.0	20.0	20.0	20.0	20.0	20.0	25.0
Limestone	3.8	3.8	3.8	3.8	3.7	3.7	2.7
Dicalcium phosphate	9.2	9.2	9.2	9.2	10.1	10.1	...
Oyster shells	...	...	...	...	...	...	45.0
L-lysine HCl	...	7.5	15.0	22.5	5.8	13.8	...
Calculated analysis[2] (g/kg)							
Metabolisable energy (MJ/kg)	11.7	11.7	11.7	11.7	11.8	11.8	11.7
Crude protein (Nx6.25)	188.0	188.0	188.0	188.0	161.0	161.0	167.0
Total lysine	5.2	6.7	8.2	9.7	7.5	9.1	7.7
Digestible lysine	4.0	5.5	7.0	8.5	6.2	7.8	6.6
Total methionine	3.9	3.9	3.9	3.9	2.8	2.8	3.6
Digestible methionine	3.3	3.3	3.3	3.3	2.3	2.3	3.0
Total methionine + cystine	8.0	8.0	8.0	8.0	5.8	5.8	6.5
Digestible meth. + cystine	6.5	6.5	6.5	6.5	4.6	4.6	5.2
Calcium	9.9	9.9	9.9	9.9	10.0	10.0	35.0
Available phosphorus	4.5	4.5	4.5	4.5	4.5	4.5	6.4

[1] Starter = 0-6 wk of age; grower = 7-18 wk of age.

[2] Formulated according to Dutch CVB-standards (1988).

Table 1. Dietary digestible lysine contents and feeding levels in each treatment.

Treatment[1]	Age (wk) 0-6	7-15	16-18
RL 85	.85 AL	.65 AL	.65 AL
RF 15^{85}	.85 AL	.81 R	.65 AL
RF 18^{85}	.85 AL	.81 R	.81 R
RL 70	.70 AL	.65 AL	.65 AL
RL 55	.55 AL	.65 AL	.65 AL
RF 15^{55}	.55 AL	.81 R	.65 AL
RF 18^{55}	.55 AL	.81 R	.81 R
RL 40	.40 AL	.65 AL	.65 AL

[1] RL: Restricted Lysine, Ad Libitum (AL) feeding level; 8 replicate cages per treatment.
RF: Restricted Feeding level (80% of AL), RF 15 = restricted from 7-15 wk of age, RF 18 = restricted from 7-18 wk of age; 10 replicate cages per treatment;
0-6 wk: starter diet; 7-18 wk: grower diet.

Data recording

BW, feed intake and, as appropiate, egg production were monitored weekly (0 to 32 wk of age), twice monthly (33 to 50 wk of age), or monthly (51 to 62 wk of age) as a mean per cage. Feed conversion ratios (FCR) during rearing (g feed per g gain) and laying (g feed per g egg mass) were calculated from these data. Rate of lay and egg weights were based on the number of normal-graded (> 30 g) eggs ('normal' eggs). Both 'normal' and 'abnormal' (broken, shell-less, very small (< 30 g) and double-yolked eggs) egg mass were determined. For 'total abnormal egg mass', broken and shell-less eggs were assumed to weigh the average egg weight of the respective cage and wk. Age at the 'onset of lay' or 'onset of egg production' was used as a measure of sexual maturity, and was calculated as the age at which a 50% rate of lay was reached (Mbugua and Cunningham, 1983; Abu-Serewa, 1979).

Statistical analysis

Observed traits for each period (0 to 18, 19 to 32, and 33 to 62 wk of age) were subjected to a one-way analysis of variance using the GLM procedure of the SAS-package (SAS Institute Inc., 1985) with treatments (8 feeding regimens; Table 1) as the main factor. Effects of tier were non-significant and therefore excluded from the model. Differences between treatment means were evaluated using Tukey's Studentized Range (HSD) Test of Multiple Comparisons. Additionally, a few contrasts were tested to determine particular

Table 3. Effect of feeding regime[1] on body weight, feed intake and feed conversion ratio during rearing.

Treatment[1]	Body weight (kg)			Cumulative feed intake (kg per hen)			Feed conversion ratio[2]		
	6 wk	15 wk	18 wk	0-6 wk	0-15 wk	0-18 wk	0-6 wk	0-15 wk	0-18 wk
RL 85	.43±.01^{c}	1.16±.01^{e}	1.27±.01^{e}	1.00±.01^{c}	4.96±.03^{e}	6.28±.04^{f}	2.54±.01^{c}	4.42±.03^{ab}	5.11±.04^{a}
RF 15^{85}	.43±.01^{c}	.97±.01^{b}	1.21±.01^{c}	1.00±.01^{c}	3.98±.02^{b}	5.71±.03^{d}	2.54±.01^{c}	4.29±.02^{c}	4.86±.03^{cd}
RF 18^{85}	.42±.01^{c}	.97±.01^{b}	1.05±.01^{a}	.99±.01^{c}	3.97±.02^{b}	5.08±.02^{b}	2.60±.03^{c}	4.29±.03^{c}	5.01±.03^{abc}
RL 70	.43±.01^{c}	1.15±.01de	1.26±.01de	1.00±.01^{c}	4.95±.05^{e}	6.27±.06^{f}	2.58±.03^{c}	4.46±.04^{a}	5.14±.05^{a}
RL 55	.33±.01^{b}	1.12±.01^{d}	1.22±.02^{cd}	.85±.02^{b}	4.72±.08^{d}	6.01±.10^{e}	2.88±.01^{b}	4.35±.03^{abc}	5.08±.02^{a}
RF 15^{55}	.34±.01^{b}	.92±.01^{a}	1.16±.01^{b}	.88±.01^{b}	3.77±.02^{a}	5.46±.02^{c}	2.87±.02^{b}	4.25±.02^{c}	4.84±.03^{d}
RF 18^{55}	.34±.01^{b}	.92±.01^{a}	1.03±.01^{a}	.86±.01^{b}	3.74±.02^{a}	4.86±.02^{a}	2.88±.03^{b}	4.25±.03^{c}	4.90±.04^{bcd}
RL 40	.19±.01^{a}	1.03±.01^{c}	1.16±.01^{b}	.58±.01^{a}	4.26±.05^{c}	5.63±.06^{cd}	3.76±.03^{a}	4.32±.03^{bc}	5.02±.04^{ab}
Probability level of contrasts[3]									
C vs RL	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.221	.456
C vs RF	...	<.001	<.001	...	<.001	<.001	...	<.001	<.001
Duration	...	...	<.001	...	...	<.001	...	...	.004

$^{a-f}$ Means (± SEM) in a column with no common superscript differ significantly ($P > .05$)

[1] RL 85: control, no restrictions;
RL ..: lysine restriction from 0-6 wk: 40, 55, and 70 = percentage digestible lysine x 100; no restrictions during the grower period (7-18 wk);
RF ..: feed restriction from 7-15 (RF 15) or 7-18 (RF 18) wk of age; superscripts indicate the digestible lysine levels in the starter diets.

[2] Feed conversion ratio = g feed consumed per g body weight gain.

[3] Contrasts between treatments:
C vs RL = effect of the lysine restriction during the starter period [3 0 0 -1 -1 0 0 -1]
C vs RF = effect of the feed restriction during the grower period [2 -1 -1 0 2 -1 -1 0]
Duration = effect of the length of the period of feed restriction [0 1 -1 0 0 1 -1 0]
...: not included in the model.

effects: 1. the effect of the lysine restriction during the starter phase of rearing (RL85 versus a combination of RL40, RL55, and RL70); 2. the effect of the feed restriction during the grower phase of rearing (RL55 and RL85 versus RF15^{55}, RF18^{55}, and RF15^{85}, and RF18^{85}, respectively); and 3. the effect of the duration of the feed restriction (RF15^{55} and RF15^{85} versus RF18^{55} and RF18^{85}). Significance, if not stated otherwise, is based on the .05 level of probability.

RESULTS AND DISCUSSION

Rearing period

A level of .55 and .40% digestible lysine in the starter diet resulted in a 23 to 56% lower BW at 6 wk of age if compared to pullets fed the .85% digestible lysine (RL85-pullets were assumed to represent the non-restricted reference group; Table 3). Pullets fed .70% digestible lysine did not differ in BW at 6 wk of age from the reference-group, suggesting that this level is already adequate for 'normal' pullet growth. Feed consumption was reduced to a lesser extent (15 to 42%) than BW in the RL40 and RL55-groups, resulting in a poorer feed conversion ratio (FCR) in these groups. Higher FCR's in pullets fed low-lysine diets have been reported more frequently (Connor *et al.*, 1977; Gous, 1978; Kwakkel *et al.*, 1988). The reduced supply of lysine in the .70, .55 and .40% lysine groups enhanced conversion of digestible lysine (dig. lysine intake per g BW gain) by 18, 28, and 39%, as can be calculated from the results on feed intake and BW in Table 3. Towards the end of the rearing period, differences in BW between the RL-groups (adequate lysine levels in the grower diet) tended to become smaller. At 18 wk of age, BW in the low-lysine groups were 91 to 99% of BW in the RL85-group, with a 0 to 10% lower feed intake (0 - 18 wk of age; Table 3).

Feed restriction during 'mid-term' rearing (7 to 15 wk of age) reduced BW by about 17% at 15 wk of age. The subsequent recovery period of 3 wk (16 to 18 wk of age) was too short to enable the birds to compensate the decreased BW gain, resulting in a 5% lower BW at 18 wk of age compared to that of the RL85-group. A feed restriction during the 'entire grower' period (7 to 18 wk of age) resulted in a 16% lower BW at 18 wk of age compared to the RL85-pullets. Feed intake during rearing (0 - 18 wk of age) of RF-pullets was 81 (RF18^{55} and RF18^{85}) to 91% (RF15^{55} and RF15^{85}) of the amount consumed by the respective *ad libitum* fed control group (RL55 and RL85). Feed restriction in both periods improved FCR in the respective period compared to the *ad libitum* fed counterparts. Previously, other work has shown that FCR's were mostly improved if pullets had been quantitatively restricted during rearing (Gous and Stielau, 1976; Connor et *al.*, 1977; Wells, 1980). A decreased amount of deposited fat as a consequence of the lower feeding level was suggested to be the most appropiate explanation for the better FCR's (Gous and Stielau, 1976; Chwalibog *et al.*, 1984). Pullets that had been fed a restricted diet from 7-15 wk of age, and were then fed *ad libitum* between 16 and 18 wk of age, had a more efficient feed conversion from 16-18 wk of age than pullets which were still restricted during this latter period. These results suggest that the change from a restricted to an *ad libitum* feeding level at the end of wk 15 allowed these pullets to gain BW at the moment when there was evidence of increased protein synthesis for the development of the reproductive tract.

There was no demonstrated effect of feeding regime on mortality during rearing (2.7%).

If treatments are compared at the end of rearing (18 wk of age), it becomes evident that a similar BW can be reached by the use of different feeding regimens. In the present study, the treatments RL40 and RF15^{55}, RL55 and RF15^{85}, and RF18^{55} and RF18^{85}, respectively, showed similar 18-wk BW. In agreement with the results of Wells (1980) and Kwakkel *et al.* (1988), our results on the rearing period showed, on average, that RL-pullets required 3 to 5% more feed than RF-pullets to reach a similar BW at 18 wk of age.

Onset of lay

The applied restriction method, as well as the severity of the restriction, significantly delayed onset of lay (see contrasts in Table 4). A lysine restriction in the starter phase delayed onset of lay (age at 50% rate of lay) by a maximum of 7 days. The application of one of the feed restrictions in the grower phase delayed onset of lay by a maximum of 6 days. Similar delays due to restricted feeding were found by Lee (1987), Hurwitz and Plavnik (1989), and Katanbaf *et al.* (1989).

RL-pullets were significantly heavier than RF-pullets at similar (delayed) ages at the onset of lay (compare RL40 with RF18^{55}, and RF15^{55} with RF18^{85}). This illustrates the relatively minor impact of BW on age at the onset of lay, particularly if comparisons are made between different restriction methods. Gous (1978) and Hurwitz and Plavnik (1989) found similar differences in response to a restriction between qualitative and quantitative methods.

Pullets in each RL-group commenced egg production at a similar BW as did pullets of the 'control' RL85-group. On the contrary, feed restricted pullets (RF) in all four groups initiated egg production at a significant (2 to 7%) lower BW than the respective *ad libitum* fed group. This is in agreement with the results reported by Gous and Stielau (1976). In addition, RF-pullets consumed 7% less feed up to the onset of lay compared to the RL-pullets.

The delay in reaching a particular body composition at start of egg production may have induced the heavier BW and concomitant larger feed intakes of the RL-pullets compared to RF-pullets with similar delays in the onset of lay. RF-pullets seemed reach this 'threshold' for any body tissue earlier, and hence, commenced egg production at a lower BW.

Table 4. Effect of feeding regime[1] on age, body weight, cumulative feed intake and egg weight at onset of lay[2].

Treatment[1]	Age (d)	Body weight (kg)	Cum. feed intake (kg per hen)	Initial egg weight (g)
RL 85	150±1^{c}	1.53±.02^{a}	8.27±.09^{a}	45.2±.3^{a}
RF 15^{85}	152±1^{bc}	1.50±.01^{abc}	7.99±.03^{ab}	45.5±.9^{a}
RF 18^{85}	153±1^{abc}	1.42±.01^{d}	7.60±.09^{b}	44.5±.5^{a}
RL 70	150±1^{c}	1.52±.02^{a}	8.27±.06^{a}	44.8±.3^{a}
RL 55	152±1^{bc}	1.52±.02^{a}	8.27±.12^{a}	44.4±.2^{a}
RF 15^{55}	153±1^{abc}	1.46±.01^{bcd}	7.81±.05^{b}	44.2±.5^{a}
RF 18^{55}	156±1^{ab}	1.45±.01^{cd}	7.69±.08^{b}	45.2±.5^{a}
RL 40	157±1^{a}	1.51±.02^{ab}	8.39±.18^{a}	44.6±.5^{a}
Probability level of contrasts[3]				
C vs RL	.004	.562	.723	.386
C vs RF	<.001	<.001	<.001	.836
Duration	.035	.001	.005	.931

[a-d] Means (± SEM) in a column with no common superscript differ significantly ($P > .05$)

[1, 3] See Table 3.

[2] Onset of lay in this paper is defined as the age at which a 50% production of normal eggs was reached.

Initial egg weight was not affected by feeding regime. Egg weight over treatments showed no relationship with either age or BW at the onset of lay. This contradicts the results reported by Johnson *et al.* (1984). They observed that restricted feeding with the subsequent delay in onset of egg production increased egg weight, due to the proposed age-egg weight relationship (Pearson and Shannon, 1979). Our results from the early laying period (Table 5) suggest the opposite to be true.

A positive empirical relationship between BW and egg weight, as suggested by Summers and Leeson (1983) and Leeson (1986), seems to exist for the RF-pullets (Table 5). However, if the RL-pullets are included, this relationship does not hold.

Table 5. Effect of feeding regime[1] on body weight at 32 and 62 wk of age, and daily feed intake, egg performance and feed conversion ratio from 19 to 32 and from 19 to 62 wk of age.

Treatment[1]	Body weight 32/62 wk (kg)	Daily feed intake (g)	Number of eggs[2]	Average egg weight[2] (g)	Egg mass (kg per hen) Total	Abnormal	Feed Conversion Ratio[4]
				Early laying period: 19 - 32 wk of age			
RL 85	$1.76 \pm .01^{ab}$	$105.0 \pm .9^{a}$	72 ± 2^{a}	$53.6 \pm .2^{a}$	$4.08 \pm .10^{a}$	$.23 \pm .05^{a}$	$2.53 \pm .05^{d}$
RF 15^{85}	$1.73 \pm .02^{ab}$	$105.2 \pm .9^{a}$	69 ± 1^{ab}	$53.0 \pm .2^{ab}$	$3.90 \pm .03^{abcd}$	$.24 \pm .04^{a}$	$2.64 \pm .01^{bcd}$
RF 18^{85}	$1.64 \pm .02^{c}$	104.5 ± 1.2^{a}	68 ± 1^{ab}	$52.5 \pm .4^{ab}$	$3.78 \pm .08^{bcd}$	$.19 \pm .01^{a}$	$2.72 \pm .05^{abc}$
RL 70	$1.76 \pm .03^{ab}$	105.6 ± 1.3^{a}	72 ± 1^{a}	$53.2 \pm .2^{a}$	$4.00 \pm .05^{ab}$	$.19 \pm .03^{a}$	$2.59 \pm .04^{cd}$
RL 55	$1.78 \pm .02^{a}$	107.1 ± 1.5^{a}	70 ± 1^{a}	$53.3 \pm .2^{a}$	$3.99 \pm .06^{abc}$	$.24 \pm .05^{a}$	$2.63 \pm .03^{cd}$
RF 15^{55}	$1.71 \pm .01^{abc}$	105.2 ± 1.2^{a}	70 ± 1^{ab}	$52.6 \pm .3^{ab}$	$3.84 \pm .05^{abcd}$	$.18 \pm .03^{a}$	$2.69 \pm .03^{bcd}$
RF 18^{55}	$1.69 \pm .01^{bc}$	$106.4 \pm .5^{a}$	67 ± 1^{ab}	$52.4 \pm .3^{ab}$	$3.73 \pm .03^{cd}$	$.23 \pm .04^{a}$	$2.80 \pm .02^{ab}$
RL 40	$1.77 \pm .01^{a}$	$107.8 \pm .8^{a}$	65 ± 1^{b}	$52.0 \pm .2^{b}$	$3.72 \pm .04^{d}$	$.35 \pm .08^{a}$	$2.85 \pm .03^{a}$
				Entire laying period: 19 - 62 wk of age			
RL 85	$1.93 \pm .02^{ab}$	$103.7 \pm .9^{ab}$	257 ± 4^{a}	$59.4 \pm .2^{a}$	$15.76 \pm .23^{a}$	$.47 \pm .10^{a}$	$2.12 \pm .03^{a}$
RF 15^{85}	$1.86 \pm .03^{abc}$	$102.6 \pm .7^{ab}$	259 ± 3^{a}	$58.0 \pm .3^{bc}$	$15.46 \pm .16^{a}$	$.47 \pm .05^{a}$	$2.14 \pm .02^{a}$
RF 18^{85}	$1.77 \pm .03^{c}$	100.8 ± 1.1^{b}	255 ± 3^{a}	$57.6 \pm .4^{bc}$	$15.08 \pm .26^{a}$	$.41 \pm .03^{a}$	$2.16 \pm .03^{a}$
RL 70	$1.91 \pm .04^{ab}$	105.1 ± 1.3^{ab}	261 ± 3^{a}	$58.9 \pm .2^{ab}$	$15.75 \pm .20^{a}$	$.40 \pm .06^{a}$	$2.15 \pm .02^{a}$
RL 55	$1.95 \pm .02^{a}$	105.0 ± 1.4^{ab}	260 ± 5^{a}	$58.7 \pm .2^{abc}$	$15.76 \pm .29^{a}$	$.50 \pm .09^{a}$	$2.15 \pm .03^{a}$
RF 15^{55}	$1.86 \pm .02^{abc}$	102.3 ± 1.0^{ab}	258 ± 3^{a}	$57.6 \pm .3^{bc}$	$15.21 \pm .20^{a}$	$.37 \pm .05^{a}$	$2.17 \pm .02^{a}$
RF 18^{55}	$1.82 \pm .03^{bc}$	$102.0 \pm .9^{ab}$	257 ± 2^{a}	$57.3 \pm .3^{c}$	$15.23 \pm .17^{a}$	$.47 \pm .06^{a}$	$2.16 \pm .02^{a}$
RL 40	$1.94 \pm .01^{ab}$	$105.9 \pm .6^{a}$	257 ± 2^{a}	$57.5 \pm .3^{c}$	$15.44 \pm .12^{a}$	$.66 \pm .13^{a}$	$2.21 \pm .01^{a}$
Probability level of contrasts[3, 5]							
C vs RL	.721 .908	.157 .187	.054 .608	.032 .005	.020 .662	.567 .618	$< .001$ .099
C vs RF	$< .001 < .001$	.419 .007	.018 .576	.001 $< .001$	$< .001$ .007	.522 .382	$< .001$ .338
Duration	.004 .019	.793 .291	.118 .519	.176 .243	.045 .376	.924 .749	.008 .833

[a-d] Means (± SEM) in a column with no common superscript differ significantly ($P > .05$)

[1, 3] See Table 3.

[2] Based on the 'normal' egg mass.

[4] g feed per g total egg mass.

[5] Per trait the probabilities for the early en entire laying period are given next to each other.

Laying period

BW gain in the early laying period was greater in the RL-groups, resulting in a similar BW at 32 wk of age compared to the 'control' RL85-group (compare Table 4 and 5). RF-hens still weighed less than the controls at 32 wk of age, particularly those that had been restricted during the entire grower phase from 7 to 18 wk of age. Daily feed intake was not different between the feeding regimens. A tendency in the direction of a higher feed intake was observed in the RL-hens, most likely necessary to sustain the concomitant increase in BW. Wells (1980) and Keshavarz (1984) found similar patterns of compensatory growth during early lay following a period of low protein feeding.

In general, no large differences between treatments in the number of eggs, egg weight, and egg mass in comparison to the control group, were observed during the early laying period. Hens that received the lowest lysine level in the starter diet (RL40), however, and those that were fed a restricted amount of feed during the entire grower phase ($RF18^{55}$, $RF18^{85}$), had significantly lower egg records (Table 5). No differences in abnormal egg mass could be demonstrated. Differences between treatments in the feed conversion ratio (g feed per g egg mass) reflected differences in the partitioning of feed towards either egg mass or body mass. Non-restricted birds had the lowest FCR's.

As a consequence of the delay in the onset of egg production, layer records in the restricted feeding regimens were somewhat lower during the early laying period. For most traits, however, differences in performance between groups diminished if the entire laying period (19 to 62 wk of age) was incorporated in the analyses (Table 5). This was with the exception of BW, where, even at 62 wk of age, RF-hens were still lighter than all other hens (Figure 1). Feed intake during lay tended to increase if the hens had been restricted qualitatively during rearing. On the other hand, hens that had been restricted quantitatively during rearing had a lower feed intake during lay, due to lower maintenance costs associated with reduced adult BW, as suggested by Hurwitz and Plavnik (1989).

Several authors reported that a delay in the onset of egg production caused by a quantitative feed restriction would result in subsequent increased average egg weights (Gous and Stielau, 1976; Robinson and Sheridan, 1982). In this study, however, smaller eggs in RF-pullets were found.

The observation in this experiment that smaller eggs were laid by hens that had been restricted in lysine during early rearing confirms the results of Connor *et al.* (1977). Moreover, it has been reported frequently that step-up protein regimens, characterized by low protein or amino acid levels during early rearing, had a major disadvantage; namely that eggs were often too small (Maurice *et al.*, 1982; Doran *et al.*, 1983).

Differences in BW at the onset of lay and subsequent BW gain during the laying period between RL- and RF-pullets suggest, in accordance with other studies (Belyavin,

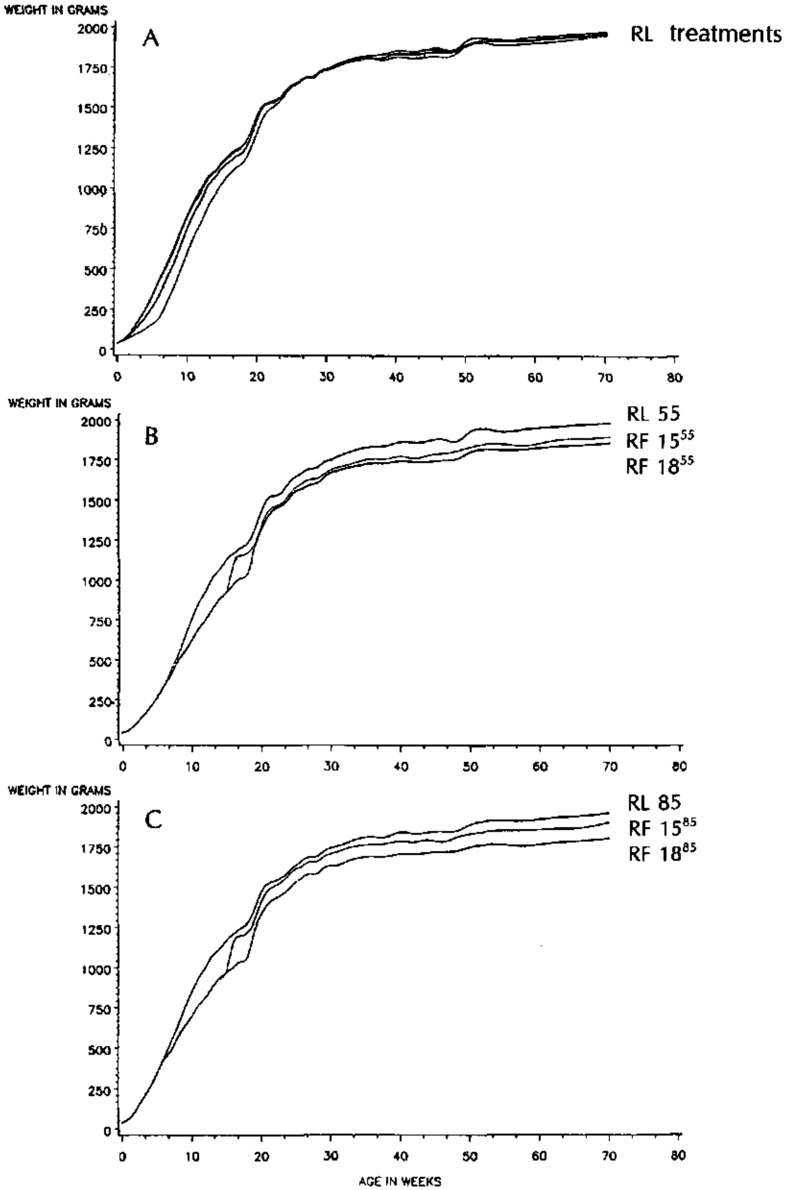

Figure 1. Body weight development during the rearing and laying period. Pullets had been restricted in lysine during the starter period (A), or in feed during the grower period (digestible lysine level in starter period: .55% (B) and .85% (C)).

1984; Leeson, 1986), that BW is not the sole critical factor in determining the response of the adult layer to previous nutrient restrictions. Body composition is perceived as being much more important. The results reported here, stress the need to study the 'mode of action' of feeding regimens in which the timing and method of the restriction are the main factors. In this experiment, timing and method of restriction were confounded in the design. Therefore, the magnitude of both effects could not be judged individually.

Secondly, it can be concluded that for all traits observed, no differences in rearing and laying performance were demonstrated between pullets that received the .70 or .85% digestible lysine level in the starter diet. In a former experiment, even a .65% digestible lysine level in the starter diet did not affect BW gain and feed intake during rearing when compared to adequate levels of lysine (Kwakkel *et al.*, 1988). It may be worthwile investigating the possibilities to reduce the recommended lysine levels of starter diets for laying-type pullets. This could reduce the nitrogen input of a pullet by as much as 5%.

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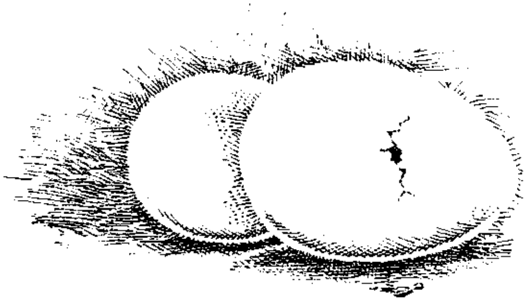

Chapter 3

EFFECT OF METHOD AND PHASE OF NUTRIENT RESTRICTION DURING REARING ON PRODUCTIVE PERFORMANCE OF LIGHT HYBRID PULLETS AND HENS

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EFFECT OF METHOD AND PHASE OF NUTRIENT RESTRICTION DURING REARING ON PRODUCTIVE PERFORMANCE OF LIGHT HYBRID PULLETS AND HENS

R.P. Kwakkel, F.L.S.M. de Koning, M.W.A. Verstegen and G. Hof

ABSTRACT

An experiment was conducted to study the effect of method (low-lysine or quantity of feed) and phase (starter or grower) of restriction during rearing on growth and laying performance in Leghorn hybrids. A reduction in body weight gain was achieved by feeding pullets a low-lysine diet in either the starter phase (0-6 wks of age: .40% digestible lysine) or the grower phase (7-18 wks of age: .30% digestible lysine). In each phase, the lysine restriction was compared with a daily feed restriction, in which pullets received a quantity of feed sufficient to pair-gain with their lysine-restricted counterparts.

The delay in onset of lay was greatest in the lysine-restricted birds, for both phases of restriction. Hens restricted during the grower phase laid heavier eggs than those restricted during the starter phase, despite similar body weights at end of rearing. This effect was not influenced by the restriction method.

Production traits varied between pullets reared on different feeding strategies up to similar body weights at 18 wk and onwards. It is concluded that body weight is not the only factor determining laying performance.

Key words: feed restriction, low lysine, performance, pullets.

INTRODUCTION

During the last 20 years, much research has been done on developing an optimal feeding strategy for laying-type pullets (Lee *et al.*, 1971; Connor *et al.*, 1977; Gous, 1978; Wells, 1980; Summers, 1983; Johnson *et al.*, 1984; Chi, 1985; Leeson, 1986; Summers *et al.*, 1987). Initially, the major aim of these investigations was to save rearing costs by restricting feed, without loss of productivity (Lee *et al.*, 1971; Balnave, 1973). Nowadays, conventional rearing programmes are applied to the modern pullet to allow the bird to express its full genetic potential. Most modern programmes are still based on the concept that body weight at onset of lay is the main determinant of performance. Hence, a physiological basis for a specific feeding strategy has yet to be demonstrated. However, there has recently been some data on ovarian responses in young hens restricted during rearing (Hocking, 1987; Hocking *et al.*, 1989).

Wells (1980) pointed out that body weight at the end of rearing is not the best predictor of laying performance. Although patterns of growth might affect egg-producing ability, the mechanism is not yet understood (Summers, 1983; Leeson, 1986). It is obvious that critical phases associated with changes in tissue deposition or organ development are likely to be important in optimising a feeding strategy for immature hens (McCance, 1977; Johnson et *al.*, 1985). For example, the application of different methods of restriction without changing the pullet's growth curve might affect development by altering deposition characteristics and these alterations may influence subsequent performance. This suggests that the concept of body weight as the determinant of laying performance is of minor value. A research programme has been initiated for the development of a feeding strategy based on these considerations (Kwakkel and Janssen, 1987).

One of the problems in evaluating the results of previous studies is the variation reported in body weight at the start of the laying period, after the application of various feeding strategies during rearing; this causes difficulty in interpreting subsequent differences in production. By fixing body weights during and at the end of rearing, one might be able to elucidate the real rearing factors that determine production.

The aim of the present experiment was to compare the performance of pullets restricted in either lysine or feed intake during part of the rearing period, under the limitation of paired weight gain. Both methods of restriction were applied during the starter (0-6 wk of age) and grower (7-18 wk of age) phases of rearing to study the effect of these distinctive growth curves on laying performance. It was also planned to have similar body weights at the end of rearing for pullets in all restricted treatment groups.

This paper deals with performance. Body composition and analysis of organ growth will be reported later.

MATERIALS AND METHODS

Birds and management

Each of 1080 1-d-old White Leghorn chicks (Lohmann Selected Leghorn, Lohmann Tierzucht Gmb, Cuxhaven, FRG) was weighed, wingbanded and allocated at random to one of 5 treatments. Each treatment consisted of 9 cages (.50 x .45 m). A cage was considered as a replicate experimental unit. Cage density decreased throughout rearing from 24 to 8 birds, because pullets were killed weekly for carcass analyses.

Management during rearing and laying was according to commercial guidelines for the strain. Light was 23 h per d for the first week, followed by a gradual reduction to 8 h per d in week 5, and this pattern was maintained until week 18. In weeks 19 and 20 respectively, 12 and 13 h light per d were given, followed by a .5 h increase each week to 16 h per d in week 25. This photoperiod was maintained till week 62. Temperature was decreased each week by 2.5 ^{0}C from 32 ^{0}C in week 1 to 22 ^{0}C in week 5, where it remained unchanged. Vaccines were given at the appropriate ages. Pullets were not debeaked to avoid the effects of any differential stress caused by variations in development among groups at the same age.

Experimental design

Pullets on the control treatment (C) were given a starter diet *ad libitum* from 0-6 wk of age, followed by a grower diet *ad libitum* from 7-18 wk of age. Pullets on treatment RLs were fed a low-lysine diet during the starter (s) phase (0-6 wk of age: .40% digestible lysine) and those on treatment RLg were fed a low-lysine diet during the grower (g) phase (7-18 wk of age: .30% digestible lysine). The low-lysine diets contained adequate amounts of all other nutrients. In each rearing phase, a quantitative feed restriction (RFs and RFg) was applied to achieve a similar body weight gain per week to that of the corresponding RL-group. Thus, each of the 4 groups was restricted in only one of the two rearing phases. The RF-groups received the appropriate control diet in the two rearing phases. After 18 wk of age, 180 hens (4 per cage) were transferred to the laying house and received a prelayer diet (with an intermediate calcium content) *ad libitum* in week 19 and 20, followed by a commercial layer diet. The compositions of the experimental diets are given in Table 1.

Data collection

Body weight and feed consumption per cage were recorded weekly during rearing and laying. Body weight gain and feed conversion ratios (FCR) were calculated from these data. Twice weekly, feed intake for RF-pullets was adjusted on the basis of the weight gained by the RL-group. Egg production per cage was recorded weekly from 19 to 32 wk of age and once every two weeks thereafter. Egg weight per cage was based on the amount of 'normal' egg mass, i.e. all clean and dirty (blood- or faecal-stained), normal graded eggs. The remaining 'abnormal' egg mass consisted of broken, cracked, shell-less, double-yolked and very small (<30 g) eggs. For the trait 'total egg mass' the entire egg mass production was calculated, assuming shell-less and cracked eggs to weigh the mean 'normal' egg weight of that specific cage and week. 'Onset of lay' was defined as the age at which 50% production of 'normal' eggs was reached. The egg weight at that age was called the 'initial egg weight'.

Statistical analysis

The design of the experiment was a '2x2 factorial plus added control' (Cochran and Cox, 1957). Analyses of variance of data were performed using the GLM procedure of

Table 1. Composition (g/kg) of the experimental diets.

Ingredients	Starter	Grower	Prelayer	Layer
Maize	372.0	350.0	451.0	460.0
Maize glutenfeed (203 g/kg)	150.0	200.0	...	...
Maize glutenmeal (635 g/kg)	102.0	80.0	20.0	20.0
Barley	...	...	55.0	55.0
Oats	...	...	50.0	50.0
Alfalfa meal (160<cp<180 g/kg)	...	...	30.0	30.0
Tapioca rootmeal	179.2 (185.0)[1]	246.8 (251.2)	103.7	57.0
Wheat middlings	37.0	...	...	...
Soybean meal(solv.extract.,cf 61 g/kg)	29.0	15.0	...	...
Soybean oil meal	...	...	192.5	192.5
Meat meal tankage(fat>120 g/kg)	7.0	...	...	...
Feathermeal, hydr.	20.0	5.0	...	...
Sunflowermeal, solv.extr.	50.0	45.0	...	...
Soybean oil	15.0	21.0	17.5	29.0
Vitamin/mineral premix	20.0[2]	20.0[2]	10.0[3]	10.0[3]
Limestone	3.8	...	48.8	75.0
Dicalcium phosphate	9.2	12.8	17.5	17.5
DL-methionine	...	...	1.0	1.0
Salt (NaCl)	...	...	3.0	3.0
L-lysine HCl	5.8 (0.0)	4.4 (0.0)	...	...
Determined analysis[4] (g/kg)				
Dry Matter	895	902	897	901
Metabolisable energy (MJ/kg)	11.7	11.8	11.7	11.7
Crude protein (Nx6.25)	192 (190)	159 (158)	168	165
Total lysine	9.8 (5.3)	7.8 (4.2)	8.0	7.9
Digestible lysine	8.5 (4.0)	6.6 (3.1)	6.5	6.5
Total methionine+cystine	8.6	7.3	7.0	6.8
Digestible meth.+cyst.	7.1	6.0	5.8	5.8
Calcium	10.2	9.1	25.0	35.1
Total phosphorus	4.1	4.4	6.3	6.4

[1] The contents in the deficient diets are given in parentheses (see 'experimental design').

[2] Contained per kg diet: 3.0 mg retinyl acetate, 37.5 mg cholecalciferol, 10 mg DL-a-tocopheryl acetate, 1.0 mg menadione sodium bisulphite, 4.0 mg riboflavin, 15 ug cobalamin, 6.5 mg d-pantothenic acid, 28 mg niacin, 200 mg choline chloride; 1.4 g sodium, 4.8 g calcium, 1.1 g phosphorus, 40 mg iron, 60 mg manganese, 4 mg copper, 40 mg zinc, .8 mg iodine and 50 ug selenium; 800 mg DL-methionine and 125 mg 3,5 dinitro-orthotoluamide.

[3] Contained per kg diet: 3.4 mg retinyl acetate, 50 mg cholecalciferol, 25 mg DL-a-tocopheryl acetate, 1.5 mg menadione sodium bisulphite, 3.5 mg riboflavin, 15 ug cobalamin, 12 mg d-pantothenic acid, 30 mg niacin, 350 mg choline chloride, 250 mg $FeSO_4.7H_2O$, 100 mg MnO_2, 100 mg $CuSO_4.5H_2O$, 150 mg $ZnSO_4.7H_2O$, 100 mg ethoxyquin and 15 ppm zincbacitracin.

[4] ME-content, calcium and available phosphorus as well as the digestibility of the amino acids in the ingredients was calculated, based on official Dutch standards (CVB, 1988).

the SAS-package (SAS Institute Inc., 1985), with cage mean as experimental unit. The following model was used to assess (1) the effect of restriction *per se* (R): examining the contrast between *ad libitum* and restricted feeding; (2) within the restricted treatments, the effect of the phase (T) of restriction: examining the contrast between the starter and grower phase; and (3) within each phase, the effect of method (Ms and Mg) of restriction: examining the contrast between the lysine and feed restrictions on rearing and laying traits:

$$Y_{ijkl} = \mu + R_i + T_{j:i} + Ms_{k:i} + Mg_{k:i} + e_{ijkl}$$

where Y_{ijkl} = observed trait, mean value of the *l*th cage; μ = overall mean; R_i = restriction effect (i = restricted, *ad lib*). Within the restricted treatments: $T_{j:i}$ = phase effect (j = starter, grower); $Ms_{k:i}$ = method effect (k = lysine, feed) within the starter (s) phase; $Mg_{k:i}$ = method effect (k - lysine, feed) within the grower (g) phase; e_{ijkl} = random error.

The model was applied to predetermined contrasts. For traits observed during only the starter phase (e.g., gain from 0 to 6 wk) or the grower phase (e.g., gain from 7 to 18 wk), the 'T' and the 'Mg' respectively 'Ms' terms were ignored in the analysis. Cages and treatments were distributed over 3 tiers during the laying period, but any effect of tier ($P>.05$) was ignored.

Mean trait values over the entire production period (19-62 wk of age) were based on 39 cages instead of 45 (5x9 replicates). Data from 6 cages were omitted because of high mortality (cannibalism) in one cage and sickness or moult in 5 other cages after 32 wk of age.

RESULTS

The results, as summarised in Tables 2, 3, and 4, show the probabilities of each predetermined contrast. By displaying the mean treatment values for all traits, however, it is possible to discuss the significance of contrasts in relation to individual treatment means.

Rearing

For most rearing traits, the differences between the *ad libitum*-fed control group and the restricted groups were highly significant (Table 2). Our effort to synchronise body weight gain between RLs and RFs in the starter phase and RLg and RFg in the grower phase was successful (Fig.1A). As a matter of fact, total body weight gain over the entire

Table 2. Effect of feeding strategy on body weight gain, feed and lysine intake and conversion ratios during rearing.

	Treatment[1]					RMSE[2]	Probability level of contrasts[3]			
	C	RLs	RFs	RLg	RFg		R	T	Ms	Mg
Body weight gain (kg)[4]										
0- 6 weeks	.42	.16	.16	.42	.42	.01	<.001	...	.245	...
7-18 weeks	.83	.94	.97	.73	.72	.03	<.001	...	...	.507
0-18 weeks	1.25	1.10	1.13	1.14	1.13	.04	<.001	.044	.074	.527
Cumulative feed intake (kg per hen)										
0- 6 weeks	1.06	.59	.49	1.05	1.05	.02	<.001	...	<.001	...
7-18 weeks	5.17	4.74	4.83	4.86	4.41	.12	<.001	...	...	<.001
0-18 weeks	6.23	5.33	5.32	5.91	5.46	.13	<.001	<.001	.793	<.001
Cumulative dig.lysine intake (g per hen)										
0- 6 weeks	9.0	2.4	4.2	8.9	8.9	.2	<.001	...	<.001	...
7-18 weeks	33.6	30.8	31.4	15.1	28.7	.6	<.001	...	...	<.001
0-18 weeks	42.6	33.2	35.5	24.0	37.6	.7	<.001	<.001	<.001	<.001
Feed conversion ratio (FCR;kg feed/kg gain)										
0- 6 weeks	2.52	3.64	3.09	2.52	2.53	.06	<.001	...	<.001	...
7-18 weeks	6.24	5.06	4.97	6.69	6.15	.18	.016	...	...	<.001
0-18 weeks	4.98	4.85	4.71	5.17	4.82	.11	.022	<.001	.006	<.001
Lysine conversion ratio (LCR;g lysine * 100/g gain)										
0- 6 weeks	2.14	1.46	2.63	2.14	2.15	.04	<.001	...	<.001	...
7-18 weeks	4.05	3.29	3.23	2.07	4.00	.09	<.001	...	...	<.001
0-18 weeks	3.41	3.02	3.15	2.10	3.32	.06	<.001	<.001	<.001	<.001

[1] C: control, no restrictions; RLs: lysine restricted in starter phase; RFs: feed restricted in starter phase; RLg: lysine restricted in grower phase; RFg: feed restricted in grower phase.

[2] RMSE = root mean square of the residual; degrees of freedom of the error term are 40 (45 observations).

[3] Contrasts between treatments:

R = effect of restriction per se 'ad lib vs restricted'; 0-18 wks: [-4 1 1 1 1]; 0-6 wks: [-2 1 1 0 0]; 7-18 wks: [-2 0 0 1 1]
T = effect of phase 'starter vs grower'; only for traits 0-18 wks: [0 1 1 -1 -1]
Ms = effect of method 'lysine vs feed' within the starter phase: [0 1 -1 0 0]
Mg = effect of method 'lysine vs feed' within the grower phase: [0 0 0 1 -1]
... : not included in the model.

[4] Body weight of 1-d-old chicks was 37 g in all groups.

rearing period (0 to 18 wk of age) differed only slightly among the four restricted groups (Table 2). The slight effect of phase of restriction on total gain during rearing ($P < .044$; Table 2) was a consequence of the small difference in body weight gain (30 g) between RLs and RFs birds, from 7 to 18 wk of age, caused by an increased voluntary feed consumption by birds on the latter treatment in week 7, immediately after the previous deprivation period.

The fixing of feed or lysine intake in each restricted group during one of the rearing phases led to the high level of significance of the subjected contrasts in these traits. For the groups RFs and RFg feed intakes (and thus lysine intakes) in the phases of actual restriction were 46.2 and 85.7%, respectively, of the intake of the control. Lysine intakes in the restricted phases by pullets of RLs and RLg were 26.7 and 43.5%, respectively, of that of the control. The total feed intakes of the RLs and RLg groups were depressed by 44.3 and 5.8%, respectively, compared to the control.

Feed and lysine conversion ratios varied considerably between treatments, indicating some differences in needs for maintenance and deposition of body components. During the starter phase, FCR was greater for the feed restricted (RFs) than for the control group. During the grower phase, however, RFg-pullets had a somewhat lower FCR than the C-pullets. Lysine utilisation, expressed as lysine(g)/gain(g), improved considerably in pullets fed the lysine-deficient diets in the appropriate phases (Table 2).

Mortality during the first two weeks of rearing was higher in treatment RFs (11.5%) than in the other treatments (2.9%). On average, mortality rate up to 7 wk of age was 5.5%. After week 7, no more pullets died during rearing.

Onset of lay

A summary of the traits associated with sexual maturity is presented in Table 3. Sexual maturity was delayed in birds on all restricted treatments ($P < .001$). Moreover, the method of restriction affected the age at maturity ($P < .038$ and $P < .001$). Lysine-restriction (RLs and RLg) delayed onset of lay by about 8 d, whereas feed restriction (RFs and RFg) resulted in a smaller delay of about 3 d (Table 3). The pronounced delay in onset of lay of the RL-groups is reflected by the subsequent low egg number output during early lay (Table 4).

Pullets in all groups started egg production at similar body weights (1.45 kg): none of the contrasts on gain from hatch to maturity was significant (Table 3). However, total feed and lysine intake until maturity varied considerably among treatments. Pullets of RFs and RFg ate about 7% less from hatch to onset of lay than pullets of the control and lysine-restricted groups. Pullets of each RL-group consumed less feed and lysine during rearing, as expected. Ultimately, however, they consumed equal amounts of feed up to maturity to the C-pullets, because of an increase in feed intake a few weeks preceding

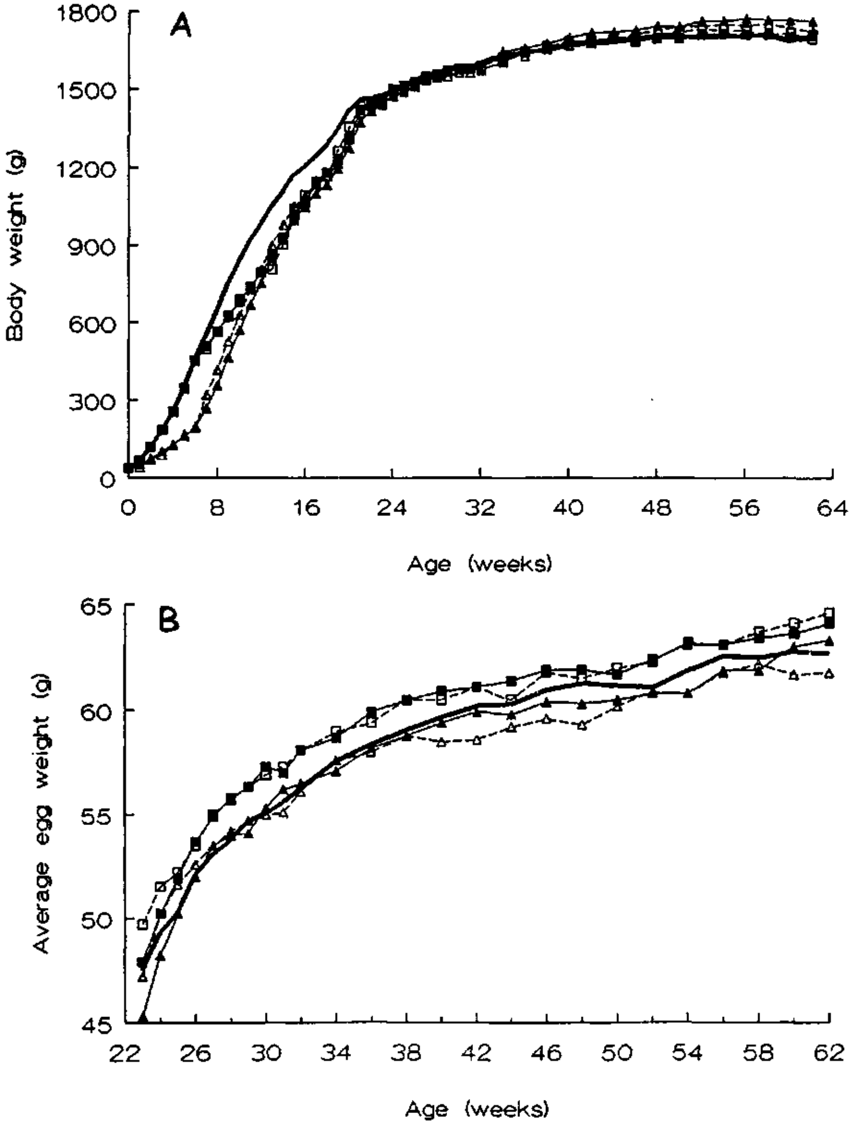

Figure 1. Production characteristics of pullets and hens, which were either fed *ad libitum* (C) or which were restricted in lysine (RL) or feed (RF) during the starter (s) or grower (g) phase; symbols are for C (▬), RLs (—▲—), RFs (--△--), RLg (—■—) and RFg (--□--). A. Body weight from 0 - 62 wk of age, B. Average egg weight from 23 - 62 wk of age.

lay (Tables 2 and 3). A small increase in feed intake during these preceding weeks was also observed in the RF-groups. Although feed intake for the RL-pullets during this stage was greater, all restricted groups showed similar weight gains from 18 wk to maturity (Table 3).

Pullets restricted during the grower phase (RLg and RFg) laid heavier eggs directly after commencing production than did pullets of all other groups ($P<.001$).

Laying

The means in Table 4 represent production traits over the early (19-32 wk of age) and the entire (19-62 wk of age) laying period. Initially, birds in each restricted group had a high rate of body weight gain, and no differences in body weight between the 5 treatments could be demonstrated at 32 wk of age (Fig.1A; Tables 2 and 4). Among the restricted treatments, weight gain during lay was affected by the phase when the restriction had been applied, pullets restricted during the starter phase gaining more rapidly than those restricted during the grower phase ($P<.013$).

During the early laying period, egg production by the lysine-restricted birds (RLs and RLg) was lower than that of the birds in the other groups ($P<.004$), reflecting the delay in maturation of the RL-groups (Table 4). No differences in number of eggs per hen between groups could be demonstrated over the entire laying period, probably because of the increasing variation between experimental units in the course of the laying period. Although it was not tested statistically, rate of lay during the second part of the laying period, however, seemed to be higher for all restricted birds than for the controls (Table 4).

Birds restricted during the grower phase laid eggs on average 1.7 g heavier over the entire laying period than did birds restricted during the starter phase ($P<.007$; Table 4; Fig.1B). Total egg mass production (19-62 wk of age) was greater for hens restricted during the grower phase than for those restricted during the starter phase ($P<.021$).

FCR (g feed/g egg mass) during the early laying period (19-32 wk of age) was closely related to the delay in onset of lay (Tables 3 and 4). Each lysine-restricted group had a higher FCR than the respective feed-restricted group ($P<.005$ and $P<.003$; Table 4), mainly because of the relatively low egg output. These differences in FCR persisted over the entire laying period, but were no longer significant. There were no differences between treatments in feed intake during lay.

During the laying period, there was no significant effect on the production of 'abnormal' egg mass (Table 4). Group RFg, however, showed a tendency to produce a relatively larger number of abnormal eggs. This was a consequence of the high incidence of broken and cracked eggs in this group (3.1%), compared with all others (1.3%), especially during the early laying period.

Table 3. Effect of feeding strategy during rearing on age and egg weight at onset of lay[1] and gain, cumulative feed and lysine intake to onset of lay.

	Treatment[1]					RMSE[2]	Probability level of contrasts[3]			
	C	RLs	RFs	RLg	RFg		R	T	Ms	Mg
Age (days)	149	157	153	156	150	4	<.001	.128	.038	<.001
Body weight gain (kg)										
wk 0-onset	1.43	1.40	1.39	1.41	1.41	.04	.119	.231	.902	.833
wk18-onset	.18	.30	.26	.27	.28	.03	<.001	.469	.032	.665
Cumulative feed intake (kg per hen)										
wk 0-onset	7.80	7.63	7.33	8.20	7.34	.32	.147	.010	.049	<.001
wk18-onset	1.57	2.30	2.01	2.29	1.88	.28	<.001	.434	.031	.003
Cumulative dig.lysine intake (g per hen)										
wk 0-onset	53.0	48.4	48.8	39.1	50.0	2.1	<.001	<.001	.663	<.001
wk18-onset	10.4	15.2	13.3	15.1	12.4	1.8	<.001	.434	.031	.003
Initial egg weight (g)										
	42.4	43.2	43.4	46.7	45.4	2.4	.016	.001	.913	.246

[1] Onset of lay in this paper is defined as the age at which 50% production of normal eggs was reached.

[2] C: control, no restrictions; RLs: lysine restricted in starter phase; RFs: feed restricted in starter phase; RLg: lysine restricted in grower phase; RFg: feed restricted in grower phase.

[3] RMSE = root mean square of the residual; degrees of freedom of the error term are 40.

[4] Contrasts between treatments:
R = effect of restriction per se 'restricted vs ad lib' [-4 1 1 1 1]
T = effect of phase 'starter vs grower' [0 1 1 -1 -1]
Ms = effect of method 'lysine vs feed' within the starter phase [0 1 -1 0 0]
Mg = effect of method 'lysine vs feed' within the grower phase [0 0 0 1 -1]

Mortality during the laying period did not differ among the treatments; average mortality was 5.8%.

DISCUSSION

Several authors have stressed that body composition is more important than body weight in preparing pullets for egg production (Wells, 1980; Johnson *et al.*, 1985; Chi, 1985; Leeson, 1986; Summers *et al.*, 1987). No investigations have been reported, however, on pair-gained pullets in fixed rearing phases using different methods of restriction, with the preconceived aim of manipulating body composition and at the same time achieving similar body weights at the start of the laying period. This was the focus of the present study.

Rearing performance

In our experiment, the low-lysine diets indirectly reduced feed intake, perhaps because of amino acid imbalances (Lee *et al.*, 1971; Lahore and Payne, 1975).

The deficient lysine concentrations chosen for each rearing phase (.40% and .30% digestible lysine) were intended to give a similar severity of restriction. The severity was greater, however, for pullets restricted in the starter phase than for those restricted in the grower phase, as shown by the differences in feed intake between the RF- and C-pullets in each phase (Table 2). Compared with the control, RFs-pullets showed a higher FCR from 0 to 6 wk, whereas RFg-pullets had a lower FCR from 7 to 18 wk. This discrepancy might have been caused by the more severe restriction in the starter phase inducing a proportional change in fat and protein deposition.

The high mortality rate in group RFs may have been related to cold stress, caused by the severity of the restriction. Most occurred during the first few wk, when some huddling was observed. Mortality in RLs was lower, however, despite similar weights. A higher heat increment in the RLs-group, because of the amino acid-imbalanced diet, may have prevented RLs-pullets from suffering from cold stress.

In each rearing phase, pullets fed the lysine-deficient diet showed more intensive feather pecking, sometimes resulting in cannibalism (Kwakkel, unpublished observations), and this agrees with earlier work (Lee *et al.*, 1971; Lahore and Payne, 1975).

As a direct consequence of increasing feed intake with advancing age, the concentration of lysine in the diet necessary to satisfy the requirement of the RLg-group decreased over time; body weight gain therefore increased. To match body weights with the RLg-group, we gradually reduced the severity of the RFg restriction. Pullets in the RFg-group were actually restricted from 7 to about 15 wk of age (Table 5). Feed intake (FI)

Table 4. Effect of feeding strategy during rearing on body weight gain, feed intake, egg production and conversion ratio during the laying period.

	Treatment[1]					RMSE[2]	Probability level of contrasts[3]			
	C	RLs	RFs	RLg	RFg		R	T	Ms	Mg
Body weight gain (kg)										
19-32 weeks	.32	.46	.43	.40	.42	.04	<.001	.034	.102	.184
19-62 weeks	.42	.63	.56	.54	.53	.07	<.001	.013	.054	.854
Daily feed intake (g per hen)										
19-32 weeks	96.4	97.5	97.3	98.4	100.0	3.0	.110	.090	.861	.281
19-62 weeks	105.0	107.5	105.3	108.0	106.8	3.0	.123	.373	.167	.448
Number of eggs per hen[4]										
19-32 weeks	74	66	72	67	72	4	.001	.597	.004	.004
19-62 weeks	255	255	263	256	260	9	.391	.919	.106	.383
Average egg weight (g)[4]										
19-32 weeks	51.8	52.3	52.3	54.1	53.8	1.4	.018	.002	.911	.684
19-62 weeks	58.2	58.2	57.8	59.8	59.5	1.5	.255	.007	.677	.841
Total egg mass (kg/hen)										
19-32 weeks	3.96	3.62	3.84	3.76	4.09	.18	.057	.003	.012	<.001
19-62 weeks	15.29	15.21	15.59	15.76	16.06	.57	.102	.021	.174	.262
Feed conversion ratio (FCR: g feed/g total egg mass)										
19-32 weeks	2.39	2.64	2.49	2.57	2.40	.11	.003	.034	.005	.003
19-62 weeks	2.12	2.18	2.08	2.11	2.05	.08	.687	.113	.018	.104
Abnormal egg mass (%)										
19-32 weeks	3.3	4.1	2.4	3.7	5.0	2.0	.481	.100	.091	.188
19-62 weeks	2.9	2.6	2.6	2.8	3.5	1.3	.928	.225	.932	.294

[1] C: control, no restrictions; RLs: lysine restricted in starter phase; RFs: feed restricted in starter phase; RLg: lysine restricted in grower phase; RFg: feed restricted in grower phase.

[2] RMSE = root mean square of the residual; degrees of freedom of the error term are 40 for traits 19-32 wks and 34 for traits 19-62 wks.

[3] Contrasts between treatments:
R = effect of restriction per se 'ad lib vs restricted' [-4 1 1 1 1]
T = effect of phase 'starter vs grower' [0 1 1 -1 -1]
Ms = effect of method 'lysine vs feed' within the starter phase [0 1 -1 0 0]
Mg = effect of method 'lysine vs feed' within the grower phase [0 0 0 1 -1].

[4] based on the 'normal' egg mass.

per metabolic body size (kgW$^{.75}$) was only 2% below that of the control group at the end of week 15. From 15 to 18 wk of age, FI per kgW$^{.75}$ was even 9% above that of the control group. The high early egg production of RFg-hens (Table 3) could be related to this period of compensatory feed intake, enabling an acceleration of ovary development. Wells (1980) also found a similar positive effect of 'rehabilitation' on production after aperiod of feed restriction, and promoted the so-called 'mid-term' feed restriction as the most favourable feeding strategy for pullets. The theory of acceleration of ovary growthis supported by the relatively high percentage of abnormal egg mass in RFg-pullets (5.0% : Table 4). Hocking (1987) stated that high feed intake just before onset of lay might stimulate the occurrence of multiple ovulation, thereby resulting in the disruption of normal egg shell formation.

Table 5. Feed intake per kgW$^{.75}$ relative to the control group (100%) Lysine intake is given in parentheses.

Week	Treatment RLs	RFs	RLg	RFg
0 - 3	97 (46)	69	99	99
3 - 6	90 (42)	85	99	100
6 - 9	109	115	97 (45)	87
9 - 12	120	113	97 (45)	89
12 - 15	115	110	110 (51)	98
15 - 18	110	103	117 (54)	109

Sexual maturity and laying performance

Despite the similarity among the four restricted groups in overall body weight gain during rearing, lysine restriction delayed onset of lay more than feed restriction, regardless of the phase when the restriction had been applied. This result is supported by those from Gous (1978) and Wells (1980). The method of restriction may have affected body composition attained just before onset of lay, and this in turn may have affected age at maturity. It seems that body composition is a critical factor in determining the onset of lay (Johnson *et al.*, 1984; Johnson *et al.*, 1985; Summers *et al.*, 1987; Zelenka *et al.*, 1987).

Our results agree with those of Connor *et al.* (1977), who found that birds on restricted feeding require less feed for onset of egg production than do birds fed *ad libitum*. Our findings also indicate that lysine-restricted pullets need more feed up to maturity than pullets fed *ad libitum* during rearing. RLg-pullets which were severely undernourished in lysine during rearing (Table 2), especially tried to compensate for their 'deficiency status' by extra intake of feed directly after cessation of restriction, and before maturity. This resulted in a greater total amount of feed being consumed by the lysine-restricted birds from hatching to maturity compared with the controls (Table 3). In agreement with our results, Connor *et al.* (1977) and Gous (1978) reported that pullets fed on a diet deficient in lysine required more feed until first egg than pullets fed on an adequate diet or those being quantitatively restricted. Hence, a total rehabilitation of the lysine-deficient status seemed not to be necessary, whereas RLg-pullets started lay in spite of a lower cumulative lysine intake to the onset of lay. Their body weights and body compositions had probably reached thresholds, after which production commenced (Summers et *al.*, 1987).

In accordance with our findings, most reports demonstrate an increased initial and/or average egg weight from restricted birds (Lahore and Payne, 1975; Johnson *et al.*, 1984), despite the sometimes lower body weights at the onset of lay. According to Johnson *et al.* (1984), this phenomenon exactly reflects the highly positive age-egg size relationship. They concluded that this relationship might be influenced by the amount of feed eaten after cessation of restriction. Some authors have already postulated that hyperphagia (Polin and Wolford, 1973) or an increased intake of energy or fatty acids (Srichai and Balnave, 1981), between cessation of restriction and onset of lay, might explain the increase in egg size. The larger initial egg size of the restricted hens compared to the *ad libitum*-fed hens ($P<.016$), is in agreement with the above-mentioned relationship between age and egg size (Johnson *et al*, 1984). Moreover, the difference of more than 1.5 g in average egg size between starter- and grower-restricted birds may be explained by some kind of "triggering" by the elevated feed intake just after cessation of restriction. The gradual increase in feed intake in the grower-restricted groups had already started at about 15 wk of age (Table 5). The hypothesis of Srichai and Balnave (1981), therefore, seems reasonable. From 7 wk onwards, however, starter-restricted birds may not be yet susceptible to such triggering. Hence, the effect on their egg size is smaller. The greater initial egg weights in RLg- and RFg-pullets cannot be attributed to differences in body weight. Both body weight and body weight gain, just before onset and during early production, were similar in the restricted groups. Several authors, however, have found egg weight to be dependent primarily on body weight (Wells, 1980; Summers, 1983; Summers and Leeson, 1983). Our results agree with those of Connor *et al.* (1977), who found that restriction (low lysine or feeding level) during the starter phase tended to reduce egg weight; partly on the basis of this finding, they rejected practising restriction at early ages.

Starter-restricted pullets gained more weight during lay than did grower-restricted pullets. Considering their average egg weight, they may have used feed for gain instead of egg mass (Table 4), or their maintenance needs may have been altered.

Restricted pullets in this experiment laid more eggs during the second part of the laying period than *ad libitum* fed pullets. Frankham and Doornenbal (1970) supposed that the improved egg production of restricted reared pullets is a consequence of an increased sensitivity of the ovary to gonadotrophins. Watson (1975) observed larger oviducts and greater rates of follicular growth possibly caused by altered gonadotrophin output. Hocking (1987), however, suggested that *ad libitum* fed pullets produce too many follicles at the beginning of lay, thereby inducing multiple and internal ovulation and causing disrupted and unsatisfactory egg production. Therefore he advised against a high energy intake just before onset of lay. This is partly in disagreement with our findings, which support the idea of some favourable effect of a gradually increasing feeding level at the end of rearing on subsequent performance (RFg: Tables 3, 4 and 5). In addition, the larger number of cracked eggs in group RFg might be the result of initiating egg production at ages similar to those of the control group, but with much larger eggs.

The better overall performance of the grower-restricted birds (RLg and RFg) is mainly attributable to the increased egg weights, as discussed above.

Wells (1980) rejected early lysine restriction as a possible feeding strategy during rearing, because his results showed comparatively poor biological and economic performance in birds so reared. Chi (1985) postulated that lysine restriction was critical in pullet development during the starter phase. He reported that pullets might overcome a body weight retardation quite well but that their capacity to perform might be irreversibly damaged.

The design of the experiment described in this paper eliminated the pure effect of body weight gain. Our results indicate that immature body weight is of minor importance in determining both the time of onset of lay and egg weights during the laying period. However, we still need to understand which 'underlying' physiological mechanisms are responsible for the observed differences in performance. Detailed investigations of the effects of feeding strategies during rearing on tissue and organ development are needed.

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Chapter 4

MULTIPHASIC ANALYSIS OF GROWTH OF THE BODY AND ITS CHEMICAL COMPONENTS IN WHITE LEGHORN PULLETS

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MULTIPHASIC ANALYSIS OF GROWTH OF THE BODY AND ITS CHEMICAL COMPONENTS IN WHITE LEGHORN PULLETS

R.P. Kwakkel, B.J. Ducro and W.J. Koops

ABSTRACT

Data of White Leghorn pullets consuming feed *ad libitum* were used to study multiphasic growth during rearing and early lay. Two mathematical functions were used. Body weight gain and growth of the chemical components DM, CP, crude fat (Cfat), and ash (Ash) were described as a function of age, using a multiphasic growth function. To describe relative growth between CP and DM, Cfat and DM, and Ash and DM, a multiphasic allometric function was applied. Body weight gain from hatching to 24 wk of age was estimated most accurately by a tetraphasic growth function. The first two growth spurts represented 82% of mature BW. Growth in these two phases seemed to be related to the development of bones, muscles, and essential metabolic organs. Body growth was further characterized by a third phase at 19 wk of age, which was related to growth of the reproductive tract. The fourth phase consisted mainly of body fat deposition.

Both multiphasic functions showed that the nature of component growth was diphasic, with a transition area between the two phases at around 11 wk of age. On the basis of the duration of growth and of allometric growth coefficients (slopes), growth of CP, Cfat, and Ash in the first phase seemed to be functionally related to each other and consisted of muscle growth, intermuscular fat deposition, and skeletal growth, respectively. After 11 wk of age, protein deposition is presumably a function of sexual maturity, i.e., the development of the reproductive tract; whereas fat growth is mainly abdominal fat deposition. The observed relationships indicate that the use of multiphasic functions might be helpful in the interpretation of changes in body growth and development of pullets.

Key words: multiphasic functions, allometry, growth pattern, components, pullets.

INTRODUCTION

For many years, much emphasis in research has been laid on BW at end of rearing as the most important feature in evaluating pullet feeding strategies with respect to subsequent laying performance (Gous and Stielau, 1976; Robinson and Sheridan, 1982; Balnave, 1984). Undoubtedly, a minimum prelay BW is necessary for the onset of lay. The shape of the pullets' growth curve, however, may give additional information on performance ability than does prelay BW when used as the sole variable (Leeson and

Summers, 1980; Wells, 1980; Kwakkel *et al.*, 1991). The body growth curve is determined by the rates of deposition of chemical body components such as DM, CP, crude fat (Cfat), and ash (Ash), which are related to the bird's age. From a biological point of view, the deposition of these body components in individual organs determines a bird's physiological age and stage of maturity. To elucidate the functional relationships between immature development and mature performance, more insight into the complexity of growth in young birds is required (Ricklefs, 1985).

Normally, if the nutrient supply is not limiting, each body component exhibits its own distinctive pattern of growth and functional maturation (Ricklefs, 1975). Consequently, there will be a variation in the nutritional demand of specific tissues and organs in the course of time, due to the biological forces in the development of these body constituents. However, in restriction programs commonly used for modern pullets, the nutrient supply is often limited. Thus, in order to ensure that the nutrition is favorable to the development of vital structures (e.g., the reproductive tract for the layer) at the right time, it becomes necessary to know exactly when these body structures develop.

An experiment was conducted to study the effects of several nutrient restriction programs during rearing on growth and laying performance, and results on egg performance have been reported by Kwakkel *et al.* (1991). In that experiment, a number of pullets had been dissected to investigate body composition in relation to body growth and nutrient supply. The current paper is focused on the body composition of control birds, which consumed *ad libitum* diets that provided sufficient nutrients to meet pullet requirements. The growth patterns of the entire body and of its chemical components were investigated by fitting the data to two mathematical functions.

A multiphasic growth function (MGF) was used to describe BW gain and body component growth as a function of age (Koops, 1986). This MGF is based on the summation of n sigmoid curves; each curve represents one growth cycle described by a logistic function. Each growth cycle might consist of growth spurts of specific body structures. The MGF was used to characterize immature growth by defining number of spurts, age at maximum gain, and magnitude and duration of growth spurts of the entire body and of the chemical components in the control pullets. Different growth spurts of CP, Cfat, and Ash are related to growth spurts of total DM. The multiphasic nature of component growth was also assessed on a relative basis by describing growth of CP, Cfat, and Ash separately as allometric functions of DM growth. In this case a multiphasic allometric function (MAF) (Koops and Grossman, 1993) was used. The objective of the current study was to investigate the usefulness of both multiphasic functions in characterizing growth of the body and its chemical components in pullets consuming feed *ad libitum*.

MATERIALS AND METHODS

Data

In 1989, an experiment was performed to study the effect of a lysine or total feed restriction during two periods of rearing on growth and egg performance in a commercial strain (Lohmann LSL, Cuxhaven, Germany) of White Leghorn (WL) pullets and hens. Results on egg production are described elsewhere (Kwakkel *et al.*, 1991). The control group, described in this paper, received from 0 to 6 wk of age a commercial starter diet (.85% digestible lysine, .71% digestible methionine + cystine, 18.9% CP, 1.0% calcium, .41% available phosphorus, and 2,800 kcal ME/kg). From 7 to 18 wk of age these pullets were fed a commercial grower diet (.65% digestible lysine, .60% digestible methionine + cystine, 15.9% CP, .9% calcium, .44% available phosphorus, and 2,800 kcal ME/kg). After 18 wk of age, the hens were fed a commercial layer diet (.65% digestible lysine, .58% digestible methionine + cystine, 16.5% CP, 3.5% calcium, .64% available phosphorus, and 2,800 kcal ME/kg). All diets were provided for *ad libitum* consumption. Pullets were not debeaked. A detailed description of diets and management is reported by Kwakkel *et al.* (1991).

Birds were housed in nine replicate cages. Body weight was recorded weekly per cage from hatch to 32 wk of age. The number of birds per cage decreased from 24 at the start of the experiment to 8 in week 24, because of weekly removal of birds for dissection purposes. Each week, one or no bird per cage was removed randomly. A total of five to eight pullets were euthanatized by cervical dislocation per week. Birds were defeathered and dissected. Gut fill was removed. Loss of body material during dissection was avoided. Water evaporation from the body was minimized by covering the pullets with wet paper. Defeathered bodies (including all viscera) of pullets of weeks 0, 3, 6, 9, 12, 15, 18, 20, 22, and 24 were frozen, minced, and freeze-dried. One-day old pullets were minced in two samples of five birds each. Pullets of weeks 3, 6, and 9 were pooled in samples of two birds. In all other weeks, birds were minced individually. The chemical components DM, CP (Kjeldahl method; N x 6.25), Cfat (ether extraction), and Ash were determined in these samples.

Multiphasic Growth Function

Mean BW of live birds in each week were used for computing the MGF. Cage was the experimental unit. In order to avoid the influence of error weights within a specific week, mean BW gain was computed by function [1].

$$G_n = (W_{n+1} - W_{n-1}) / 2 \qquad [1]$$

where G_n is the average weekly gain in week n; W_{n+1} is the average BW in week n+1; and W_{n-1} is the average BW in week n-1.

Chemical component data on the basis of live birds were estimated by calculating the ratio between the weight of a component and BW of a dissected bird in that week. Multiplying the average ratio in that week with the live birds' BW provided an estimate for the weight of the component in the live birds. Chemical composition of birds in weeks 0, 3, 6, 9, 12, 15, 18, 20, 22, and 24 were available for computations. Analogous to the calculation of weekly BW gain (see [1]), component growth in week n was computed as the difference in component weight between week n+3 and week n-3 divided by 6 wk for $n < 18$. For $n > 18$, component growth was computed by (n+2) - (n-2), divided by 4 wk. The composition of a normal egg without the shell (13.7 g DM, 6.6 g CP, 6.7 g Cfat, and .6 g Ash) was assumed to be representative for the pullets' body composition at start of embryonic development (week -3). Component gain in weeks -3 and 24 were assumed to be zero. Finally, gain data of weeks -3, 0, 3, 6, 9, 12, 15, 17.5, 20, 22, and 24 (n=11) were used in the analyses for the fitting of the MGF curves.

The MGF (Koops, 1986) describes the gain-age relationship as the first derivative of the weight-age curve. For a better interpretation of curve parameters, the function has been reparameterized (Kwakkel and Koops, 1992):

$$y_t = \sum_{i=1}^{n} \left(2A_iB_i^{-1} \left\{ 1 - \tanh^2 \left[4B_i^{-1} (t - C_i) \right] \right\} \right) \qquad [2]$$

where y_t is the predicted value of mean weight gain in week t; n is the number of phases; and in each Phase i, A_i is the asymptotic weight gained (grams); B_i is duration of phase [(weeks); according to Koops and Grossman (1991a)]; tanh is hyperbolic tangent; and C_i is age at maximum gain (weeks).

Multiphasic Allometric Function

Average data of component weights in weeks 0, 3, 6, 9, 12, 15, 18, 20, 22, and 24 (n = 10) were used in the analyses of the MAF. Each linear segment in the relation from one body component to another can be described by the simple allometric function of Huxley (Koops and Grossman, 1991b):

$$y_x = \alpha x^{\beta} \qquad \text{or} \qquad \ln(y_x) = \ln(\alpha) + \beta \ln(x) \qquad [3]$$

where y_x is the weight of the body component (CP, Cfat, or Ash); x is the weight of DM; α is the scale parameter [$\ln(\alpha)$ is intercept]; and β is the allometric growth coefficient (slope).

However, a simple allometric relationship between two body structures is not valid if the nature of growth is multiphasic. Multiphasic allometry can be described by a function (the MAF) allowing smooth transitions from one allometric level to another (Koops and Grossman, 1993).

The MAF is an extension of Equation [3], providing more than one linear segment, connected to each other by smooth transitions:

$$\ln(y_x) = \ln(\alpha_{i-1}) + \beta_i \ln(x) - \sum_{i=1}^{n-1} \{(\beta_i - \beta_{i+1})\, r_i \ln[1 + (x/c_i)^{1/ri}]\} \qquad [4]$$

where y_x, x, and α are as in Equation [3]; n is the number of phases; and in each phase i, β_i is the allometric slope, c_i is the estimated breakpoint between phase i and i + 1, and r_i is a smoothness parameter.

For both types of multiphasic functions, curves were fitted using a nonlinear regression procedure PROC NLIN (Method Dud; convergence criterion 10^{-8}; SAS Institute, 1991). Goodness of fit was checked on four criteria: 1) asymptotic SE (ASE) for each parameter; 2) residual SD (RSD); 3) coefficient of determination (CD); and 4) Durbin-Watson statistic (DW; a test on first-order autocorrelation).

Diphasic functions of DM, CP, Cfat, and Ash growth were compared to monophasic functions in order to verify the multiphasic nature of component growth. The significance of the addition of an extra phase (n-phasic towards (n + 1)-phasic functions) was assessed by an *F* test. The difference in residual MS (RMS) between the n and (n + 1) phasic function was tested against the RMS of the (n + 1)-phasic function.

RESULTS AND DISCUSSION

Body growth

Parameter estimates for asymptotic weight, duration, and age at maximum gain in each phase i for a tri-, tetra-, and pentaphasic model are listed in Table 1. For the triphasic function, large ASE on most of the parameter estimates show that the data fitted poorly. The tetra- and pentaphasic functions showed better results according to their ASE and goodness of fit criteria. Both functions estimated equal total asymptotic weights (1,669 g for the tetraphasic and 1,671 g for the pentaphasic function), whereas a higher total asymptotic weight of 1,756 g was estimated by the triphasic function. For the tetra- and pentaphasic function, differences in parameter estimates between both functions were negligible in the first three phases. The partitioning of the fourth phase in the tetraphasic

Table 1. Parameter estimates and asymptotic SE (in parentheses) of growth of the entire body, described by tri-, tetra-, and pentaphasic growth functions[1].

Number of phases	Phase	Parameter estimates Asymptotic weight gained Absolute (g)	 Cumulative[2] (%)	 Duration (wk)	 Age at max. gain (wk)	Goodness of fit RSD[3]	 CD[4]	 DW[5]
Triphasic	1	862 (1026)	49	22.3 (8.9)	6.6 (.7)			
	2	808 (1255)	95	48.4 (24.8)	13.0 (14.1)			
	3	86 (22)	100	4.1 (.9)	19.2 (.1)			
						5.80	.9932	1.54
Tetraphasic	1	1150 (102)	69	22.4 (1.7)	6.6 (.3)			
	2	215 (98)	82	13.1 (4.0)	14.0 (.4)			
	3	169 (24)	92	5.8 (.7)	19.2 (.1)			
	4	135 (21)	100	13.9 (2.8)	25.6 (.4)			
						3.97	.9972	2.41
Pentaphasic	1	1155 (88)	69	22.4 (1.5)	6.6 (.3)			
	2	207 (84)	82	12.7 (3.4)	14.0 (.4)			
	3	185 (22)	93	6.2 (.6)	19.2 (.1)			
	4	49 (36)	96	5.3 (2.3)	24.2 (.3)			
	5	75 (43)	100	10.7 (5.5)	27.5 (1.2)			
						3.57	.9981	2.82

[1] $y_t = \Sigma\ [2A_iB_i^{-1}\{1-\tanh^2(4B_i^{-1}(t-C_i))\}]$, where, in each phase i, A_i is the asymptotic weight gained (grams); B_i is the duration (wk); tanh is hyperbolic tangent; and C_i is the age at maximum gain (wk).

[2] Cumulative weight gained as a percentage of mature BW.

[3] Residual SD (n in all fits was 31).

[4] Coefficient of Determination.

[5] Durbin Watson statistic: a value less than 2 indicates a positive autocorrelation, a value greater than 2 a negative autocorrelation.

model into two phases in the pentaphasic model seemed irrelevant, with regard to the large ASE on the asymptotic weights in the two split up phases. The addition of an extra phase to the triphasic model improved the fit significantly ($P < .001$; $F(3,19) = 9.32$). However, the assumption of an additional fifth phase to the tetraphasic model was not confirmed by the *F* test (NS; $F(3,16) = 2.50$). On the basis of these considerations, it was concluded that the body growth data were described most accurately by the tetraphasic growth model.

Almost 70% of mature BW has been gained at around 14 wk of age (see Table 1: percentage asymptotic weight gained in the first phase; tetraphasic model). As 80% of adult weight is being reached, body growth per unit of time decreases rapidly, to attain its lowest value around 16 wk of age. Hereafter, a sharp increase (short duration:

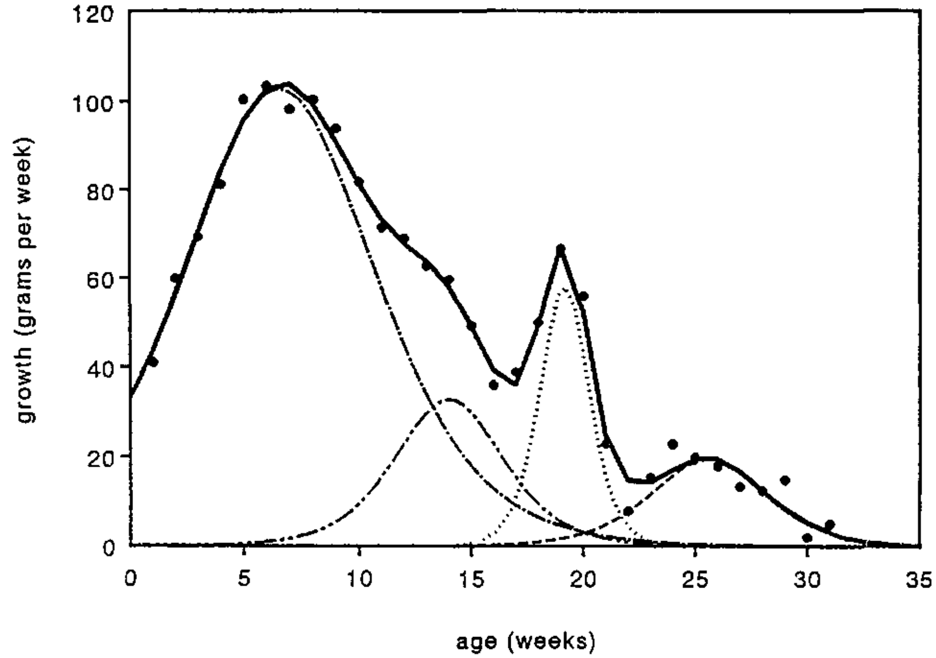

Figure 1. Gain pattern of body weight from hatch to 32 wk of age of White Leghorn pullets and hens consuming feed *ad libitum*, fitted by a tetraphasic growth function (● , observations; ———————— , tetraphasic; —— • —— , first phase; —— • • —— , second phase; • • • • •, third phase; — — — — — , fourth phase).

B_3 = 5.8 wk) in BW, with its peak at around 19 wk of age, marks a third clearly identified spurt (Figure 1). Only 8% of total BW is accrued after 22 wk of age.

Grossman and Koops (1988) fitted body growth data of White Leghorn layers to demonstrate the usefulness of a multiphasic growth function for poultry, and the results summarized in Table 2. Grossman and Koops (1988) detected two phases of growth, whereas in the present paper data were fitted to a tetraphasic function. The difference in number of estimated phases could be explained partially by the higher variance in the model as illustrated by the RSD in Table 1 compared with the one in Table 2. The lower RSD in the present model are probably the result of the procedure for computing gain in week n, using weights of week (n + 1) and week (n-1). This may have had a smoothing effect on the data structure.

Table 2. Parameter estimates of growth of the entire body of White Leghorn hens, described by a diphasic growth function[1] by Grossman and Koops (1988)

Phase	Parameter estimates				Goodness of fit	
	Asymptotic weight gained		Duration	Age at max. gain	RSD[3]	DW[4]
	Absolute (g)	Cumulative[2] (%)	(wk)	(wk)		
1	1499	82	33.3	1.8		
2	334	100	11.9	27.3		
					13.2	2.1

[1] $y_t = [2A_1B_1^{-1}\{1\text{-}\tanh^2(4B_1^{-1}(t\text{-}C_1))\}] + [2A_2B_2^{-1}\{1\text{-}\tanh^2(4B_2^{-1}(t\text{-}C_2))\}]$, where, in each phase, A is the asymptotic weight gained (grams); B is the duration (wk); tanh is hyperbol tangent; and C is the age at maximum gain (wk). Reparameterized: in Grossman and Koops (1988): a, b, and c; in this paper: A, B, and C; $A = 2*a$, $B = 4*b^{-1}$, $C = c$.

[2] Cumulative weight gained as a percentage of mature BW.

[3] Residual SD (n in all fits was 43).

[4] Durbin Watson statistic: a value less than 2 indicates a positive autocorrelation, a value greater than 2 a negative autocorrelation.

From Tables 1 and 2, it can be hypothesized that the first phase in the work of Grossman and Koops (1988) is a combination of the first and second phase of the present work. This is enhanced by the respective parameter estimates: total asymptotic weight gained, as a percentage of mature weight cumulated over the first two phases was 82% (Table 1). That value is equal to that observed by Grossman and Koops in 1988 (Table 2; first phase). Estimates for age at maximum gain of 6.6 and 14.0 wk for the first and second phases, respectively in Table 1 could be combined easily to the estimate of age at maximum gain for the first phase in Table 2 (11.8 wk). Duration was almost comparable: a total of 35.5 (22.4 + 13.1) wk in the current results (Table 1) was only slightly different to the duration of the first phase in Table 2 (33.3 wk).

The first two posthatch growth phases seem to represent general growth: skeletal development, feathering, development of muscles, and other essential organs (e.g., digestive tract) necessary for maintenance (Lilja et *al.*, 1985). Grossman and Koops (1988) hypothesized that their second growth phase was associated with sexual maturity. That growth spurt is comparable to the third phase in the present work. Consequently, growth in this maturity phase (169 g) should be the result of an increase in growth of the reproductive tract. This is consistent with observations on ovary and oviduct growth (about 95 g; Kwakkel, unpublished data). Abdominal fat deposition (about 40 g in this stage; Kwakkel, unpublished data) is the other body constituent representing growth within this phase. Age at maximum gain of the maturity phase in the current study (19.2

wk) differed from the 27.3 wk found by Grossman and Koops (1988); part of this difference can be explained by the genetic progress for earlier sexual maturity (1967 to 1989; 1 day/yr: Leeson, 1986). Duration of the maturity phase in the present study and that of Grossman and Koops (1988) was about 6 and 12 wk, respectively. A genetically enhanced rate of development of the reproductive system in the modern Leghorn may account for the observed difference in duration. Although the modern hen is quite different genetically from the one in production in 1967, the pullet still needs to complete 82% of mature weight before the maturity spurt starts. Growth of the reproductive tract and deposition of adipose tissues at same ages is likely, because it is widely assumed that there is a physiological relationship between sexual maturity and increased fat metabolism, induced by changing sex hormone levels.

The fourth phase in the present model was also recognized by Grossman and Koops (1988). However, the addition of an extra phase in their model did not improve the fit significantly, so the phase was not added. Because of this omission, the duration of their maturity phase (12 wk) was probably overestimated. In the current study this phase was fitted seperately. Gain in this phase most likely consists of abdominal fat deposition.

Component growth

Parameter estimates for growth phases of body components, using the MGF and the MAF, are presented in Table 3. For the MGF, all goodness of fit criteria point towards the existence of a diphasic model in all components. Different ages at maximum gain for CP and Cfat in the monophasic growth model (8.4 and 14.0 wk, respectively) indicate the diphasic nature of total DM growth. Moreover, the individual components also show a better diphasic than a monophasic fit (Figure 2). However, due to the small number of observations ($n = 11$) compared to the large number of parameters that have to be estimated for a diphasic growth function, the ASE are quite large. Therefore, it is not surprising that the assumption of an extra phase to the monophasic growth model improved only the fit for Ash significantly [$P < .025$; $F(3,5) = 8.76$]. For DM, CP, and Cfat, $F(3,5)$-values were 1.18, 4.82, and .15, respectively. Although fit criteria and plots (Figure 2) evidently show the diphasic nature of growth of the components, examination of the MAF must strengthen this hypothesis.

The assumption of simple allometry did not fit the data very well. Residuals for the simple model were not randomly distributed around the zero axis. They showed periodic deviations, characteristic for data fitted by a simple allometric function for which a broken stick curve is expected (Figure 3). In all cases the allometric fit was significantly improved by assuming a diphasic allometric function instead of a monophasic one. The $F(2,6)$-values for CP-DM, Cfat-DM, and Ash-DM were 39.29 ($P < .001$), 7.31 ($P < .025$), and 28.50 ($P < .001$), respectively. In the present paper, only four parameters of the

Table 3. Parameter estimates and asymptotic SE (in parentheses) of growth of the chemical components DM, CP, crude fat (Cfat) and Ash, described by both the Multiphasic Growth Function[1] and the Multiphasic Allometric Function[2].

		Multiphasic Growth Function						
Chemical component	Phases	Parameter estimates				Goodness of fit		
		Asymptotic weight gained		Duration	Age at max. gain	RSD[4]	CD[5]	DW[6]
		Absolute (g)	Cumulative[3] (%)	(wk)	(wk)			
DM	Mono 1	579 (60)	100	34.3 (4.5)	11.4 (.8)	.776	.8493	1.25
	Di 1	380 (94)	69	25.2 (5.7)	8.3 (1.2)			
	2	170 (82)	100	14.4 (4.3)	17.0 (.7)	.526	.9567	2.26
CP	Mono 1	234 (17)	100	28.8 (2.6)	8.4 (.5)	.240	.9356	1.54
	Di 1	212 (23)	91	25.2 (3.3)	7.9 (.5)			
	2	20 (16)	100	10.0 (6.2)	17.6 (1.2)	.224	.9649	1.99
Cfat	Mono 1	265 (29)	100	29.4 (4.1)	14.0 (.7)	.411	.8537	1.28
	Di 1	123 (31)	47	22.6 (4.8)	8.5 (1.1)			
	2	137 (29)	100	15.5 (2.0)	16.7 (.4)	.170	.9843	1.84
Ash	Mono 1	48 (8)	100	25.7 (5.3)	7.2 (.9)	.120	.7107	1.63
	Di 1	42 (3)	82	20.6 (2.0)	6.8 (.3)			
	2	9 (8)	100	6.1 (3.8)	18.9 (1.7)	.038	.9815	2.36

		Multiphasic Allometric Function						
y/x	Mono/Di phasic	Parameter estimates				Goodness of fit		
		Scale parameter	Allometric slope Phase 1	Phase 2	Breakpoint	RSD[4]	CD[5]	DW[6]
CP/DM	Mono	-.20 (.16)	.91 (.03)	-	-	.117	.9917	.64
	Di	-.51 (.07)	1.00 (.02)	.46 (.11)	5.66 (.14)	.036	.9994	3.51
Cfat/DM	Mono	-1.79 (.16)	1.15 (.03)	-	-	.114	.9950	1.17
	Di	-1.53 (.13)	1.08 (.03)	1.51 (.19)	5.61 (.33)	.071	.9986	3.46
Ash/DM	Mono	-1.71 (.24)	.91 (.04)	-	-	.174	.9816	.65
	Di	-2.21 (.12)	1.05 (.03)	.40 (.13)	5.46 (.18)	.062	.9983	2.77

[1] $y_t = [2A_1B_1^{-1}\{1\text{-}\tanh^2(4B_1^{-1}(t\text{-}C_1))\}]$ (monophasic, Mono)
$y_t = [2A_1B_1^{-1}\{1\text{-}\tanh^2(4B_1^{-1}(t\text{-}C_1))\}] + [2A_2B_2^{-1}\{1\text{-}\tanh^2(4B_2^{-1}(t\text{-}C_2))\}]$ (diphasic, Di)
where, in each phase i, A_i is the asymptotic weight gained (grams), B_i is the duration (wk), tanh is *hyperbolic tangent, and* C_i *is the age at maximum gain* (wk).

[2] $\ln(y_x) = \ln(\alpha) + ß_1\ln(x)$ (monophasic, Mono)
$\ln(y_x) = \ln(\alpha) + ß_1\ln(x) - .1\,(ß_1 - ß_2)\ln[1 + e^{(\ln(x)-\ln(c))/.1}]$ (diphasic, Di)
where $\ln(\alpha)$ is the scale parameter, $ß_1$ and $ß_2$ are the allometric slope of phase 1, and 2, respectively, ln (c) is the breakpoint.

[3] Cumulative weight gained as a percentage of mature weight of that component.

[4] Residual SD: n in all fits for the MGF and MAF was 11 respectively 10.

[5] Coefficient of Determination.

[6] Durbin Watson statistic: a value less than 2 indicates a positive autocorrelation, a value greater than 2 a negative autocorrelation.

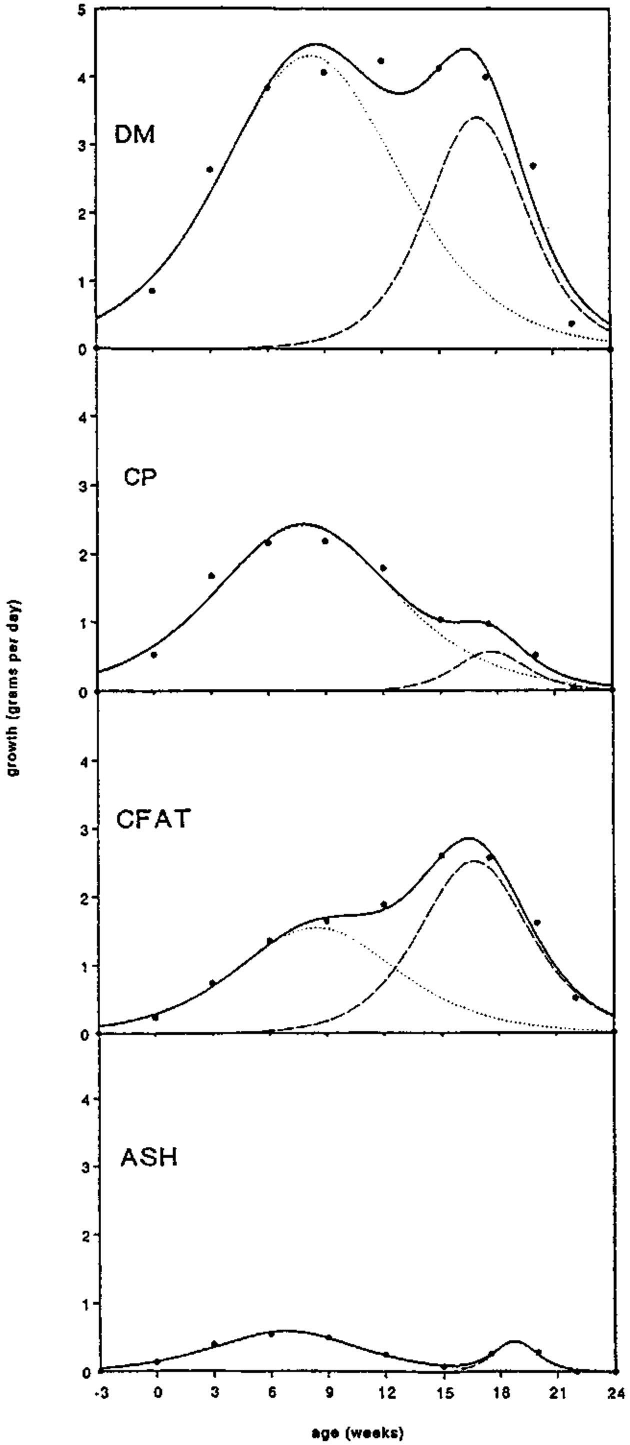

Figure 2.
Component growth [DM, CP, crude fat (Cfat), Ash; markers are observed means] from hatch to 27 wk of age of White Leghorn pullets and hens consuming feed *ad libitum*, fitted by a diphasic growth function (• • • • first phase; — — — , second phase; ———————— , diphasic).

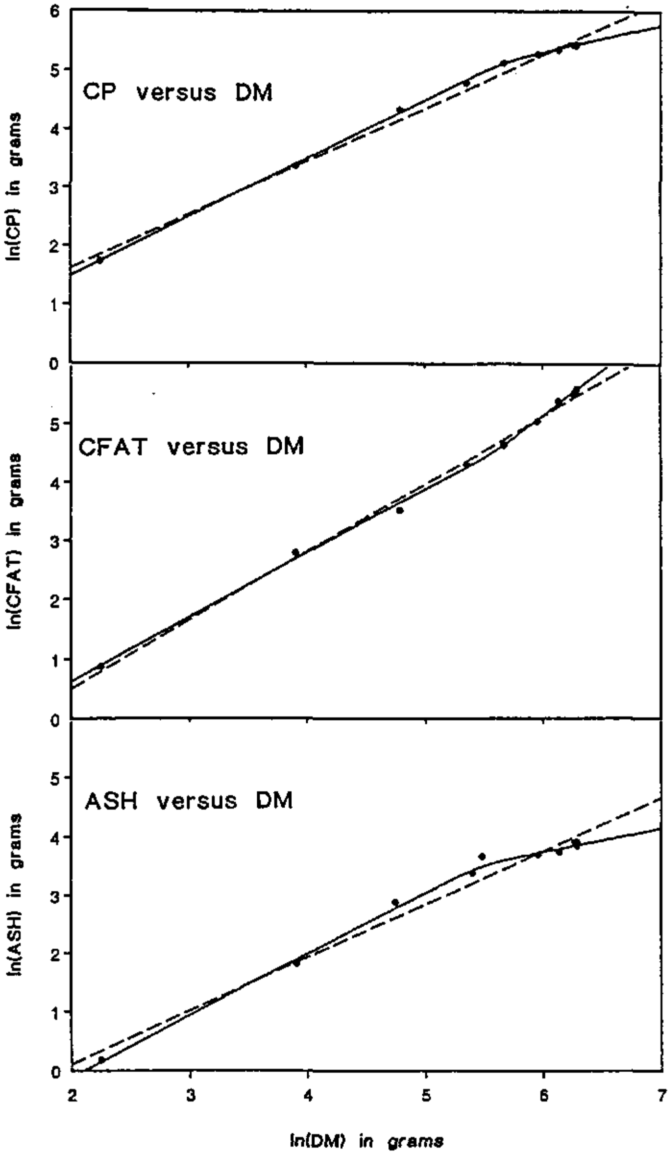

Figure 3. Allometric relationship (markers are observations) between CP and DM, crude fat and DM, and Ash and DM. Each relationship is described by a monophasic (— — — —) and diphasic (————————) allometric function.

MAF were estimated. With only 10 observations, the multiphasic allometric function could not estimate the smoothness parameter r accurately. The lack of observations in the transition area prevented an exact study of the bending curve. Value for r was fixed at .10, suggesting a quite abrupt transition from the first to the second linear segment. In this way all data points could be used for estimating both linear segments. Moreover, when the r was varied between .01 and .20 only slight differences in estimations of the other parameters were observed.

The sum of asymptotic weights of CP, Cfat, and Ash in the first and second phases of the MGF (377 and 166 g, respectively) is almost equal to the total asymptotic weight of DM in the respective phases (380 and 170 g), which validate the diphasic nature of growth of these components.

In the first phases of CP, Cfat, and Ash, similar durations (25.2, 22.6, and 20.6 wk, respectively) and approximately the same age at maximum gain (7.9, 8.5, and 6.8 wk, respectively) suggest a functional relationship between these components during the first 15 wk posthatch. Moreover, such a biological relationship is strengthened by the observation that the allometric slopes in the first segment of CP, Cfat, and Ash were all the same and close to unity (1.00, 1.08, and 1.05, respectively), suggesting isometry in growth pattern.

A marked change of the ß-values in the allometric relationship is noted, assuming first and second phases. Observations on component gain within the different phases of the MGF showed that 56% of DM growth in the first phase is CP growth (91% of total CP growth), and only 33% is Cfat growth (47% of total Cfat growth). On the contrary, in the second phase, only 12% of DM growth consists of CP growth (10% of total CP) whereas almost 83% of DM growth is Cfat growth (53% of total Cfat growth). Thus, the difference in composition of DM growth in the first and second phase of the MGF illustrates the changes in levels of protein and fat deposition, which is reflected by the ß-values in the MAF. The large $ß_2$-value of fat illustrates the late maturation of this component.

The allometric slopes of the MAF can be predicted by some parameter values of the MGF. The ratio of duration of growth spurts of two components within one phase (i.e., with similar ages at maximum gain) gives a rough estimate of the allometric slope in the respective phases (Koops and Grossman, 1991b). For the relation between CP and DM, Cfat and DM, and Ash and DM, ratios are 1.00 (25.2:25.2), .90, and .82, respectively, in the first phase and .69, 1.08, and .42, respectively, in the second phase. Allometric slopes in both phases were 1.00, 1.08, and 1.05 (first phase) and .46, 1.51, and .40 (second phase), respectively. Although the estimates do not seem precise, magnitudes are of the same order. This illustrates the comparability of both multiphasic models.

The point of transition is at similar ages in each of the diphasic allometric relations, varying between 5.46 and 5.66. This equals a DM weight of about 250 g, which reflects pullets at about 11 wk of age. This is the point in the MGF plots (Figure 2) at which the influence of the second phase starts to increase. In literature of other species, transition areas in growth patterns have often been associated with the moment of susceptibility towards body signals announcing physiological changes like sexual maturity (Cantier et *al.*, 1969; Robelin *et al.*, 1977).

It is speculated that phases of component growth are related to growth of different body structures (Table 4). Growth of protein and fat in the first phase presumably consists of general muscle growth and deposited functional fat (e.g., intermuscular and skin fat) (Walstra, 1980; Koops and Grossman, 1991a). Ash growth in the first phase is assumed to be mainly skeletal growth (calcification) on a functional basis related to protein and fat growth (allometric slopes all around 1). In the second phase, protein growth may be attributed to growth of the reproductive tract (compare age at maximum gain in the second phase (17.6 wk) with C_3 of the BW curve (Table 1: 19.2 wk). Fat growth in the second phase most likely consists of abdominal fat deposition associated with sexual maturity. The diphasic nature of fat growth has been recognized in other animal species (Robelin *et al*, 1977, in cattle; Walstra, 1980, in pigs). Bone growth in the second phase (after 11 wk of age) is almost complete. Most bones have reached their mature weight and length around 12 wk of age (unpublished data on shank, tibia, and keel length). The small amount of ash growth during the second phase (19% of total ash) is expected to be mineralization of the medullary bone and ash growth related to muscle growth.

Table 4. Expectation of growth of body structures in the different phases of each component.

Component	First phase	Second phase
CP	Muscles	Reproductive tract
Crude fat	Intermuscular / Functional	Functional / Depot (abdominal)
Ash	Calcification (bones)	Mineralization

In human research, the use of multiphasic growth functions is widely accepted and incorporated in field studies. In contrast, growth in farm animals (e.g., poultry) has usually been described by monophasic functions. This was not because animal researchers did not believe in the concept of growth as consisting biologically of several distinguishable growth cycles instead of a single one. The main reason for the use of monophasic functions was a lack of detailed observations on growth in course of time (Koops, 1986).

Recently, some researchers in the field of animal science applied the concept of multiphasic growth. They distinguished different phases assuming breakpoints on the basis of visual inspection between phases and then further fitted the phases separately (Ricklefs, 1975; Lilja *et al.*, 1985). Others tried to develop mathematical functions including such transition areas between growth phases (Bacon and Watts, 1971; Koops, 1986; Koops and Grossman, 1993).

The number of observations is a critical aspect in curve fitting. In this paper, the data on component growth in course of time made a diphasic model the most feasible. Weekly observations might have extended the number of separate phases. In that case, a better comparison between total body and component growth could have been made.

From the data, it is concluded that BW gain in rearing pullets can be described by a tetraphasic growth function. The proposed model enables a precise determination of the age at which reproductive organs start to develop. This point marks the beginning of the maturity stage, a period of enormous importance for the prelayer. A lack of nutrients at this stage could be critical for the development of the respective body structure (McCance, 1977; Ricklefs, 1985). A multiphasic approach of pullet growth can be helpful in evaluating different nutritional conditions during several rearing stages.

The growth pattern of body components in WL pullets consuming feed *ad libitum* was described accurately by a diphasic model. Both the diphasic growth function and the diphasic allometric function supplied comparable information on the existence of at least two phases in component growth. Studying growth of a body component (or any other body structure) in course of time (gain-age relationship) or studying its dependency on growth of another component (allometric relationship) determines which model should be used (MGF versus MAF).

The observed relationships indicate that the use of multiphasic functions might be helpful in the interpretation of changes in body development of pullets. Such changes may be due to both intrinsic and extrinsic factors.

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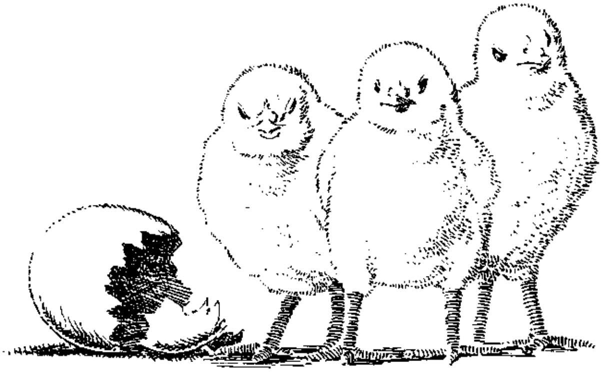

Chapter 5

ONSET OF LAY RELATED TO MULTIPHASIC GROWTH AND BODY COMPOSITION IN *AD LIBITUM* AND RESTRICTED FED WHITE LEGHORN PULLETS

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ABSTRACT

Growth of the body as a whole and of parts of the reproductive tract of *ad libitum* and restricted fed White Leghorn pullets were analyzed by a multiphasic growth function. Parameter estimates were related to onset of lay. Chemical body compositions at defined stages of sexual maturity were related to the development of the reproductive organs. Data of pullets were used that had been restricted in one of two rearing periods (0 to 6 and 7 to 18 wk of age). In each period, pullets were fed a low-lysine diet or a daily restricted amount of feed, on a pair-gain basis.

In all mathematical fits, a well distinguishable, so-called 'maturity growth spurt' at around 19 wk of age was found. About 40 to 70% of total growth within this phase consisted of growth of the reproductive tract (the ovary covered 19 to 35%, the oviduct 15 to 23%, and the uterus 7 to 11% of total growth). Age at maximum gain of the maturity growth spurt was 20.1 wk for lysine-restricted pullets and 19.6 wk for feed-restricted pullets. This difference reflected differences in onset of lay (50% rate of lay) between lysine- and feed-restricted birds (22.3 and 21.6 wk, respectively). An interval of 14 to 15 days existed between maximum gain of the maturity growth spurt and onset of lay. It was suggested that the occurrence of the maturity growth spurt can serve as a predictor for subsequent onset of lay.

Body compositions (crude protein, crude fat, water, and ash) at defined moments during the maturity growth spurt were calculated by interpolation from chemical analyses of wk 15, 18, 20, 22, and 24. Cfat content in the body at start of the maturity growth spurt varied considerably between the feeding regimens (112 to 179 g Cfat). The fat-free body (including CP, ash, and water) showed less variation between the treatments at this stage (807 to 870 g fat-free tissue). It is concluded that a particular amount of fat-free tissue is critical in pullet development, and may be required before sexual development starts.

Keywords: multiphasic growth, feed restriction, body composition, sexual development, onset of lay, pullets.

INTRODUCTION

Ample research has been conducted to elucidate determinants for onset of lay in light hybrids and even more in broiler breeders (among others: Dunnington *et al.*, 1983;

Bornstein et *al.*, 1984; Brody *et al.*, 1984; Yu *et al.*, 1992). Several authors stressed the importance of body composition over BW as the primary determinant for onset of lay (Brody *et al.*, 1984; Soller et *al.*, 1984; Chi, 1985; Johnson *et al.*, 1985; Leeson, 1986; Zelenka *et al.*, 1986; 1987; Summers *et al.*, 1987). However, the physiological pathways are not yet well understood (Dunnington and Siegel, 1984). Onset of lay may be determined by a number of interrelated factors such as age and body weight as threshold factors (Brody *et al.*, 1980; Dunnington *et al.*, 1983; Dunnington and Siegel, 1984), body fat (Brody et *al.*, 1984), lean tissue (Brody et *al.*, 1980; Soller *et al.*, 1984; Zelenka *et al.*, 1986), body size (Summers *et al.*, 1987), and light stimulation as a management factor (Eitan and Soller, 1992). It was stated by Dunnington and Siegel (1984) and Summers *et al.* (1987) that to be profitable, a young hen must probably attain a minimum body weight in combination with a 'particular' body composition in order to initiate egg production. Most of the above mentioned reports based their findings on dissected birds and subsequent chemical analyses, carried out at age of onset of lay. One of the factors hindering a clear reviewing of the literature concerning 'onset of lay', is the problem of definition. Several authors considered 'age at first egg' as setpoint for egg production (Dunnington and Siegel, 1984), while others contemplated 'age at 10% production' (Gous and Stielau, 1975; Gous, 1978) or 'age at 50% production' (Abu-Serewa, 1979; Wells, 1980; Mbugua and Cunningham, 1983) as a reliable parameter for onset of lay.

The approach in the present paper is somewhat different. It is proposed here that *not* 'age at onset of lay' but rather 'age at which the reproductive tract develops' may provide the cue for the biological events that play a critical role in determining onset of lay. This implicates that the moment of development of the reproductive tract is an important feature and needs to be assessed. The 'timing' of growth of the reproductive tract might be influenced by a certain nutrient supply during particular stages of rearing. Hammond and colleagues of the Cambridge school (Hammond, 1932; Palsson, 1955) suggested by their 'classical' growth order theory that growth is the result of sequential growth waves, each of which represents distinguishable stages of development of individual, functional body parts. As a consequence, these functional body tissues and/or organs will probably display a competing demand for essential nutrients during their 'own' critical growth stage (McCance, 1977; Ricklefs, 1985). A lack of nutrients at that stage might delay the initiation of adult egg performance.

Most feeding programs for rearing modern layer stock do not take these critical stages into account. Modern programs are still based on the concept of 'saving feed costs' during rearing (Kwakkel, 1993). Breeders' advice focusses on the application of a certain 'moderate' feed restriction throughout the *entire* rearing period up to a particular target weight along particular growth curves, developed as being 'unique' for the breed. Such strategies then guarantee the producer that the hen will quickly initiate production. In practice, however, layer records sometimes disappoint through insufficient daily feed intakes, 'dips' around peak production and/or problems of prolapse (Leeson, pers.comm). Thus, it seems necessary to analyze pullet growth and development in order to elucidate possible determinants for onset of lay and to adjust pullet feeding regimens accordingly.

Recently, it was demonstrated that growth in several animal species could be described in terms of distinguishable growth phases by a multiphasic growth model (Koops, 1986). A clear body growth spurt at around 82% of adult BW was identified in pullets of different strains (Grossman and Koops, 1988). Kwakkel *et al.* (1993) found this growth spurt at a similar stage of maturity in *ad libitum* fed pullets as did Grossman and Koops (1988). They speculated that this, so-called, 'maturity growth spurt' represents mainly growth of the reproductive tract.

In the current paper, it was aimed to assess the maturity growth spurt in pullets that had been fed either a low-lysine diet or a restricted daily amount of an adequate diet, on a pair-gain basis, during one of two rearing periods (0 to 6 or 7 to 18 wk of age; Kwakkel *et al.*, 1991). Additionally, growth of parts of the body representing the reproductive tract was assessed. Results on chemical body composition were used to predict amounts of crude protein (CP), crude fat (Cfat), ash and water (Wtr) at predetermined times during the maturity growth spurt.

The objective of this study was to find clues in multiphasic body growth and chemical body composition that determine directly the physiological 'readiness' for egg production (Watson, 1975; Foxcroft, 1980; Leeson, 1986).

MATERIALS AND METHODS

Experimental design and diets

Data on BW, weights of the reproductive tract, and chemical compositions of pullets came from an experiment reported by Kwakkel *et al.* (1991). Briefly, in this experiment White Leghorn (WL) pullets of a commercial strain (Lohmann GmbH, Cuxhaven, Germany) were either restricted in lysine (RL) or in amount of feed (RF), on a pair-gain basis, during the starter (s; RLs and RFs: 0 to 6 wk of age) or grower (g; RLg and RFg: 7 to 18 wk of age) phase of rearing. A fifth control group (C) of pullets received *ad libitum* a normal starter (.85% digestible lysine, .71% digestible methionine + cystine, 18.9% CP, 1.0% calcium, .41% available phosphorus, and 2,800 kcal ME/kg) and grower diet (.65% digestible lysine, .60% digestible methionine + cystine, 15.9% CP, .9% calcium, .44% available phosphorus, and 2,800 kcal ME/kg) throughout rearing. In each of the two rearing phases, the diets for RLs and RLg contained .40% and .30% digestible lysine, respectively. All other nutrients were provided in amounts believed to be adequate (NRC, 1984). In the unrestricted phase the respective control diet was supplied *ad libitum*. Both feed-restricted groups (RFs and RFg) were allowed to eat daily a quantity of feed of the starter and grower diet, respectively, so that they matched BW of the respective RL-pullets. After 18 wk of age, all hens were fed *ad libitum* a commercial layer diet (.65% digestible lysine, .58% digestible methionine + cystine, 16.5% CP, 3.5% calcium, .44% available phosphorus, and 2,800 kcal ME/kg). Each treatment group was tested in nine replicate cages (24 pullets per cage). Details of the experiment have been described previously (Kwakkel *et al.*, 1991).

The multiphasic growth function

A Multiphasic Growth Function (MGF; Kwakkel *et al.*, 1993) was applied in each treatment group to describe weekly gain of total BW and of the reproductive organs. The model was fitted to BW gain data from 12 to 31 wk of age to identify the so called 'maturity growth spurt' (Kwakkel *et al.*, 1993). Gain in each of the reproductive organs, ovary, oviduct (minus uterus), and uterus, was assessed to determine the respective growth phase. The MGF [1] gives a mathematical description of several successive growth cycles, each of them represented by a logistic equation:

$$y_t = \sum_{i=1}^{n} [2A_iB_i^{-1}\{1\text{-}\tanh^2(4B_i^{-1}(t\text{-}C_i))\}] \qquad [1]$$

where y_t is the predicted value of weight gain in week t; n is the number of phases; for each phase i, A_i is the asymptotic weight gained (g), B_i is the duration (wk), and C_i is the age at maximum gain (wk).

Stages of sexual development

Three stages of sexual development (in wk) were defined: (1) 'Start of sexual development'(S), estimated by subtracting half the duration (B) from the age at maximum gain of the maturity growth spurt (C) within each treatment: S = C - 0.5*B, and var(S) = var(C) + var(B)/4 - Cov(C,B)/2 (Snedecor and Cochran, 1979); (2) 'Peak of sexual development', defined by the age at maximum gain of the maturity growth spurt (C); and (3) 'End of sexual development'(E), estimated by adding half the duration (B) to age at maximum gain of the maturity growth spurt (C): E = C + 0.5*B, and var(E) = var(C) + var(B)/4 + Cov(C,B)/2.

Data collection

BW was recorded weekly per cage. According to the procedures described by Kwakkel *et al.* (1993), BW gain was calculated from these data and used for curve-fitting to assess the maturity growth spurt.

To obtain data on organ and component growth, birds were sacrificed during the experiment; the number of pullets per cage decreased from 24 in wk 0 to 8 in wk 24. Each week, 5 to 8 pullets per treatment group were randomly removed from different cages (to a maximum of one bird per week per cage) and killed by cervical dislocation. After killing, birds were plucked and the gut contents removed. Every week certain skeletal bones and organs associated with food processing (gut, heart, liver) were examined individually. These results will be reported in a companion paper. For the present paper, growth of the reproductive organs, i.e., the ovary (wk 12 to 24), the oviduct minus the uterus (wk 18 to 23), and the uterus separately (wk 18 to 23), were examined.

Defeathered pullets (including all dissected organs and viscera but without gut fill) of wk 15, 18, 20, 22, and 24, were stored in plastic bags and frozen to -20^0C. Pullets were plucked to reduce the heterogenity of the sample composition. Feathers were not included in the analyses. Before mincing, pullets were thawed for 3 to 5 hours (within their closed plastic bag) and cut into pieces with a small axe. Pullets were minced individually. Each minced sample was mixed with 20 to 30% celite to stabilize fat separation, the amount depending on the expected fat content of the sample. They were then freeze-dried, ground on a 1 mm sieve, and stored in plastic bottles for subsequent chemical analyses. Dry Matter (DM), Ash, CP (Kjeldahl; Nx6.25) and Crude Fat (Cfat; ether extraction) were determined in duplicate samples. To ensure a justified comparison between pre-pubertal body development and subsequent egg performance, body composition of dissected birds was adjusted for differences in BW between live and dissected birds (Kwakkel *et al.*, 1993).

Results of the chemical analyses were used to calculate body composition Y_t at a defined stage of sexual development by a linear interpolation between the nearest lower (t-) and upper (t+) observation. For example, body composition at t = 16.3 wk of age ('start of sexual development') was calculated using data of wk 15 (t-) and 18 (t+). The general equation was: $Y_t = Y_{t+} - b * (Y_{t+} - Y_{t-})$, where $b = [(t+) - (t)]/[(t+) - (t-)]$. Variances were calculated by: $\mathrm{var}(Y_t) = (1-b)^2 * \mathrm{var}(Y_{t+}) + b^2 * \mathrm{var}(Y_{t-})$, where b is a constant for each stage and treatment, and has no variance. The covariances between Y_{t+} and Y_{t-} in this serial-slaughter experiment were assumed to be zero. Differences between treatment groups were assessed by the Studentized t-test (Snedecor and Cochran, 1979).

Curve-fitting

The data were fitted using nonlinear regression from the SAS package (Method Dud; convergence criterion 10^{-8}; SAS Institute Inc., 1991). Goodness-of-fit criteria were: the asymptotic SE (ASE) for each parameter, the residual SD (RSD), the coefficient of determination (CD), and the Durbin-Watson (DW) test for first-order autocorrelation.

Treatment effects were tested by F-tests. If there were no treatment differences, parameter estimates of the simple model (including all data) were tabulated. However, if a 'full' model (each treatment has a unique set of parameter estimates) improved the fit significantly ($P<.05$), then all treatments were fitted individually and the parameter estimates displayed accordingly. To investigate the origin of treatment differences, parameters were grouped for testing contrasts subsequently.

RESULTS

The maturity growth spurt and growth of the reproductive tract

Parameter estimates of the maturity growth spurt of the most accurate fit on BW gain for each treatment are presented in Table 1. Asymptotic gain (on average 224 g) and duration (on average 5.7 wk) during the maturity growth spurt did not differ significantly between the feeding regimens. This may be additional evidence for the maturity growth spurt representing the same group of organs in each treatment group. Age at maximum gain of the maturity growth spurt, however, differed significantly between RF- and RL-pullets (19.6 and 20.1 wk of age; $P<.05$; $F(1,69)=5.53$).

Table 1. Parameter estimates and asymptotic SE (in parentheses) of the maturity growth spurt for each treatment[1], fitted by a multiphasic growth function[2] on body weight gain data from 12 to 32 wk of age.

Treatment	Parameter estimates			Goodness of fit		
	Asymptotic weight gained (g)	Duration (wk)	Age at max. gain (wk)	RSD[3]	CD[4]	DW[5]
C[6]	169 (24)	5.8 (.7)	19.2 (.10)	3.97	.9972	2.41
RLs	255 (28)	6.7 (.6)	20.1 (.07)	3.41	.9918	3.11
RFs	240 (34)	5.3 (.7)	19.9 (.10)	7.19	.9672	2.43
RLg	193 (20)	5.2 (.4)	20.1 (.05)	6.77	.9669	2.52
RFg	263 (52)	5.7 (1.0)	19.4 (.14)	9.03	.9531	2.64

[1] Treatments: C – control group, no restrictions; RLs – lysine restriction in starter period; RFs – pair-gained feed restriction in starter period; RLg – lysine restriction in grower period; RFg – pair-gained feed restriction in grower period.

[2] $y_t = \Sigma\ [2A_iB_i^{-1}\{1\text{-}\tanh^2(4B_i^{-1}(t\text{-}C_i))\}]$, where, in each phase i, A_i is the asymptotic weight gained (g), B_i is the duration (wk), and C_i is the age at maximum gain (wk).

[3] Residual SD (n in all fits was 20).

[4] Coefficient of Determination.

[5] Durbin Watson statistic: a value around 2 indicates no autocorrelation.

[6] Values of the Control group were adapted from Kwakkel *et al.* (1993). The fit was based on n – 31.

Table 2. Parameter estimates and asymptotic SE (in parentheses) of gain in the reproductive organs of WL-pullets, described by a monophasic growth function[1].

Reproductive organ		Parameter estimates						Goodness of fit		
		Asymptotic weight gained (g)		Duration (wk)		Age at max. gain (wk)		RSD[2]	CD[3]	DW[4]
Oviduct[5]		39.2	(3.0)	7.7	(.7)	20.1	(.1)	1.39	.7353	2.52
Uterus		18.3	(1.3)	8.6	(1.1)	19.8	(.1)	.64	.6157	2.51
Ovary	C	59.2	(7.9)	10.5	(1.8)	21.4	(.3)	1.53	.9135	2.30
	RLs	72.2	(14.3)	12.6	(2.2)	22.8	(.6)	.97	.9635	2.43
	RFs	48.7	(3.9)	6.4	(.6)	20.7	(.1)	1.33	.9434	2.18
	RLg	56.7	(7.0)	9.4	(1.5)	21.6	(.2)	1.47	.9272	2.54
	RFg	50.5	(3.1)	8.1	(.6)	21.2	(.1)	.84	.9735	2.36

[1] $y_t = 2AB^{-1}\{1-\tanh^2(4B^{-1}(t-C))\}$, where A is the asymptotic weight gained (g), B is the duration (wk), and C is the age at maximum gain (wk). Oviduct and uterus were fitted ignoring treatment (n = 20), whereas ovary was fitted with treatment (n = 12).

[2] Residual SD.

[3] Coefficient of Determination.

[4] Durbin Watson statistic: a value less than 2 indicates a positive autocorrelation, a value greater than 2 a negative autocorrelation.

[5] Oviduct - Total oviduct minus the uterus.

Parameter estimates for growth of the ovary, the oviduct minus the uterus, and the uterus separately are summarized in Table 2. Growth patterns of the respective reproductive organs could be described accurately by a monophasic growth function. The simple model (no treatment differences) was compared to the full model (including treatment differences). For the oviduct and uterus, the full model did not improve the fit significantly (NS; $F(12,5) = 0.44$ and $F(12,5) = 0.34$). The absence of any treatment effect was largely due to the small number of observations (n = 4) and the variation in trait values. For the ovary, on the other hand, the inclusion of individual sets of parameters per treatment in the model improved the fit significantly ($P < .01$; $F(12,45) = 3.41$). Differences between lysine and feed restricted pullets in ages at maximum gain (22.2 and 20.9 wk of age, respectively) and duration (11.0 and 7.2 wk, respectively) accounted largely for this improvement. No significant differences in total ovary growth between the treatments were demonstrated. Growth of the total oviduct (including the uterus) occurred about 1.5 wk earlier than growth of the ovary (compare the ages at maximum gain in Table 2).

Table 3. Predicted body composition[1] (Means (SEM)) at defined stages of sexual development[2] of pullets on the different treatments[3].

Treatment	Age (wk)	Body composition (g)			
		Ash	Crude Protein	Water	Crude fat
		Start of sexual development (wk)			
C	16.3 (.40)	41.1 (.8)^{a}	201.5 (3.6)^{a}	627.1 (10.3)	178.7 (12.2)^{a}
RLs	16.7 (.29)	37.7 (.6)^{b}	176.7 (2.7)^{b}	592.0 (10.5)	125.4 (9.8)^{bc}
RFs	17.3 (.35)	38.3 (.4)^{b}	187.9 (2.0)^{b}	602.6 (7.2)	154.3(11.5)^{ac}
RLg	17.5 (.21)	38.1 (1.0)^{ab}	187.1 (4.9)^{ab}	599.3 (16.2)	177.5(21.9)^{ac}
RFg	16.6 (.52)	38.7 (.7)^{ab}	192.1 (2.3)^{ab}	620.9 (8.0)	112.4 (5.3)^{b}
		Peak of sexual development (wk)			
C	19.2 (.10)	46.5 (1.5)	221.0 (5.7)	681.9 (16.0)	230.7 (23.9)
RLs	20.1 (.07)	47.3 (1.4)	211.8 (5.3)	661.9 (18.7)	180.1 (20.5)
RFs	19.9 (.10)	46.2 (1.5)	215.8 (6.9)	684.5 (21.8)	168.5 (20.2)
RLg	20.1 (.05)	45.7 (1.3)	218.7 (6.5)	686.2 (23.0)	181.4 (13.3)
RFg	19.4 (.14)	46.6 (1.0)	214.9 (4.1)	673.3 (13.1)	184.9 (8.3)
		End of sexual development (wk)			
C	22.1 (.33)	49.8 (2.1)	225.2 (7.3)	713.0 (21.9)	257.6 (21.7)
RLs	23.5 (.28)	53.1 (1.9)	226.3 (4.5)	723.8 (12.9)	242.2 (13.0)
RFs	22.5 (.34)	48.8 (1.4)	225.8 (5.3)	732.1 (17.9)	225.0 (12.5)
RLg	22.7 (.20)	49.6 (1.8)	227.6 (4.1)	730.4 (11.8)	235.2 (15.1)
RFg	22.2 (.53)	51.2 (1.5)	230.2 (6.1)	732.3 (21.2)	219.4 (13.6)

[1] Body composition Y_t at a defined stage t of sexual development was calculated by interpolation between the nearest lower t- and upper t+ observation, using data on chemical body composition of wk 15, 18, 20, 22, and 24 of age (Kwakkel, Unpublished: n in each wk was based on 6 - 8 observations): $Y_t = Y_{t+} - b * (Y_{t+} - Y_{t-})$, where $b = (X_{t+} - X_t)/(X_{t+} - X_{t-})$; Variances were calculated by $\mathrm{var}(Y_t) = (1-b)^2 * \mathrm{var}(Y_{t+}) + b^2 * \mathrm{var}(Y_{t-})$ (Snedecor and Cochran, 1979).

[2] Sexual development is defined here as the period in which the maturity growth spurt appears. 'Start of sexual development'(S) has been estimated by subtracting half the individual duration (B) from the age at maximum gain of the maturity peak (C; see Table 2), thus $S = C - 0.5*B$; The variation has been calculated by $\mathrm{var}(S) = \mathrm{var}(C) + \mathrm{var}(B)/4 - \mathrm{Cov}(C,B)/2$. 'Peak of sexual development' has been defined by age at max. gain of the maturity peak (C) itself, and 'End of sexual development' (E) has been estimated by adding half the individual duration (B) to age at max. gain of the maturity peak (C), thus $E = C + 0.5*B$; The variation has been calculated by $\mathrm{var}(S) = \mathrm{var}(C) + \mathrm{var}(B)/4 + \mathrm{Cov}(C,B)/2$ (Snedecor and Cochran, 1979).

[3] Treatments: C = control group, no restrictions; RLs = lysine restriction in starter period; RFs = pair-gained feed restriction in starter period; RLg = lysine restriction in grower period; RFg = pair-gained feed restriction in grower period.

[a,b] Means (SEM) of body composition in a column with no common superscript differ significantly ($P < .05$); if none of the treatments differed from one another, no superscripts are displayed; significancies are within stage of development.

For each treatment, total growth of the reproductive tract and growth partitioning among the individual structures is related to growth during the maturity growth spurt (Figure 1). The maximum growth rates ('ages at maximum gain', *see* Tables 1 and 2) of the oviduct and the uterus appeared prior to the maximum growth rate of the ovary, but coincided with the maximum growth rates of the maturity growth spurt of the different treatments. On the basis of asymptotic weights (Tables 1 and 2), ovary growth covered 19 to 35% of growth during the maturity growth spurt for the different treatments; the oviduct and the uterus covered 15 to 23 and 7 to 11%, respectively (in total 40 to 70%). In most groups, however, the maturity growth spurt tended to precede growth of the reproductive organs by .3 to 1.2 wk, resulting in a small non-overlapping part (Figure 1). As a consequence, the 40 to 70% growth during the maturity growth spurt that is allocated to growth of the reproductive tract is slightly overestimated. That part of total growth during the maturity growth spurt that remains (more than 30 to 60%) can probably be allocated to abdominal fat deposition during sexual development (Kwakkel et *al.*, 1993). It is clear from Figure 1, that the rapid increase in gain during the maturity growth spurt occurs at the same age as the sexual organs develop.

Body composition and sexual development

In the previous section, it was suggested that growth of the reproductive tract is related to the maturity growth spurt. It is therefore important to know what mechanism triggers the initial growth during the maturity growth spurt. This can be rephrased as what kind of body conformation or composition is necessary to allow a rapid development of the reproductive tract ?

If the maturity growth spurt reflects to a major extent growth of the sexual organs, it can be regarded as an important, critical growth phase of sexual development. Thus, three different stages during the maturity growth spurt ('stages of sexual development') were defined. For all treatments, body composition was calculated at 'start', 'peak', and 'end' of sexual development, to investigate changes in the distribution of deposited body components during these stages (Table 3).

At 'start' of sexual development, predicted amounts of ash (on average 38.8 g), CP (189.1 g), and water (608.4 g) were similar for all feeding regimens, although the control group tended to show somewhat higher values. Predicted amounts of Cfat of pullets at this stage of development varied significantly ($P < .05$) among the treatments, ranging from 112.4 to 178.7 g, with an average of 149.7 g. Body composition per 100 g defeathered body mass was, on average, 3.9 g ash, 19.2 g CP, 61.7 g water, and 15.2 g Cfat.

At 'peak' of sexual development, amounts of ash (46.5 g), CP (216.4 g), and water (677.6 g) were each increased by about 15% (varying over treatments from 10 - 20%), compared to the situation at 'start' of sexual development. No differences in body

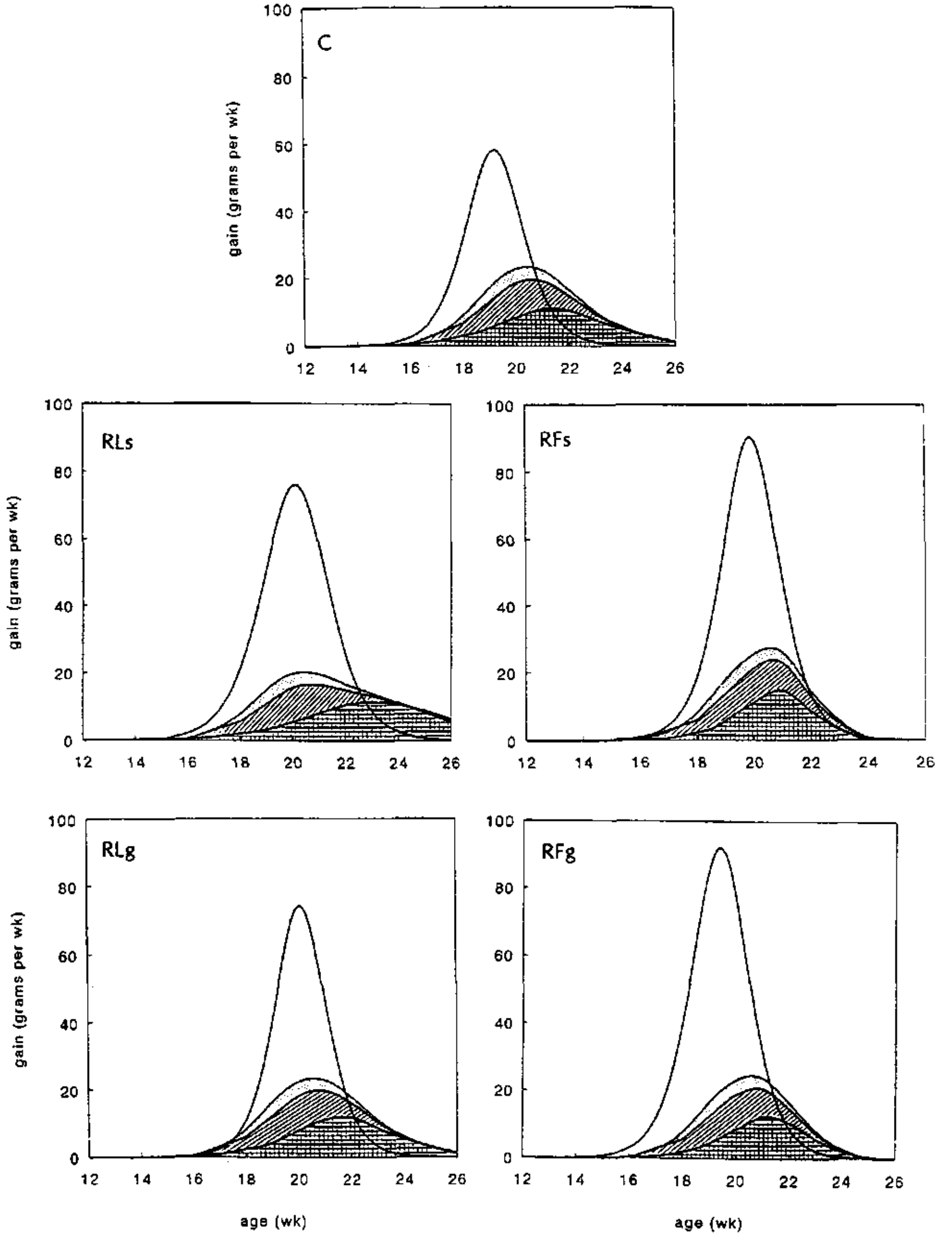

Figure 1. The partitioning of growth during the maturity growth spurt to growth of the individual organs of the reproductive tract, in WL-pullets fed different feeding regimens; each growth spurt is described by a monophasic logistic function; ▥ ovary, ▨ oviduct minus uterus, ░ uterus.

composition between the treatments could be found at this stage. The amount of Cfat did not vary among the four restricted groups (178.7 g). Only the C-pullets had still a, non-significant, larger amount of fat in the body (230.7 g). Fat growth had increased relatively more (26%) than fat-free growth, although large variations in fat deposition existed among the treatments (2 - 65%). At 'peak', body composition per 100 g defeathered body mass was, on average, 4.1 g ash, 19.2 g CP, 60.0 g water, and 16.7 g Cfat.

From 'peak' to 'end' of sexual development, Cfat still gained by 25%, whereas gain of the defeathered fat-free body mass was almost over (approximately 6%). Predicted amounts of ash, CP, and water were, on average, 50.5, 227.0, and 726.3 g at 'end' of sexual development. Again, no differences between the treatments in defeathered fat-free body were obvious. Pullets of the control group had still larger amounts of Cfat in the body (257.6 g), but, at this stage the predicted amounts of Cfat in the restricted groups also differed in a range of 219.4 to 242.2 g, with an average of 230.4 g. Body composition per 100 g defeathered body mass was, on average, 4.1 g ash, 18.3 g CP, 58.6 g water, and 19.0 g Cfat.

DISCUSSION

Unjustified curve fitting

Mathematical fitting of data for restricted fed birds have to be considered critically. Computer procedures may lead to some problems if changes in feed allowance interfere with inherent growth spurts. In that case, it might be difficult to fit the data of restricted fed birds adequately e.g. to the logistic curve. Ricklefs (1968) stated that "growth curves of birds under conditions of severe starvation are often so distorted that they can no longer be meaningfully fitted by a mathematical function". For that reason, BW gain of the restricted fed birds was not assessed by the MGF before 12 wk of age. The change in dietary treatment at week 7 may distort the 'bell-shape' form of the logistic curve and thus interfere with the intrinsic growth spurts. Moreover, our particular interest was the 'maturity growth spurt' around puberty. Weights of the reproductive organs before 12 wk of age were negligible.

The maturity growth spurt as a marker for sexual development and onset of lay

It is clear from Tables 1 and 2 and Figure 1, that the occurrence of the maturity growth spurt coincides well with growth of reproductive organs. The maturity growth spurt, however, preceded in some cases the growth spurts of the reproductive organs (Figure 1). In the restricted groups, this might be explained by some compensatory lean tissue growth at a somewhat earlier age than growth of the sexual organs occurs, yet both located underneath the maturity growth spurt. The *ad libitum* feeding level in the control pullets may have resulted in excessive amounts of fat deposition already before sexual development, thus complementing the maturity growth spurt in the same way as for the restricted groups (*see* Table 3 and Kwakkel *et al.*, 1993). Based on our findings and the comparisons among the treatments, it seems still justified to conclude that the maturity growth spurt is a fair indicator for sexual development, i.e., growth of the reproductive tract.

In this experiment, RL-pullets delayed onset of lay (in terms of 50% rate of lay) by about 5 days compared to the 'pair-gained' RF-pullets (22.3 versus 21.6 wk of age; $P<.01$; Kwakkel *et al.*, 1991). Differences between the RL- and RF-pullets in onset of lay were clearly reflected by differences in the occurrence of the maturity growth spurt. A strict interval of 14 to 15 days was observed between the peak of the maturity growth spurt and onset of lay. One has to remind, however, that none of the pullets had actually been restricted during this 15 days-interval. Low-lysine levels were no more limiting at that stage and consequently access to feed for the RFg-group was increased to *ad libitum* (Kwakkel *et al.*, 1991). It can be concluded from our results that onset of lay is related to the occurrence of the maturity growth spurt.

Body composition controlling onset of puberty

The system of reaching puberty is very complicated, and it seems therefore unlikely that only one factor is involved (Foxcroft, 1980; Bronson and Manning, 1991; Widdowson and Lister, 1992). The elucidation of the controlling factor(s) that are involved, has been a difficult task because in many studies 'cause' and 'effect' were not always clearly distinguished. Correlated observations were explained as the mechanistic events (Bronson and Manning, 1991). Reports in this field in both human and poultry literature suggested that body fat is the critical controlling factor for onset of puberty and lay, respectively (e.g., in humans: Baker, 1985; Whitworth and Meeks, 1985; in chickens: Brody *et al.*, 1984). It has been demonstrated by the work of Frisch in the early seventies that fat in the body of the rat accumulated to some 'threshold ratio' between fat and fat-free body mass before onset of puberty could be initiated (Frisch, 1984).

This observation, however, could be due to the already enhanced fat deposition, occurring just after the female reproductive system has been triggered. At the time of initial sexual development, nutrients, particularly energy, will merely be predestinated towards fat deposition (Widdowson, 1980; Baker, 1985). This phenomenon might have obscured results in which was concluded that fat had to be the main regulator. These changes in deposition characteristics are governed by circulating estrogens, produced by the yet to be developed ovary (Leat and Cox, 1980) and possibly some non-glandular body sites with aromatization (conversion of androgens to estrogens) activity (Whitworth and Meeks, 1985). This phenomenon is also clear from this study: the amount of fat increased by about 58% in the period from 'start' to 'end' of sexual development, whereas each component within the fat-free tissue increased by about 20% in the respective period (Table 3).

This implicates that there is still some primary 'unknown' factor (or factors) that alters the sensitivity of the hypothalamic-adenohypophyseal-ovarian axis. The altered sensitivity ultimately initiates the setting of the endocrine feedback mechanisms and induces the release of the brain and gonadal sex hormones (Foxcroft, 1980; Bronson and Manning, 1991; Yu *et al.*, 1992).

Bronson and Manning (1991) reviewed human and animal literature on search for the evidence 'for' and 'against' the hypothesis that first ovulation is regulated by a critical amount of fat. They rejected the 'fat hypothesis' as did also Soller *et al.* (1984), Johnson *et al.* (1985), and Zelenka *et al.* (1986) in reports on poultry. The findings of these researchers are in good agreement with the present results: amounts of fat in the pullet body varied considerably among the different feeding regimens at 'start' of sexual development. This indicates that the amount of body fat is not the key 'setting' factor for sexual maturity.

Particularly in poultry literature it is proposed that onset of lay is determined by a number of interrelated 'critical' factors such as age, BW, body fat, lean tissue, both in absolute amounts or as percentages of BW (Brody *et al.*, 1980, 1984; Dunnington *et al.*, 1983; Soller *et al.*, 1984; Zelenka *et al.*, 1986; Summers *et al.*, 1987). Johnson *et al.* (1985) proposed 'feed intake prior to first egg' as the main determinant for onset of lay. More recently, Yu *et al.* (1992) provoked a more biological integrating approach to search for 'pubertal initiators'. In their scenario, it was suggested that the maturation of brain tissue that controls steroidogenesis could interact with onset of lay, this process being mediated by feed intake and growth during earlier stages of juvenile development.

However, the main problem of these studies is that relationships between body composition and onset of lay were mostly assessed at 'age of first egg'. To our opinion, that moment is beyond the true 'setting' age.

In this study, there was no variation within each component of the fat-free body mass between the treatments at 'start' of sexual development. It appears that growth of the fat-free components largely depends upon each other. It is concluded that a particular amount of fat-free tissue seems to be critical in determining the moment of development of the reproductive tract and, as a consequence, will determine onset of lay, some 5 wk weeks later. These findings are in line with those of Wilen and Naftolin (1977), quoted by Foxcroft (1980). They proposed protein, as part of the fat-free body mass, to be the 'metabolic trigger'.

At 'peak' of sexual development, amounts of Cfat in the body were quite similar in the different treatments. The assumption that all restricted birds reached some 'equilibrium status' of fat in the body seems the only explanation for the large differences (2 to 65%) in fat deposition between 'start' and 'peak' stage of sexual development. At 'end' of sexual development, Cfat again varied among the restricted treatments (Table 3). It is suggested that at this stage of sexual development, when nearly all hens were in production, fat mobilization to sustain energy requirements for egg production played an interfering role. This may be the explanation for the lower amounts of Cfat in the 'early-maturing' RF-pullets.

Verification of sexual organ growth during the maturity growth spurt

For the major part, the maturity growth spurt and the growth phases of the reproductive organs overlapped. In that situation, both chemical and morphological calculations of tissue growth during the maturity growth spurt must correspond to a large extent, and morphological values may not be larger than the chemical values. A chemical estimate of total gain in the reproductive organs can be made by the calculation of the amount of 'soft' lean tissue (reproductive tract: CP + water; ash to represent minerals has been ignored) accrued during the maturity growth spurt (see Table 3: CP+Wtr at 'end' minus CP+Wtr at 'start'). Cumulated gains in these components (CP + Wtr =Y) were 109.6 (C), 181.4 (RLs), 167.4 (RFs), 171.6 (RLg), and 149.5 g (RFg). Total gain of the reproductive tract (=X) has been estimated without large differences between the treatments (see Table 2: 116.9, 130.3, 106.3, 115.0, and 107.0 g, for the respective treatments). Differences (Y minus X) are -7.3 (C), 51.1 (RLs), 61.1 (RFs), 56.6 (RLg), and 42.5 g (RFg). These values are somewhat underestimated, as a consequence of the small shift in sexual organ growth with respect to the maturity growth spurt. The discrepancy between, on one hand, the restricted pullets and, on the other, the control pullets, suggests some compensatory growth of other body structures in restricted birds still occurring during the period of sexual development.

Practical implications

Starter diets that allow an optimal development of the pullet's frame size (skeleton), its musculature and organs that sustain maintenance and growth processes (the fat-free body) will create large pullets during the first part of the rearing period. It can be stated from the results of this study that this approach will stimulate biological 'readiness' for egg production. Moreover, it is suggested that the occurrence of the maturity growth spurt may predict onset of lay. This could provide a practical application for the producer. On the basis of a weekly recording of flock BW by random sampling and a direct calculation of this growth spurt, poultry farmers may decide to change from a grower diet to a pre-layer diet, providing those nutrients that allow an optimal development of the reproductive tract.

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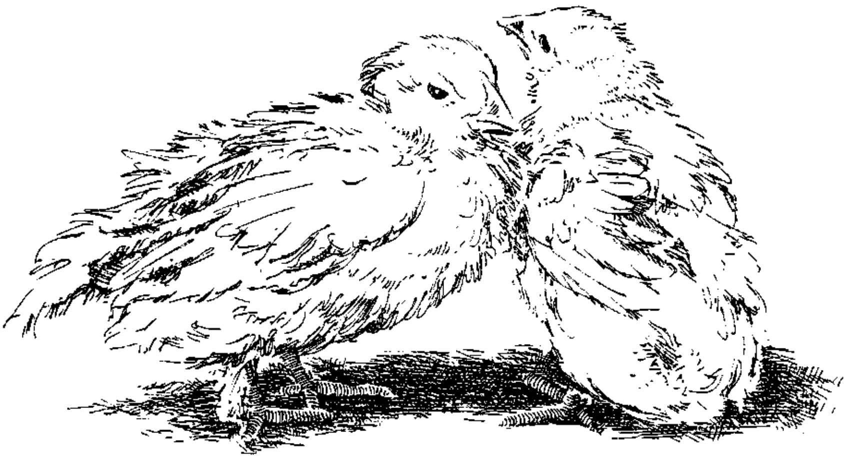

Chapter 6

MULTIPHASIC ALLOMETRY IN *AD LIBITUM* AND RESTRICTED FED WHITE LEGHORN PULLETS.
1. GROWTH OF CHEMICAL BODY COMPONENTS

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ABSTRACT

A multiphasic allometric function was used to describe the growth of crude fat (Cfat), CP, ash, and water, each as a function of plucked empty body mass (EBM) or fat-free plucked empty body mass (FFEBM) in White Leghorn pullets. Pullets had been restricted in lysine or feed intake daily, on a pair-gain basis, during early (0 to 6 wk of age) or late rearing (7 to 18 wk of age).

The allometric relationships between each chemical body component and EBM were clearly diphasic for all treatments, with different slopes for the first and second phases. The diphasic relationships, however, were, to a large extent, due to the diphasic growth pattern of fat *per se*. If FFEBM instead of EBM was chosen as the independent variable in the allometric relationship between each fat-free body component and total FFEBM, differences between the allometric slope of the first and second phase became smaller. Up to approximately 300 to 400 g FFEBM (the first allometric phase), a slope of 1 (proportional growth) between each fat-free component and FFEBM was found, regardless of the applied feeding regime. This means a constant composition of the fat-free body in that first growth phase. Between, on average, 350 and 1000 g FFEBM (the latter amount being the final observation in time: 24 wk of age; maturity was assumed), CP grew relatively faster than ash and water. However, again, no differences between the feeding regimens were observed. These results suggest that relative gain within the FFEBM is *not* influenced by feeding regime, but it is affected by aging of the fat-free body. In this study, relative fat growth (towards FFEBM) was affected much more by a low feeding level than was any other chemical body component. Fat seemed to serve as an independent buffer, that could be easily mobilized in situations of undernourishment. For that reason, FFEBM is proposed to be a better independent variable than EBM or BW in relative growth studies in the field of nutrition.

During the first growth phase, proportional growth between Cfat and FFEBM was observed in pullets fed *ad libitum* and in pullets fed a low-lysine diet. It is suggested, that accretion in adipose tissue during the first growth spurt mainly represents functional fat deposition, related to growth of the fat-free body at that stage. In spite of some extra energy intake, no extra fat was deposited in the low-lysine birds.

Keywords: multiphasic allometry, feeding regime, restriction, body composition, pullets.

INTRODUCTION

The pullet's body growth curve has a remarkable influence on subsequent layer performance (Wells, 1980; Kwakkel *et al.*, 1991). The body growth curve is determined by the deposition of particular amounts of body components to 'build' essential body structures, a process that is governed by the birds' inherent growth plan (Kyriazakis and Emmans, 1992). The genetically defined growth curve, however, may be modified by extrinsic constrains such as a sub-optimal environment. A nutrient restriction may be one of these constrains. According to McCance (1977), the effect of a nutrient restriction during rearing on subsequent egg performance is directly related to insufficient supplies of essential nutrients for synthesis and formation of important body structures at so-called critical stages of development.

If a growth retardation of body constituents is under study, the weight-age relationship is less relevant for assessing the effects of a nutrient restriction. The essential point is whether the development of some particular body constituents is affected *more* by the applied restriction than others. Hence, a description of relative growth is a relevant approach.

In previous studies, the allometric relationship has often been used to study the relative growth of important body constituents in several species: a.o., pigs (Davies, 1983), cattle (Robelin and Daenicke, 1980), birds (Ricklefs, 1967; Shebaita *et al.*, 1977; Lilja, 1983; Lilja *et al.*, 1985; Katanbaf *et al.*, 1988), and fish (Alami-Durante, 1990). Two problems, however, may arise when the simple allometric function of Huxley (1932) is used in nutritional studies.

Firstly, if the allometric growth of a body constituent is described as a function of BW or empty body mass (EBM), the effects of feeding regime on growth of the respective constituent may be falsely interpreted. Elsley (1964) and Davies (1983) reanalyzed data on pig growth of McMeekan (1940, 1941). They suggested that the effects of nutrient restrictions on the growth of body organs or tissues could easily lead to misinterpretations, when these body constituents were expressed relative to BW or EBM (e.g., in an allometric equation or as a weight measure per 100 g BW or EBM). This was due to the fact that there can be a large variation in fat deposition due to the variation in the feeding plane. Such representations, still reported until very recently (Katanbaf *et al.*, 1989; Yu *et al.*, 1992), may obscure the 'true' level of retardation, and therefore lead to false conclusions. For example, McCance (1977) and Widdowson (1980) stated that, after cessation of a nutrient restriction, catch-up growth may consist for a greater part of fat deposition, ultimately resulting in a fatter body at equal BW compared to the controls.

Secondly, on the basis of their results, most authors assumed that there are no changes in the allometric relationship between two body constituents with advancing age (e.g., Shebaita et *al.*, 1977; Alami-Durante, 1990). Others, however, did actually expect some kind of a 'multiphasic' allometric relationship (Ricklefs, 1967; Lohse *et al.*, 1971; Katanbaf *et al.*, 1988). They fitted two or more linear segments with different allometric slopes, and estimated the transition area by eye (Ricklefs, 1967; Lilja, 1983; Lilja et *al.*, 1985).

In the present study these problems were tackled. A multiphasic allometric function (Koops and Grossman, 1993; Kwakkel *et al.*, 1993) was used to assess the growth of crude fat (Cfat), CP, ash, and water, each as a function of EBM or fat-free plucked EBM (FFEBM). Data were obtained from pullets that had been fed either a low-lysine diet or a restricted daily amount of an adequate diet, on a pair-gain basis, during the starter or grower period of rearing (Kwakkel et *al.*, 1991).

The objective of the present study was to assess the effects of different feeding regimens on the growth of body components by use of a multiphasic allometric approach. The aim was to investigate the role of body fat as an 'independent' component during underfeeding.

This study is part of a wider investigation which has as its general objective to address empirical relationships to quantify biological principles of pullet growth. These may serve as a step forward in the elucidation of causal relationships between immature growth and mature performance.

MATERIALS AND METHODS

Experimental design

A detailed description of the design of the experiment, from which the data in the present paper were obtained, has been reported elsewhere (Kwakkel et *al.*, 1991). In brief, one-day-old White Leghorn (WL) pullets of a commercial stock (Lohmann GmbH, Cuxhaven, Germany) were allocated to one of five feeding regimens. A control group (C) of pullets had full access to adequate starter (0 to 6 wk of age: .85% digestible lysine, .71% digestible methionine + cystine, 18.9% CP, 1.0% calcium, .41% available phosphorus, and 2,800 kcal ME/kg) and grower diets (7 to 18 wk of age: .65% digestible lysine, .60% digestible methionine + cystine, 15.9% CP, .9% calcium, .44% available phosphorus, and 2,800 kcal ME/kg). In each of these two rearing phases (0 to 6 and 7 to 18 wk of age), pullets were restricted by providing them with a low lysine diet (.40% or .30% digestible lysine in the starter (RLs) or grower (RLg) phase, respectively) or by a restricted daily amount of feed (RFs and RFg). The feed restriction was based on 'pair-gain' feeding: pullets of groups RFs and RFg were fed daily such an amount of feed of the starter and grower diet, respectively, that they matched the BW of the respective RL-group. All other nutrients were supplied in sufficient amounts (NRC, 1984). During the laying period (19 to 62 wk of age, all hens were fed *ad libitum* a commercial diet (.65% digestible lysine, .58% digestible methionine + cystine, 16.5% CP, 3.5% calcium, .44% available phosphorus, and 2,800 kcal ME/kg).

Data

The number of pullets per cage (nine replicate cages per treatment) decreased from 24 birds in wk 0, to 8 birds in wk 24. Each week 5 to 8 pullets per treatment were sacrificed by cervical dislocation and subsequently dissected. Birds were defeathered and the gut was emptied. The results of several organ determinations are reported elsewhere (Kwakkel *et al.*, Subm. B).

In wk 0, 3, 6, 9, 12, 15, 18, 20, 22, and 24, the plucked empty bodies of pullets (including all dissected organs and viscera) were minced individually (wks 12 to 24) or as a pooled sample of 2 birds (wks 3 to 9). One-day-old pullets were pooled in 2 samples of 5 birds each. After the samples had been freeze-dried and ground they were ready for chemical analyses of dry matter (DM), ash, CP (Kjeldahl; Nx6.25) and crude fat (Cfat; ether extract). Water was calculated as empty body mass (EBM) minus DM, and FFEBM as EBM minus Cfat. The detailed procedures for proximate analysis have been outlined in Kwakkel *et al.* (Subm. A).

Plumage was not included in the samples since there was a close linear relationship between plucked EBM and BW, similar for all treatments ($EBM = .87 * BW$: $R^2 = .99$). Moreover, feather protein contains 1.6% lysine whereas other protein parts in the body contain 9.6% (Scott *et al.*, 1982). It was assumed that our low-lysine diets, in which all other amino acids were provided in adequate amounts, would not interfere to any extent with feather growth (Singsen *et al.*, 1965). Additionally, feathers would have increased the heterogenity of the minced samples.

The multiphasic allometric function

The Multiphasic Allometric Function (MAF; Kwakkel *et al.*, 1993), developed by Koops and Grossman (1993), has been applied to describe the growth of Cfat, CP, ash and water, each as an allometric function of EBM or FFEBM, among treatments. The MAF [1] is based on the allometric model of Huxley which describes the monophasic log-log relationship between two body constituents (Huxley, 1932). However, the MAF allows for changes in the allometric relationship with advancing age (Kwakkel *et al.*, 1993):

$$\ln(y_x) = \ln(\alpha_{i=1}) + \beta_i \ln(x) - \sum_{i=1}^{n-1} \{(\beta_i - \beta_{i+1})\, r_i \ln[1 + (x/c_i)^{1/r_i}]\} \qquad [1]$$

where, ln is the natural logarithm; y_x is the weight of a body component (CP, Cfat, ash or water), and x is the weight of EBM or FFEBM, both in grams; α is the scale parameter ($\ln(\alpha)$ is the intercept); n is the number of phases; and in each phase i, β_i is the allometric slope, c_i is the estimated breakpoint between phase i and i+1, and r_i is a smoothness parameter.

Average component weights per treatment and sample week (n=10) were transformed to their natural logarithm for use in the curve-fitting procedures in order to determine mono- and diphasic relationships. Due to the small number of observations within the breakpoint area, the curve-fitting did not accurately estimate the smoothness parameter r_i. Therefore, all diphasic curves were initially fitted with fixed r_i-values of .01, .05, .1, .5, and 1.0. If r_i varied between .1 and .01, none of the parameter estimates changed substantially. Outside this range, in approximately 90% of the cases, no convergence was reached even after 75 iteration steps. The lowest SE's were derived by fixing the smoothness parameter at .05, which is a rather abrupt transition (Koops and Grosmann, 1993).

Statistics

All curves were fitted using the nonlinear regression algorithm procedures from the NONLIN package (Method Levenberg-Marquardt (Dennis *et al.*, 1981); convergence criterion 10^{-10}). Goodness-of-fit criteria were the asymptotic SE (ASE) for each parameter, the residual SD (RSD), the coefficient of determination (CD), and the Durbin-Watson (DW) statistic on first-order autocorrelation.

Mono- and diphasic allometric relationships of each component versus EBM and FFEBM were tabulated per treatment. The fitted results were displayed per treatment since in this way even small - and non-significant - differences between treatments can be judged individually. Breakpoints between phases were related to chronological ages for each treatment by plotting the natural logarithm of FFEBM versus age.

Statistics were carried out according to the following procedure. Firstly, the significance of the addition of a second phase to the monophasic allometric model was assessed by an F-test, in order to verify the diphasic nature of the relationship (Kwakkel et *al.*, 1993). Subsequently, for the best model (mono- or diphasic), differences between treatment groups or combinations of treatments were also investigated by F-tests using indicator variables (Neter *et al.*, 1990).

Table 1. Parameter estimates and SE (in parentheses) of growth of the body component Cfat, as a function of EBM[1] or fat-free EBM of WL-pullets, fed different feeding regimens[2], described by a Multiphasic Allometric *Function*[3].

Mono/Di phasic	Trmt[2]	Parameter estimates Scale parameter	Allometric slope phase 1	 phase 2	Breakpoint	Goodness of fit RSD[4]	 CD[5]	 DW[6]	F(2,6)[7]
				Cfat growth as a function of EBM					
Mono	C	-3.63 (.40)	1.26 (.06)	-	-	.226	.9806	.72	
	RLs	-3.34 (.42)	1.20 (.07)	-	-	.264	.9746	.90	
	RFs	-4.46 (.65)	1.67 (.10)	-	-	.410	.9552	1.69	
	RLg	-3.49 (.32)	1.24 (.05)	-	-	.178	.9868	1.61	
	RFg	-3.45 (.57)	1.20 (.09)	-	-	.322	.9564	.43	
Di	C	-2.68 (.24)	1.05 (.05)	1.98 (.15)	6.34 (.14)	.088	.9978	3.02	23.28
	RLs	-2.60 (.35)	1.03 (.07)	2.67 (.71)	6.65 (.17)	.162	.9929	2.86	7.65
	RFs	- .09(1.26)	.28 (.32)	1.80 (.12)	4.77 (.26)	.202	.9918	2.55	13.48
	RLg	-2.77 (.38)	1.07 (.08)	1.54 (.14)	5.97 (.47)	.123	.9953	3.47	5.36
	RFg	-2.42 (.23)	.98 (.04)	2.48 (.26)	6.57 (.10)	.101	.9968	2.33	37.49
				Cfat growth as a function of FFEBM					
Mono	C	-3.69 (.50)	1.30 (.08)	-	-	.278	.9707	.66	
	RLs	-3.34 (.50)	1.23 (.08)	-	-	.312	.9646	.86	
	RFs	-4.56 (.74)	1.41 (.12)	-	-	.462	.9432	1.65	
	RLg	-3.54 (.39)	1.27 (.06)	-	-	.214	.9808	1.54	
	RFg	-3.45 (.68)	1.22 (.11)	-	-	.378	.9398	.42	
Di	C	-2.60 (.29)	1.05 (.06)	2.42 (.24)	6.28 (.13)	.111	.9965	2.89	22.15
	RLs	-2.24 (.52)	.97 (.11)	2.40 (.53)	6.25 (.24)	.208	.9882	2.59	6.00
	RFs	- .03(1.41)	.26 (.37)	1.94 (.15)	4.76 (.28)	.240	.9885	2.49	11.82
	RLg	-2.70 (.46)	1.08 (.10)	1.68 (.19)	5.88 (.45)	.149	.9931	3.44	5.29
	RFg	-2.32 (.28)	.98 (.05)	3.20 (.44)	6.50 (.09)	.123	.9952	2.55	34.46

[1] EBM: plucked empty body mass.

[2] Feeding regimens (Trmt): C = no restrictions; RLs - lysine restriction from 0-6 wk of age; RFs - feed restriction, pair-gained to RLs; RLg = lysine restriction from 7-18 wk of age; RFg - feed restriction, pair-gained to RLg.

[3] $\ln(y_x) = \ln(a) + ß_1 \ln(x)$ (monophasic, Mono)
$\ln(y_x) = \ln(a) + ß_1 \ln(x) - .05\,(ß_1 - ß_2)\ln[1 + e^{(\ln(x)-\ln(c))/.05}]$ (diphasic, Di)
where $\ln(a)$ is the scale parameter, $ß_1$ and $ß_2$ are the allometric slope of phase 1, and 2, respectively, ln (c) is the breakpoint.

[4] Residual SD: n in all fits was 10; Each observation is represented by 5 to 8 pullets.

[5] Coefficient of Determination.

[6] Durbin Watson statistic: a value around 2 indicates no autocorrelation.

[7] $F(2,6)$ represents the significancy of the addition of a second phase; Critical $F(2,6)$-values were 5.14 ($P<.05$) and 10.92 ($P<.01$).

RESULTS

In order to relate the breakpoints between the allometric levels in the Tables 1, 2, and 3, to chronological ages, the natural logarithm of FFEBM versus age is displayed in Figure 1. At 24 wk of age (which was the final observation in time), amounts of FFEBM were similar for all treatments (Figure 1; 1026.4 g ± 7.3 (mean±SEM): R.P. Kwakkel, Unpublished results).

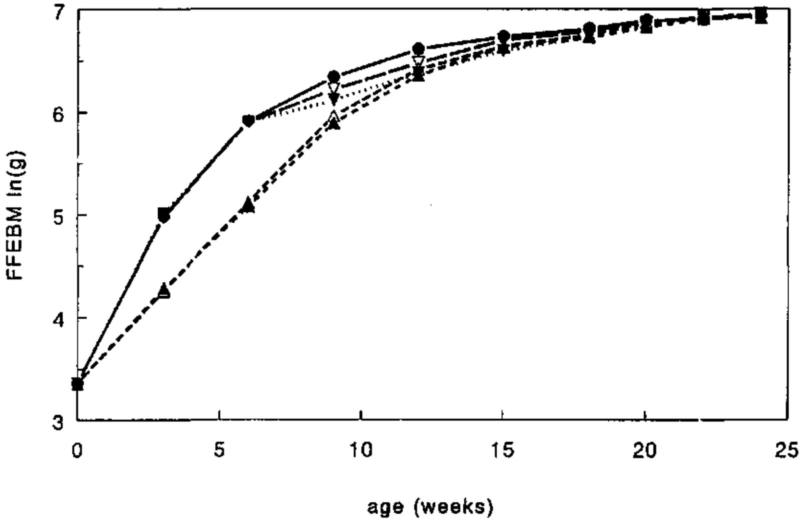

Figure 1. The relationship between the natural logarithm of fat-free plucked empty body mass and age in WL-pullets fed different feeding regimens (C: ● ; RLs: ▲ ; RFs: △ ; RLg: ▼ ; RFg: ▽ ; markers are observed means).

The parameter estimates of the mono- and diphasic allometric relationships between Cfat and EBM, and Cfat and FFEBM, for all feeding regimens, are listed in Table 1. The goodness of fit was significantly improved if a diphasic instead of a monophasic function was fitted to the data, for both allometric relationships (see *F*-values in Table 1). Therefore, comparisons between treatments were made for the diphasic model.

No large differences in parameter estimates were observed between both diphasic models (Cfat versus EBM and Cfat versus FFEBM, respectively). The relationship between Cfat and FFEBM, however, tended to have overall larger intercepts (scale parameter), similar $ß_1$'s, larger $ß_2$'s, and breakpoints at lower weights than the relationship between

Table 2. Parameter estimates and SE (in parentheses) of growth of the body components CP, ash and water, each as a function of EBM[1] of WL-pullets, fed different feeding regimens[2], described by a Multiphasic Allometric Function[3].

Mono/Di phasic	Trmt[2]	Parameter estimates				Goodness of fit			F(2,6)[7]
		Scale parameter	Allometric slope phase 1	phase 2	Breakpoint	RSD[4]	CD[5]	DW[6]	
		CP growth as a function of EBM							
Mono	C	-1.72 (.07)	1.01 (.01)	-	-	.038	.9991	1.07	
	RLs	-1.82 (.06)	1.02 (.01)	-	-	.041	.9991	1.83	
	RFs	-1.65 (.07)	1.00 (.01)	-	-	.041	.9991	2.15	
	RLg	-1.74 (.05)	1.01 (.01)	-	-	.026	.9996	2.38	
	RFg	-1.75 (.07)	1.01 (.01)	-	-	.042	.9989	.69	
Di	C	-1.79 (.06)	1.02 (.01)	.72 (.23)	6.92 (.18)	.031	.9996	2.33	3.31
	RLs	-1.85 (.08)	1.03 (.01)	.43(4.81)	7.02 (.69)	.044	.9992	2.16	.39
	RFs	-1.69 (.08)	1.00 (.02)	.66(2.37)	6.97 (.87)	.045	.9992	2.56	.43
	RLg	No convergence reached							
	RFg	-1.75 (.06)	1.03 (.01)	.66 (.27)	6.94 (.17)	.030	.9996	1.63	4.98
		Ash growth as a function of EBM							
Mono	C	-3.25 (.16)	1.01 (.02)	-	-	.089	.9952	.92	
	RLs	-3.23 (.11)	1.01 (.02)	-	-	.072	.9973	1.08	
	RFs	-3.08 (.15)	.99 (.02)	-	-	.096	.9951	1.38	
	RLg	-3.23 (.13)	1.01 (.02)	-	-	.073	.9966	.93	
	RFg	-3.25 (.15)	1.01 (.02)	-	-	.085	.9955	1.42	
Di	C	-3.49 (.15)	1.06 (.03)	.68 (.17)	6.61 (.26)	.063	.9982	2.33	5.02
	RLs	-3.58 (.16)	1.09 (.04)	.86 (.06)	5.91 (.40)	.044	.9992	1.60	7.71
	RFs	-3.79 (.19)	1.15 (.04)	.86 (.05)	5.26 (.38)	.049	.9990	2.81	12.18
	RLg	-3.46 (.12)	1.05 (.02)	.82 (.10)	6.37 (.33)	.051	.9987	2.07	5.21
	RFg	-3.48 (.18)	1.06 (.03)	.83 (.13)	6.36 (.46)	.073	.9975	2.35	2.47
		Water growth as a function of EBM							
Mono	C	-.12 (.11)	.95 (.02)	-	-	.062	.9974	.59	
	RLs	-.16 (.09)	.95 (.02)	-	-	.058	.9980	.47	
	RFs	-.10 (.08)	.95 (.01)	-	-	.052	.9984	.84	
	RLg	-.15 (.09)	.95 (.01)	-	-	.049	.9983	.81	
	RFg	-.16 (.12)	.96 (.02)	-	-	.065	.9970	.46	
Di	C	-.38 (.04)	1.00 (.01)	.73 (.03)	6.39 (.09)	.016	.9999	2.18	57.39
	RLs	-.40 (.06)	1.01 (.01)	.74 (.05)	6.33 (.13)	.022	.9998	1.78	25.54
	RFs	-.47 (.08)	1.03 (.02)	.85 (.02)	5.49 (.23)	.022	.9998	1.76	19.95
	RLg	-.38 (.05)	1.00 (.01)	.85 (.02)	6.03 (.17)	.017	.9998	3.16	30.42
	RFg	-.39 (.03)	1.01 (.01)	.72 (.03)	6.49 (.07)	.014	.9999	2.48	87.86

[1-7] As in Table 1.

Table 3. Parameter estimates and SE (in parentheses) of growth of the body components CP, ash and water, each as a function of fat-free EBM[1] of WL-pullets, fed different feeding regimens[2], described by a Multiphasic Allometric Function[3].

Mono/Di phasic	Trmt[2]	Parameter estimates				Goodness of fit			*F*(2,6)[7]
		Scale parameter	Allometric slope phase 1	Allometric slope phase 2	Breakpoint	RSD[4]	CD[5]	DW[6]	
CP growth as a function of FFEBM									
Mono	C	-1.81 (.07)	1.05 (.01)	-	-	.037	.9992	1.13	
	RLs	-1.85 (.07)	1.05 (.01)	-	-	.043	.9991	1.57	
	RFs	-1.77 (.04)	1.04 (.01)	-	-	.023	.9997	1.76	
	RLg	-1.80 (.05)	1.04 (.01)	-	-	.029	.9995	1.14	
	RFg	-1.79 (.06)	1.04 (.01)	-	-	.032	.9994	.93	
Di	C	-1.60 (.10)	1.00 (.02)	1.10 (.03)	5.44 (.45)	.027	.9997	1.33	4.62
	RLs	-1.58 (.10)	.98 (.02)	1.12 (.03)	5.35 (.41)	.029	.9997	3.05	5.64
	RFs	-1.67 (.05)	1.02 (.01)	1.09 (.03)	5.96 (.46)	.016	.9999	2.92	5.46
	RLg	-1.61 (.04)	1.00 (.01)	1.09 (.01)	5.37 (.21)	.012	.9999	.80	19.80
	RFg	-1.61 (.05)	1.00 (.01)	1.11 (.01)	5.66 (.24)	.015	.9999	.88	15.83
Ash growth as a function of FFEBM									
Mono	C	-3.34 (.10)	1.05 (.02)	-	-	.058	.9980	1.49	
	RLs	-3.26 (.10)	1.04 (.02)	-	-	.064	.9979	1.45	
	RFs	-3.20 (.11)	1.03 (.02)	-	-	.071	.9973	1.44	
	RLg	-3.30 (.10)	1.04 (.02)	-	-	.057	.9979	1.47	
	RFg	-3.31 (.12)	1.04 (.02)	-	-	.066	.9973	2.08	
Di	C	-3.44 (.14)	1.07 (.03)	.88 (.21)	6.49 (.49)	.058	.9985	2.14	1.01
	RLs	-3.47 (.22)	1.09 (.05)	.96 (.07)	5.68 (.86)	.063	.9984	1.44	1.07
	RFs	-3.60 (.18)	1.12 (.04)	.93 (.06)	5.36 (.52)	.053	.9989	2.09	4.10
	RLg	-3.42 (.13)	1.07 (.03)	.92 (.13)	6.26 (.57)	.054	.9986	2.11	1.52
	RFg	-3.37 (.19)	1.05 (.04)	.99 (.15)	6.21(1.93)	.074	.9974	2.17	.16
Water growth as a function of FFEBM									
Mono	C	-.20 (.04)	.98 (.01)	-	-	.025	9996	.89	
	RLs	-.19 (.04)	.98 (.01)	-	-	.025	.9996	1.06	
	RFs	-.21 (.03)	.98 (.01)	-	-	.021	.9997	.91	
	RLg	-.21 (.04)	.98 (.01)	-	-	.022	.9996	.71	
	RFg	-.21 (.04)	.98 (.01)	-	-	.023	.9996	.68	
Di	C	-.31 (.03)	1.01 (.01)	.89 (.02)	6.19 (.16)	.012	.9999	2.06	15.70
	RLs	-.30 (.03)	1.01 (.01)	.90 (.03)	6.09 (.22)	.013	.9999	2.65	12.14
	RFs	-.31 (.02)	1.01 (.01)	.93 (.01)	5.93 (.16)	.006	.9999	2.20	42.97
	RLg	-.31 (.01)	1.01 (.01)	.93 (.01)	5.97 (.08)	.004	.9999	2.47	117.36
	RFg	-.31 (.02)	1.01 (.01)	.92 (.01)	6.05 (.15)	.009	.9999	1.39	24.17

[1-7] As in Table 1.

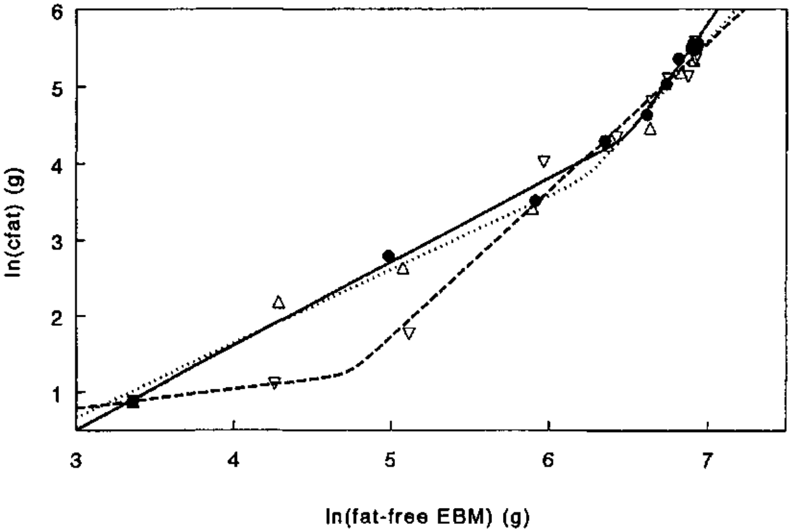

Figure 2. Diphasic allometric relationships between crude fat and fat-free plucked empty body mass in WL-pullets of the Control (●), RLs (△) and RFs (▽) treatments (markers are observed means).

Cfat and EBM. For each group, the allometric slope of the first growth phase ($ß_1$) was about 1, for both Cfat versus EBM as well as Cfat versus FFEBM, except for the RFs-group. These pullets had significantly ($P<.01$) lower $ß_1$'s (.28 and .26, respectively, and illustrated in Figure 2). The slope of the second growth phase ($ß_2$) was estimated as a value above 1.50 for all treatment groups. For both the EBM and the FFEBM model, RFs and RLg had significantly ($P<.01$) lower $ß_2$'s than the other groups. Breakpoints in the allometric relationship between Cfat and EBM, and Cfat and FFEBM for the control pullets were estimated at 6.34 and 6.28, respectively. RLs-pullets followed a similar diphasic allometric pattern in both phases for Cfat versus FFEBM (Table 1; Figure 2). For Cfat versus EBM, the similarity was less clear. RFs- and RLg-pullets had a breakpoint at a significantly ($P<.01$) lower weight (RFs: 4.76 (both EBM and FFEBM) and RLg: 5.97 (EBM) and 5.88 (FFEBM)) than the C-pullets. The breakpoint for the RFg-group tended to a somewhat higher value than the one for the C-group (EBM: 6.57 vs. 6.34, and FFEBM: 6.50 vs. 6.28).

In Tables 2 and 3, the parameter estimates of the allometric relationships of CP, ash, and water, each as a function of EBM (Table 2) or FFEBM (Table 3) are given. The diphasic nature of the relationship between CP and EBM could not be proved statistically, due to the large variations in the second slope. For the RLg-group even no convergence could be reached. For the relationship between CP and FFEBM, all diphasic fits were significantly better than the monophasic fits, except for the control group. The allometric fits between ash and EBM, and between water and EBM or FFEBM improved significantly if a second phase was included in the model (see *F*-values and 'goodness of fit'-criteria).

When the relative growth of CP, ash, and water was fitted against FFEBM instead of EBM, differences between the allometric slope of the first and second phase became smaller (compare *F*-values in Tables 2 and 3; *see* Figures 3A and 3B). For ash versus FFEBM, differences became so small, that no diphasic relationship could be significantly detected anymore.

Tables 2 and 3 showed that the diphasic relationships between each fat-free body component and EBM or FFEBM had $ß_1$'s of about 1, on average. The $ß_2$'s in the EBM model were far below 1 (ranging from .50 to .85 for all three fat-free components), whereas in the FFEBM model the $ß_2$'s were significantly larger than 1 for CP (on average 1.10), not significantly different from 1 for ash (.94) and significantly lower than 1 for water (.91).

Significant ($P<.01$) differences in parameter estimates between the early-restricted groups (in particular RFs), on the one hand, and the other groups, on the other, for the diphasic functions of ash versus EBM, and water versus EBM, were evident. Differences in parameter estimates between feeding regimens, however, could not statistically proved in the diphasic relationships between each of the fat-free components (including protein) and FFEBM (Table 3 and Figure 4).

DISCUSSION

The feeding regimens in this study were designed to impose growth retardation and, as a consequence of changed dietary compositions, alterations in chemical body composition (Kwakkel *et al.*, 1991). After the first period of restriction (0 to 6 wk of age), BW of the RLs- and RFs-pullets (pair-gained) was 43% of the C-pullets (on average 197 versus 457 g BW, respectively; see Kwakkel et *al.*, 1991). On the basis of this difference, it was expected that the low-lysine diets would have changed the body composition of the RL-birds compared to the controls.

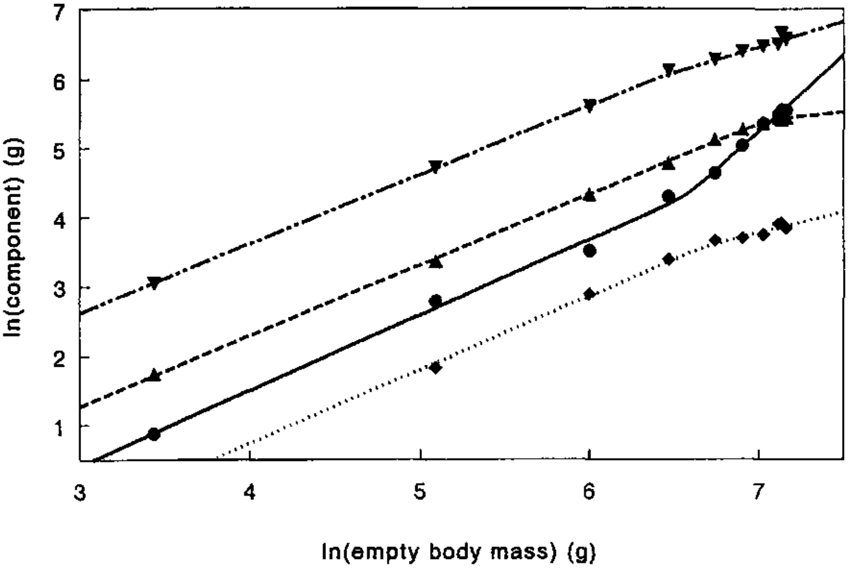

Figure 3a. Diphasic allometric relationships of crude fat (●), CP (▲), ash (◆), and water (▼), each as a function of plucked empty body mass in WL-pullets fed adequate diets *ad libitum* (markers are observed means).

The interpretation of slopes and breakpoints in the diphasic model

In the diphasic model of Cfat versus EBM or FFEBM (Table 1), the late maturation of the fat component in the body is clearly demonstrated by the large allometric slopes of the second growth phase. In the control pullets (as a reference), this second fat growth spurt started at around 567 g EBM and 534 g FFEBM (=breakpoint). Both values reflect an age of 8 wk for the C-pullets (Figure 1). Due to their restricted feeding, pullets of the RFs-treatment showed a decreased relative growth of fat during the first phase (Figure 2). The earlier change from the first to the second phase for fat growth in these pullets compared to the C-pullets (at about 120 g EBM *and* FFEBM) was most likely a consequence of the cessation of the restriction at the end of wk 6. A similar breakpoint in the EBM and FFEBM model for RFs suggests that the amount of FFEBM was equal to the amount of EBM at the end of the restrictive period for this group. The early change, however, lowered the second slope for fat growth, since the fat-free body still grew relatively fast at that stage.

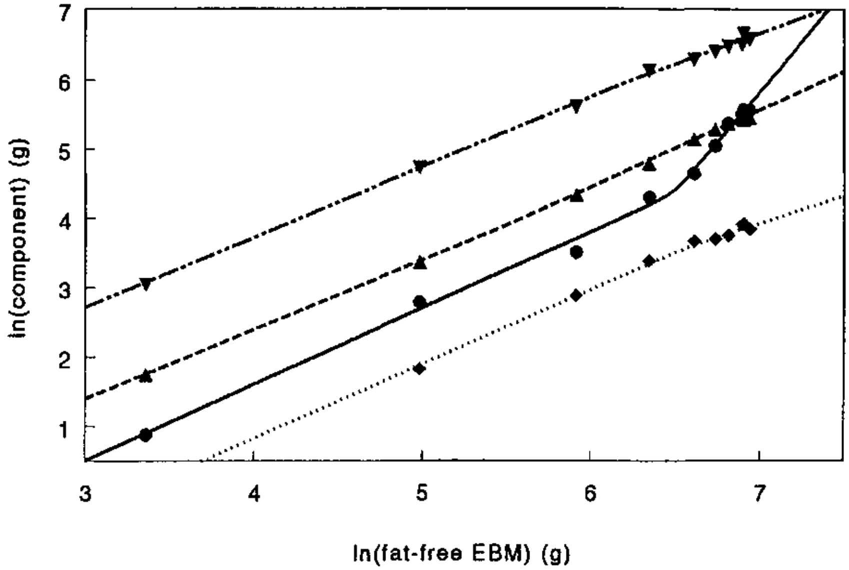

Figure 3b. Diphasic allometric relationships of crude fat (●), CP (▲), ash (◆), and water (▼), each as a function of fat-free plucked empty body mass in WL-pullets fed adequate diets *ad libitum* (markers are observed means).

Pullets that had been restrictedly fed from 7 wk onwards (RFg) demonstrated a delay in the attainment of the second fat growth spurt, compared to the C-pullets (breakpoints were for RFg at 713 g EBM and 665 g FFEBM, respectively). It is most likely that this delay was due to the feeding regime: a restricted feeding level hindered the bird to increase fat deposition at the appropiate 'physiological' age (a particular amount of EBM or FFEBM). The larger $ß_2$ in the RFg-pullets compared to the C-pullets seems to be directly related to this delay: growth of the fat-free body had already decreased to a larger extent than when the C-group entered the second fat growth phase.

The earlier breakpoint in the fat-curve of the RLg-pullets (Table 1: at 392 g EBM and 358 g FFEBM) coincided with the moment at which the birds were confronted the low-lysine grower diet beyond wk 7. The lower slope of the second fat growth phase in the RLg-pullets, in contrast to the C-pullets, might be related to the earlier breakpoint. Resulting in a situation, in which fat-free tissue deposition had not yet decreased to a major extent.

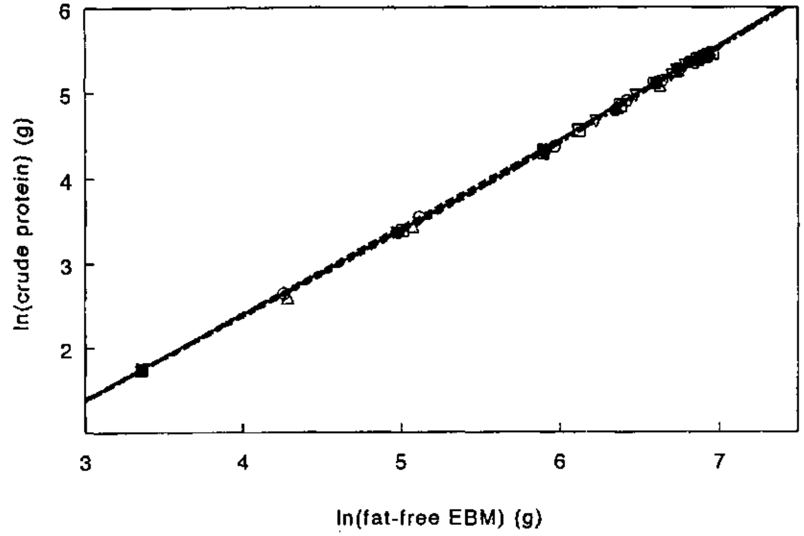

Figure 4. Diphasic allometric relationship between CP and fat-free plucked empty body mass in WL-pullets fed different feeding regimens (C: ● ; RLs: Δ ; RFs: o ; RLg: □ ; RFg: ∇ ; markers are observed means).

No large differences in parameter estimates could be demonstrated if Cfat versus EBM was compared to Cfat versus FFEBM. The latter model, however, showed overall larger intercepts (less negative), larger β_2's, and breakpoints that appeared at an earlier weight. On the other hand, the β_1's were, on average, similar. This illustrates that fat growth during the first growth spurt is related to growth of the fat-free body (similar slopes for fat vs EBM or FFEBM). During the second fat growth spurt, fat seems to act more independently from the fat-free body, which is illustrated by the divergence of the β_2's between the fat-EBM and fat-FFEBM model, respectively.

The allometric relationship between each chemical fat-free body component and EBM (Table 2) was diphasic for *all* treatments, with widely different slopes. This diphasic relationship, however, was to a large extent due to the diphasic pattern of fat growth *per se*: the allometric slope of the second phase between each of the fat-free body component and total FFEBM (Table 3) tended towards 1 (see also Figures 3A and 3B).

This does not imply that there were no changes in the relative growth rate within the fat-free body throughout the entire rearing period: most relationships still remained diphasic (Table 3). Isometry was found in the first allometric phase for each fat-free body component and for each feeding regime. This implies a constant fat-free body composition in the first growth phase, regardless the applied treatment. The breakpoint for CP, however, was at a lower FFEBM (260 g, on average) than the ones for ash (400 g FFEBM) and water (420 g FFEBM). Between, on average 350 and 1000 g FFEBM (FFEBM reached an asymptote in all treatments, assuming maturity; Figure 1), CP grew relatively faster than ash and water.

Allometric slopes found in the present study and those reported for chickens by Shebaita *et al.* (1977) and for pigs by Davies (1983) and Tess *et al.* (1986) were of the same magnitude. In Fayoumi chickens, aging 0 to 9 wk old, Shebaita *et al.* (1977) found slopes for monophasic allometric functions between fat, water, ash, and protein, each as a function of the fat-free body, of .72, .98, 1.21, and 1.07, respectively. The low ß-value for fat versus FFEBM may be the result of the short age-interval and the obviously early fat-maturing Fayoumi bird. In pigs, Davies (1983) reported slope values of .83 and 1.05 for bone and muscle, respectively, each as an allometric function of 'muscle + bone'. Tess *et al.* (1986) reported values of 1.10, 1.07, and .96 for protein, ash, and water, each as a function of the fat-free body, respectively.

Fat mobilization and inherently ruled fat deposition

The results for fat growth in the RF-pullets confirmed the theory that fat is easily mobilized or not deposited to the required functional levels, as a result of a restricted nutrient intake (Palsson, 1955; Gous and Stielau, 1976; Connor *et al.*, 1977; Leat and Cox, 1980; Burgess, 1986).

RLs-pullets consumed 46% more energy and 30% less digestible lysine (first limiting amino acid in this experiment) per gram BW gain, than did the control pullets from 0 to 6 wk of age (Kwakkel *et al.*, 1991). The required protein to energy ratio in the diet of the RLs-pullets, however, has to be lower than in the control diet, due to the larger part of maintenance and thus the increased relative demand for energy, if growth is substantially reduced. Additionally, maintenance requirements may be increased as a consequence of the imbalanced diet in order to deaminate the excess of surplus amino acids. However, the RLs-pullets still consumed about 5 to 10% less energy per metabolic BW ($kJ.kg^{.75}$) than did the control pullets (R.P. Kwakkel, Unpublished results). Because of the extra energy available, these pullets were expected to become fatter than the C-pullets (Gous, 1972; Fuller *et al.*, 1973; Leeson and Summers, 1980). Their body composition, however, did not change if compared to the *ad libitum* fed group. On the basis of these findings, it was deduced that early fat growth is closely related to growth of the fat-free body. The young pullet at that stage of biological development probably does not deposit fat for energy reserve purposes. In other words: the deposition of surplus, non-functional, fatty tissue has no priority at very young ages in layer pullets. This is in line with the ideas of Gous *et al.* (1990), who, based on the Edinburgh Growth Model, suggested that "at a given degree of maturity in body protein, a given kind of chicken 'seeks' a particular lipid-to-protein ratio". Additionally, our findings agree with those of McLachlan *et al.* (1977), who reported that after feeding pullets a low protein diet (130 g/kg CP) from 5 wk of age onwards, fat levels were decreased at 20 wk of age compared to the control fed birds. Kyriazakis and Emmans (1992) presented a set of propositions that may lead to a theory on growth retardation and compensatory growth. They suggested, as did many others, that animals subjected to a low CP/energy diet will deposit relatively more lipid tissue.

It is suggested in the present paper that some young and 'lean' animals may have an inherent constrain to deposit excessive amounts of lipid during particular stages of juvenile growth. By characterizing growth features of a particular type of animal during several stages of development, valuable theories like the one of Kyriazakis and Emmans (1992) could be refined.

No constant fat-free body composition throughout rearing

The suggestion that there would be a constant fat-free body composition (proportional growth of ash, water and protein) throughout the entire rearing period is an oversimplification. This is only valid for the first growth spurt. The differences in the second slope between the curves of CP vs FFEBM on the one hand, and water vs FFEBM on the other, illustrate the general observation that the proportion of water in fat-free tissue decreases as the animal grows older (Ricklefs, 1967; Dunn and Brisbin, 1980 (quoted by Katanbaf *et al.*, 1988); Tess *et al.*, 1986). This means that there is a declining water content in the fat-free body (irrespective of the increasing amount of fat) with advancing age. The change in water content seems to be rather abrupt, related to some particular physiological stage.

Components within FFEBM respond inseparately to nutrient restrictions

Hammond and colleagues from the Cambridge school, stated about 40 years ago (Palsson, 1955), that a severe feed restriction would result in mobilization of body tissue, in the reverse order in which it had been deposited. Thus, a moderate restriction would affect fat deposition first, whereas more severe nutritional deficiencies would also lead to a mobilisation of muscle tissue, but still maintain bone growth (Palsson, 1955). The findings reported here disagree with this proposition of a sequential response of muscles and bones to severe nutrient restrictions. From the results shown in Table 3 it can be concluded that muscle (in our study: 'CP + water') and bone ('ash') tissue responded inseparately to underfeeding situations. This is in agreement with data on fat-free body composition in cattle (Berg and Butterfield, 1976) and pigs (Walstra, 1980; Davies, 1983). This means that relative gain within the FFEBM is not influenced by feeding regime. Burgess (1986) also found a decreased protein mass in line with a smaller skeletal body in layer pullets stunted by a feed restriction. In contrast, Yu *et al.* (1992) reported that the response to an underfeeding situation in broiler breeders was less for bone growth than for protein growth.

Ash is a major component of the skeleton. The main biological functions of the skeleton during early growth are support of the body structure, and being the place of attachment for muscles (Vaughan, 1980). This may clarify biologically, the close relationship that seems to exist between CP, water, and ash during the period of the first growth spurt (Table 3). The assumption of protein/ash (in)-dependencies is often discussed in literature on growth (Kyriazakis and Emmans, 1992). In contrast to other farm animals, birds are covered with feathers. Therefore, the protein part of the whole body need to be separated into 'plumage' protein and protein of the 'rest of the body' (e.g. *see* Fisher and Scougall, 1982). This separation may explain the discrepancy found between several farm species on the relationship between protein and ash.

It could be argued that the functional relationship (allometric slopes of around 1) between protein and ash as found in this study, independent of the applied feeding regime, is associated with the removal of the feathers to obtain a *defeathered* FFEBM. E.g., Yu *et al.* (1992) actually found a smaller protein to ash ratio after feed restriction in broiler breeders, when they included feathers in the empty bodies. This could mean that feather protein is relatively more affected than body protein on a weight-weight basis if a feed or protein restriction is applied. Another explanation might be the extra loss of feathers due to an enhanced feather pecking, a phenomenon that has been observed frequently during the feeding of protein deficient diets (Lee *et al.*, 1971; Lahore and Payne, 1975; Kwakkel, Unpublished observations).

Advantages of multiphasic versus monophasic allometry

The present study provides evidence that, under an adequate feeding regime, the body components Cfat, CP, ash, and water grow proportionally during the first stage of rearing (up to wk 8 or 9). During the second stage of rearing, fat increases enormously, and within the fat-free body, only protein still grows.

In literature, fat has always been regarded as a late-maturing component, based on the relatively large fat growth spurt during later rearing stages. Kwakkel *et al.* (1993) already suggested that fat growth could be separated into a phase which represents mainly functional fat growth and a subsequent phase that represents depot fat growth.

The present results confirm those observations. The use of the term 'late-maturing' for *total* adipose tissue in the body is therefore somehow misleading.

Ash and water mature earlier than protein. These small changes in relative growth rate within the fat-free body are possibly mediated by hormonal changes which are due to the attainment of some particular stage of development. Such changes had not been found if a monophasic allometric model had been used.

Lysine-restricted pullets apparently deposited an amount of protein (or fat-free tissue) associated with a certain amount of fat, at a similar ratio as did the control group on an adequate diet. This optimal ratio may be regulated by the inherent growth plan, related to the process of multiphasic growth.

FFEBM as a measure of physiological age

Fowler (1980) and others stated that a part of the total fat acts independently from the rest of the body, being an energy store buffering nutritional fluctuations to maintain body energy supply. The 'rest of the body' is considered to be the basic animal, i.e., a total of functional units, including some adipose tissue that is metabolically active.

It can be concluded from our results that when EBM (including fat) is used as the independent variable, the 'real' effects of the different feeding regimens on the deposition of protein, ash and water are masked, due to the interfering role of fat, being mobilized or deposited continously, dependent on the nutritional situation. It was clear from the present study that no effects of feeding regime (especially RFs) were any longer evident, if body component data were expressed as a function of FFEBM.

Widdowson and Lister (1992) stated from their own results and from the literature that, "fat, well known to be the most variable tissue in the carcass, seems not to vary wholly independently of the lean body". From this point of view, in combination with our results, the best approximation of the 'basic animal' should be the entire body excluding the amount of fat accrued in the second fat growth spurt. Since both fat growth spurts overlap (Kwakkel *et al.*, 1993) the separation is difficult to make. However, whereas functional fat growth (first fat growth spurt) is fixed to the growth of the fat-free body, the use of FFEBM as an estimate of the 'functional body' for the layer pullet seems appropiate. This supports the use of FFEBM as the independent variable (thus as a measure of 'physiological' age) to determine the retarded growth of body organs in nutritional studies.

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ago, who integrated animal production and biology in such a way, that the ideas still attract workers on growth and development in farm animals.

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Chapter 7

MULTIPHASIC ALLOMETRY IN *AD LIBITUM* AND RESTRICTED FED WHITE LEGHORN PULLETS.
2. GROWTH OF SOME SKELETAL BONES AND BODY ORGANS

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ABSTRACT

Data of body constituents of layer pullets that had been restricted from 0 to 6 or 7 to 18 wk of age by one of two restriction methods, were used in a multiphasic allometric growth study. The restriction methods were: a low-lysine diet and a daily restricted amount of feed. Pullets restricted by means of the latter method were fed such an amount of feed that they increased similar weight gains as the low-lysine group (pair-gained).

Some skeletal bones (shank, tibia, and keel), the digestive tract (gizzard separately), the heart, the liver, and the ovary were subjected to multiphasic allometric analyses with the fat-free empty body mass (FFEBM) or a functional entity related to the respective body constituent as the independent variable. It was aimed to find differences in relative growth pattern between the treatments.

Relative organ growth towards FFEBM was described most accurately by a diphasic model. Allometric slopes of the first growth phase were almost identical for all treatments per constituent, suggesting that the variation in organ growth rate due to the applied regime, follows the variation in growth rate of the FFEBM. A breakpoint in the diphasic model towards a second allometric slope of around zero was related to the moment of attainment of maturity for the respective constituent. Similar curves for the different treatments suggested that the growth pattern and attainment of maturity for most organs depended upon a certain amount of FFEBM. The keel, however, grew relatively faster (priority) if an early nutrient restriction was applied. The attainment of maturity reflected the original growth sequence studies by the Hammond School. No differences in mature organ weights between the feeding regimens were observed.

The results confirmed the 'classical' observation that inhibited growth of the skeleton and the digestive tract, as a consequence of a nutritional induced growth retardation, is related to the decrease in growth rate of the fat-free body. It is concluded that probably most of the effects on organ weight reported in literature (e.g., as weight per 100 g BW) are simply a consequence of the mobilization of fat *per se*.

Keywords: multiphasic allometry, feeding regime, restriction, organ growth, layer pullets.

INTRODUCTION

The 'quality' of a young hen (i.e., her potential to show a good layer record) is for a large part determined by nutritional conditions and related growth characteristics during rearing (Leeson and Summers, 1980; Wells, 1980; Leeson, 1986). Thus, a better knowledge of growth processes in the pullet is necessary to improve the quality of the young layer and may even lead to a reduction in the proportion of nutrients used for its rearing.

Within the framework of a larger project, experiments have been conducted to determine physiological relationships between pullet growth and egg performance. The results on feed intake and egg performance of an experiment in which pullets were restricted during early (0 to 6 wk of age) or late (7 to 18 wk of age) rearing have been reported by Kwakkel *et al.* (1991). In this trial, in each rearing phase, a restricted feeding level for a group of pullets (RF) was adjusted weekly to match BW gain of a group that was restricted in lysine (RL).

If a restricted nutrient supply during developmental stages is under study, one of the most interesting questions is whether particular body constituents have been hindered *more* than others and/or have been retarded irreversibly with respect to their mature weights within the concept of the inherent growth plan. The appropiate way to investigate such a problem is by means of a relative growth study. Another problem arises then: which independent variable has to be chosen? In growth studies of poultry, the most commonly used independent variate is BW (a.o., Plavnik and Hurwitz, 1982; Lilja, 1983; Lilja *et al.*, 1985; Katanbaf *et al.*, 1989; Yu *et al.*, 1992; Nir *et al.*, 1993).

Recently, relative growth of the chemical body components of the pullets of the above mentioned study has been investigated by means of a multiphasic allometric function (Kwakkel *et al.*, Subm. B). Evidence was provided that relative growth of CP, ash, and water, each as a function of fat-free empty body mass (FFEBM) was not influenced by feeding regime. It was stated that lipid tissue acts as an 'independent' energy buffer: its deposition and mobilization is highly influenced by the applied feeding regime, but it basically does not affect organ growth. For that reason, Kwakkel *et al.* (Subm. B) suggested, following the 'classical' propositions of the Cambridge School, to use FFEBM as the independent variable in relative growth studies to judge the effects of nutrient restrictions correctly and to avoid wrong conclusions. FFEBM as an estimate for the 'basic animal' (Fowler and Livingstone, 1972) would be a far better alternative for a measure of physiological age than BW or EBM, particularly in studies on nutritional deprivation. Recalculation of organ growth data in restricted fed broiler breeders, as reported by Katanbaf et *al.* (1989) and Yu *et al.* (1992), revealed that differences in relative organ weights between *ad libitum* and restricted fed birds reduced by 50 to 100% if weights were expressed as a percentage of fat-free BW instead of BW.

In particular, growth responses of the skeleton and digestive and reproductive organs to feed restriction have been reported frequently (Leeson and Summers, 1980, 1984; Burgess, 1986; Katanbaf *et al.*, 1989). It was investigated whether a permanent smaller body frame could be induced by our feeding regimens, in order to reduce 'maintenance requirements', and whether possible changes in relative gut weights would increase absorptive capacity throughout lay.

The aim of this study was to assess differences between feeding regimens in relative growth of several body organs. A multiphasic allometric function (Kwakkel *et al.*, 1993), developed by Koops and Grossman (1993), was used for this purpose. Growth of three skeletal bones (shank, tibia and keel), metabolic organs (total digestive tract, gizzard, intestines, liver), heart, and ovary, were related to FFEBM and/or a 'functional related entity'.

MATERIALS AND METHODS

Birds, management, and feeding regimens

Detailed descriptions of the experimental diets and management procedures were presented in earlier papers of this series (Kwakkel *et al.*, 1991; Kwakkel *et al.*, Subm. B). Briefly, each of a total of 1080 White Leghorn (WL) pullets (Lohmann GmbH, Cuxhaven, Germany) were assigned to one of five feeding regimens (nine replicate cages per treatment), four restricted feeding regimens and an *ad libitum* fed control group. Pullets of the control group (C) consumed *ad libitum* a starter diet (.85% digestible lysine, .71% digestible methionine + cystine, 18.9% CP, 1.0% calcium, .41% available phosphorus, and 2,800 kcal ME/kg) from 0 to 6 wk of age and a grower diet (.65% digestible lysine, .60% digestible methionine + cystine, 15.9% CP, .9% calcium, .44% available phosphorus, and 2,800 kcal ME/kg) from 7 to 18 wk of age. Pullets of the other four feeding regimens were restricted during the starter (s: 0 - 6 wk) or grower (g: 7 - 18 wk) phase by either feeding a low-lysine diet (RLs and RLg: .40% and .30% digestible lysine, respectively) or a restricted daily amount of feed (RFs and RFg). Pullets in each RF-group were fed such an amount of feed of the starter or grower diet, respectively, to match BW of the respective RL-group (pair-gained). All other nutrients were formulated according the specifications of the NRC (1984). The control diet was given *ad libitum* in the rearing phase in which the respective group of pullets was not restricted. All hens were fed *ad libitum* a commercial diet (.65% digestible lysine, .58% digestible methionine + cystine, 16.5% CP, 3.5% calcium, .44% available phosphorus, and 2,800 kcal ME/kg) from 19 to 62 wk of age. Water was provided *ad libitum* throughout.

Traits measured

At weekly intervals, five to eight pullets per treatment were randomly selected from different cages and killed by cervical dislocation. Immediately after death, birds were plucked by use of hotwater scald and dissected subsequently. Gut fill was removed (plucked empty body mass: EBM).

The following body structures were examined individually:

(1) three skeletal bones were recorded as a measure of frame size: length of the right shank (tarsometatarsal; measured between the bottom of the foot pad and the top of the bend hock joint) and shank thickness; length of the right tibia (tibiotarsus); length of the keel (sternum; following the procedures described by Leeson and Summers, 1984); all measures were done with a vernier caliper to the nearest .1 mm;

(2) weights of organs associated with metabolism and reproduction: total digestive tract (including crop, proventriculus, gizzard, and intestines, excluding the pancreas); the gizzard (without adhering fat) was weighed separately; heart (empty ventricles); liver (excluding the gall bladder); and the ovary. All weights to the nearest .01 g. Some organs were not detectable at very young ages (ovary < 4wk; abdominal fat pad < 15 wk). In this paper, intestines are defined as 'total digestive tract minus gizzard'.

Chemical body composition was determined following the procedures described by Kwakkel *et al.* (Subm. A). Briefly, after dissection, plucked empty bodies of pullets (all dissected organs and viscera including) of wk 0, 3, 6, 9, 12, 15, 18, 20, 22, and 24 were frozen and subsequently minced. Chemical analyses were assessed on an individual bird basis from wk 12 to 24, and as a pooled sample of two birds from wk 3 to 9. One-day-old pullets were pooled in two samples of five birds each. Chemical analyses of Dry Matter (DM), Ash, CP (Kjeldahl; Nx6.25) and Crude Fat (Cfat; ether extraction) were conducted with freeze-dried samples. Fat-free plucked empty body mass (FFEBM) was calculated as EBM minus Cfat.

Analyses of data: the multiphasic allometric function

Average bone lengths or organ weights per treatment were plotted versus FFEBM or a related body entity on a natural log-log basis. Basically, the allometric model of Huxley was used for the fitting procedures (Huxley, 1932). However, Huxley's monophasic equation has been extended recently to a multiphasic function (Koops and Grossman, 1993). This Multiphasic Allometric Function (MAF; [1]) allows changes in the allometric relationship with advancing age (Kwakkel *et al.*, 1993):

$$\ln (y_x) = \ln(\alpha_{i-1}) + ß_i \ln(x) - \sum_{i=1}^{n-1} \{(ß_i - ß_{i+1})\, r_i \ln[1 + (x/c_i)^{1/r_i}]\} \qquad [1]$$

where, ln is the natural logarithm; y_x is the weight of a body component (CP, Cfat, ash or water) or a body organ, and x is the weight of FFEBM, both in grams (or mm); α is the scale parameter (ln(α) is the intercept); n is the number of phases; and in each phase i, β_i is the allometric slope, c_i is the estimated breakpoint between phase i and i+1, and r_i is a smoothness parameter.

All organ data were transformed to their natural logarithm prior to the curve-fitting procedures. Due to the small number of observations within the breakpoint area, the smoothness parameter r could not be estimated accurately. In a previous paper, it was found that the most appropiate value for r in this material was .05 (Kwakkel *et al.*, Subm. B). Curves were fitted by the nonlinear regression algorithm of the NONLIN package (Method Levenberg-Marquardt (Dennis *et al.*, 1981); convergence criterion 10^{-10}). Goodness-of-fit was judged by the SE's of the parameter estimates, residual SD's (RSD) of the model, coefficients of determination (CD), and the Durbin-Watson (DW) test. Additionally, the Student's T-test gave information on the probability of the allometric slope being not significantly different from zero. Differences in curves or curve parameters (slopes, breakpoints) between treatments were investigated by F-tests according to procedures reported in detail by Kwakkel *et al.* (Subm. A, Subm. B). The significance of the addition of a second phase to the monophasic allometric model was assessed by an F-test (Kwakkel *et al.*, 1993). If the diphasic model was the best one, the discussion has been focused on the diphasic parameter estimates instead of the monophasic ones.

For the skeletal bones and the digestive tract, the fits per treatment were tabulated. For all other body constituents, overall estimates were presented if the full model (including treatment) did not improve the simple model (excluding treatment).

RESULTS AND DISCUSSION

Relative growth in length of the shank, the tibia, and the keel, as well as growth in thickness of the shank, is presented by mono- and diphasic allometric functions towards FFEBM (Tables 1A and 1B). Growth of organs associated with food processing and digestion is summarized in the Tables 2 and 3. In Table 2, the total digestive tract and the gizzard are separately fitted relative to FFEBM, whereas in Table 3, the gizzard and intestines are fitted relative to the total digestive tract. Results on growth of the heart, liver, and ovary, relative to FFEBM are presented in Table 4.

It was questioned whether the adult organ weights would differ between treatments. According to statistical procedures, as outlined by Neter *et al.* (1990), for predicting a new observation, mature lengths of skeletal bones and mature weights of digestive tract and gizzard were calculated for each treatment at one of two values (m_1 and m_2) for the independent variable: the breakpoint (m_1: in cases where the β_2 was zero: mature) or at

Table 1a. Parameter estimates and SE (in parentheses) of growth of some skeletal bones as a function of fat-free EBM[1] of WL-pullets, fed different feeding regimens[2], described by a monophasic allometric function[3].

y	Trmt[2]	Parameter estimates			Breakpoint	Goodness of fit		
		Scale parameter	Allometric slope phase 1	phase 2		RSD[4]	CD[5]	DW[6]
Shank (length)	C	-.35 (.09)	.39 (.02)	-	-	.052	.9880	.54
	RLs	-.28 (.09)	.38 (.01)	-	-	.055	.9883	.77
	RFs	-.33 (.07)	.39 (.01)	-	-	.044	.9927	.71
	RLg	-.33 (.13)	.38 (.02)	-	-	.070	.9779	.54
	RFg	-.33 (.13)	.38 (.02)	-	-	.070	.9782	.49
Shank (thickness)	C	-2.62 (.14)	.35 (.02)	-	-	.075	.9703	.72
	RLs	-2.56 (.14)	.34 (.02)	-	-	.088	.9632	.81
	RFs	-2.59 (.14)	.34 (.02)	-	-	.086	.9663	.78
	RLg	-2.58 (.17)	.34 (.03)	-	-	.095	.9504	.62
	RFg	-2.58 (.18)	.34 (.03)	-	-	.097	.9489	.63
Tibia	C	-.19 (.07)	.38 (.01)	-	-	.041	.9924	.70
	RLs	-.14 (.05)	.38 (.01)	-	-	.033	.9956	.76
	RFs	-.16 (.04)	.38 (.01)	-	-	.026	.9974	1.06
	RLg	-.19 (.10)	.38 (.02)	-	-	.056	.9853	.81
	RFg	-.18 (.10)	.38 (.02)	-	-	.054	.9866	.67
Keel	C	-.97 (.06)	.48 (.01)	-	-	.032	.9970	1.53
	RLs	-.78 (.13)	.45 (.02)	-	-	.081	.9822	1.73
	RFs	-.81 (.12)	.45 (.02)	-	-	.073	.9856	1.74
	RLg	-.97 (.07)	.48 (.01)	-	-	.038	.9958	1.37
	RFg	-.94 (.08)	.47 (.01)	-	-	.045	.9939	1.77

[1] EBM: plucked empty body mass.

[2] Feeding regimens (Trmt): C = no restrictions; RLs = lysine restriction from 0-6 wk of age; RFs = feed restriction, pair-gained to RLs; RLg = lysine restriction from 7-18 wk of age; RFg = feed restriction, pair-gained to RLg.

[3] $\ln(y_x) = \ln(\alpha) + ß_1\ln(x)$ (monophasic, Mono)
$\ln(y_x) = \ln(\alpha) + ß_1\ln(x) - .05\,(ß_1-ß_2)\ln[1+e^{(\ln(x)-\ln(c))/.05}]$ (diphasic, Di)
where $\ln(\alpha)$ is the scale parameter, $ß_1$ and $ß_2$ are the allometric slope of phase 1, and 2, respectively, ln (c) is the breakpoint.

1000 g FFEBM (m_2: mean FFEBM at 24 wk of age (final observation): 1026.4 g ± 7.3 (n = 5)). To discriminate between differences in mature weights, a 95% confidence interval for each predicted value was calculated.

No differences were found statistically between treatments and therefore we decided not to tabulate the mature weights per treatment. Overall mature weights (means ± SEM) will be given below per constituent.

Table 1b. Parameter estimates and SE (in parentheses) of growth of some skeletal bones as a function of fat-free EBM[1] of WL-pullets, fed different feeding regimens[2], described by a diphasic allometric function[3].

y	Trmt[2]	Parameter estimates				Goodness of fit			*F*(2,6)[7]
		Scale parameter	Allometric slope phase 1	Allometric slope phase 2	Breakpoint	RSD[4]	CD[5]	DW[6]	
Shank (length)	C	-.49 (.04)	.42 (.01)	.01 (.10)	6.60 (.08)	.019	.9988	2.01	27.23
	RLs	-.46 (.05)	.42 (.01)	.04 (.13)	6.45 (.14)	.025	.9982	2.42	16.51
	RFs	-.46 (.03)	.42 (.01)	.06 (.09)	6.55 (.08)	.016	.9993	2.86	26.14
	RLg	-.54 (.07)	.43 (.01)	.02 (.14)	6.49 (.13)	.033	.9962	2.03	14.39
	RFg	-.53 (.07)	.43 (.01)	.04 (.11)	6.49 (.14)	.033	.9964	1.68	15.03
Shank (thickness)	C	-2.94 (.09)	.42 (.01)	.09 (.07)	6.18 (.15)	.032	.9959	1.51	18.94
	RLs	-2.90 (.15)	.42 (.01)	.06 (.14)	6.12 (.28)	.058	.9882	2.05	6.37
	RFs	-2.92 (.13)	.42 (.02)	.06 (.12)	6.17 (.24)	.051	.9910	2.20	8.21
	RLg	-2.87 (.06)	.41 (.01)	-.26 (.12)	6.54 (.06)	.026	.9971	1.87	48.10
	RFg	-2.92 (.08)	.42 (.02)	-.03 (.08)	6.33 (.10)	.033	.9957	2.47	32.36
Tibia	C	-.26 (.07)	.40 (.01)	.07 (.22)	6.69 (.16)	.031	.9966	1.77	3.78
	RLs	-.22 (.04)	.40 (.01)	.04 (.16)	6.63 (.11)	.018	.9990	2.31	10.94
	RFs	-.22 (.03)	.39 (.01)	.11 (.15)	6.67 (.12)	.015	.9994	3.07	9.70
	RLg	-.30 (.10)	.41 (.02)	.04 (.32)	6.64 (.25)	.047	.9924	1.72	2.76
	RFg	-.28 (.09)	.40 (.02)	.01 (.30)	6.69 (.19)	.041	.9943	1.54	4.05
Keel	C	-1.00 (.06)	.48 (.01)	.13 (.59)	6.83 (.25)	.031	.9978	1.79	1.16
	RLs	-1.85 (.22)	.73 (.06)	.39 (.02)	4.34 (.21)	.032	.9978	2.08	21.79
	RFs	-1.75 (.19)	.70 (.05)	.39 (.02)	4.40 (.19)	.032	.9980	2.16	18.04
	RLg	-1.09 (.05)	.51 (.01)	.33 (.08)	6.38 (.24)	.021	.9991	2.70	10.33
	RFg	-1.01 (.08)	.49 (.01)	.03 (.46)	6.78 (.19)	.037	.9969	3.02	2.98

[4] Residual SD: n in all fits was 10; Each observation is represented by 5 to 8 pullets.

[5] Coefficient of Determination.

[6] Durbin Watson statistic: a value around 2 indicates no autocorrelation.

[7] *F*(2,6) represents the significancy of the addition of a second phase; Critical *F*(2,6)-values were 5.14 ($P<.05$) and 10.92 ($P<.01$).

Skeletal growth: shank, tibia, and keel

For most bones and most treatments, the diphasic allometric function improved the fit significantly if compared to the monophasic function (see the *F*(2,6)-values in Table 1B). The difference, although very small, between the monophasic allometric slopes of length (.38) and thickness (.34) of the shank (Table 1A) disappeared completely when the diphasic function was fitted (.42; Table 1B). This indicates a proportional increase in length and thickness within the shank bone.

No significant effects of treatment were observed in the diphasic model for shank length and thickness, and tibia length, each as a function of FFEBM. In all three traits, the β_2's were not significantly different from zero (as proved by the Student's T-test), suggesting that the respective bone structure had reached the stage of maturity when the curve bended (the 'breakpoint' as a marker for the attainment of bone maturity). Late-restricted pullets tended to delay the maturity of the shanks' thickness towards a heavier FFEBM (6.44 (626 g) vs. 6.16 (473 g) for the other groups). Shank and tibia length, and, to a minor extent, shank thickness, matured at a particular stage of the fat-free empty body (on average at 675, 784, and 527 g FFEBM, respectively), independent of the feeding regime that had been followed. The time (age)-sequence in which these bones matured was (with the *ad libitum* fed pullets as reference) at around 7 wk of age for shank thickness, at 10-12 wk of age for shank length, and at 12-13 wk of age for the tibia. To relate log (FFEBM) to age, one has to consult Figure 1 in Kwakkel *et al.* (Subm. B).

For the keel, parameter estimates between early-restricted pullets (RLs and RFs) and pullets of the other treatments differed significantly ($P<.01$). The early-restricted pullets showed lower intercepts, larger β_1's and β_2's, and breakpoints at much lower FFEBM (Table 1B). An explanation could be that there is some age-effect: just after hatching, the young pullet has to prepare for precocial life: an increase in volume of the chest cavity for necessary lung growth and/or preparing sites for the attachment of the pectorals may have obliged the fast growth rate of the keel, irrespective the slower fat-free body growth. So, this is an example of a 'priority' situation in which keel growth predominates growth of the fat-free body (FFEBM), without having the confusing effects of depleted fat. For example, Palsson and Verges (1952) found that the bones of the head (brain development) of a sheep were not *that much* affected by nutrition as total bone. It seems that changes in form are likely to be related to functional priorities.

A good estimate of the age of maturity of the keel couldn't be made, because the 'zero'-slope for keel growth (= mature) was attained outside the observation range. However, at the end of rearing, keel growth will, similar to FFEBM, be over (at around wk 17-18).

The sequence in bone maturation (shank -> tibia -> keel) is in agreement with the 'centripal' growth theory, which suggests that there is a distal to proximal growth gradient in the limbs. Bones that are most distal from the body are expected to mature first (Hammond, 1932; McMeekan, 1940).

Overall mature lengths of shank (m_1), tibia (m_1) and keel (m_2), as well as shank thickness (m_1), were 9.52 ± .08, 11.00 ± .10, 10.11 ± .09, and .74 ± .02 cm, respectively. Though keel relative to FFEBM showed different slopes for the early-restricted pullets in contrast to the other groups, it did not affect mature keel length.

In previous studies, the skeletal bones, shank, tibia, and keel had been used as markers for the hen's body frame size (Brody et *al.*, 1984; Schaten-Romberg, 1985; Burgess, 1986). Morris et *al.* (1966) related tibia weights (x) to weights of the total skeleton (y), and found them to be highly related: $y = 2.997 + 6.601\,x$; $r = .94$). It is suggested in literature that the development of the body frame of maturing pullets may be related to subsequent performance (Leeson and Summers, 1984; Burgess, 1986). Burgess (1986) suggested that a permanent smaller body size (not BW) could possibly reduce egg sizes. In this experiment, hens restricted during the grower period laid a 1.5 g heavier egg ($P < .01$) than those restricted during the starter period (Kwakkel *et al.*, 1991). From our results on skeletal growth relative to FFEBM, however, it was concluded that growth curves and adult lengths of the 'frame' were unaffected by feeding regime.

In the *ad libitum* fed pullets, the two long bones (shank and tibia) that were recorded, reached their mature size at around 13 wk of age, as suggested above. Matsuzawa (1981) reported also that the tibia did not increase further in length beyond 14 wk of age in WL-chicks. To reach the stage of maturity, the cessation of bone growth is induced by the inhibition of linear bone growth and the fusion of the epiphyseal plates, initiated by the increase of circulating levels of estrogens at start of sexual development (Short, 1980). Some delay in the fusion of the plates might have occurred in the late-restricted pullets (tibia RLg and RFg: 785 g FFEBM corresponded to an age of about 15 wk). Early restrictions will not have a permanent effect on mature bone size, but this will depend on the total period of restriction. Burgess (1986) stated that if a nutrient restriction is still being applied after the epifyseal plates have fused (even after a delay), the bone will remain shorter. Nutrient restrictions during late rearing would not give permanent smaller bones (Burgess, 1986; Yu *et al.*, 1992).

The allometric slopes of the three skeletal bones towards FFEBM were all far below unity, indicating early-maturing structures. In the precocial chicken, the bones for locomotion purposes (shank and tibia) constitute already a large weight proportion of BW at hatch. That means that the growth spurt occurred already prior to hatch. Possibly dictated by some biological 'economic' law, these bones develop during early post-hatch at a much slower rate in order to provide nutrients available for other 'maintenance' organs like the gut (Katanbaf *et al.*, 1988; Nir *et al.*, 1993).

Growth of the total digestive tract, gizzard, and intestines

The diphasic model fitted the data much better than did the monophasic model ($F(2,6)$-values in Table 2). For both the total digestive tract and the gizzard, no differences could be demonstrated in parameter estimates between individual treatments or groups of treatments. The total digestive tract showed β_1's near to unity (.94, on average), which

Table 2. Parameter estimates and SE (in parentheses) of growth of the total digestive tract and gizzard, separately, each as a function of fat-free EBM[1] of WL-pullets, fed different feeding regimens[2], described by a Multiphasic Allometric Function[3].

Mono/Di phasic	Trmt[2]	Parameter estimates				Goodness of fit			F(2,6)[7]
		Scale parameter	Allometric slope phase 1	Allometric slope phase 2	Breakpoint	RSD[4]	CD[5]	DW[6]	
		Growth of the digestive tract as a function of FFEBM							
Mono	C	-.94 (.26)	.74 (.05)	-	-	.142	.9759	1.40	
	RLs	-.78 (.25)	.71 (.04)	-	-	.159	.9727	1.06	
	RFs	-.79 (.24)	.72 (.04)	-	-	.150	.9762	1.53	
	RLg	-1.00 (.24)	.75 (.04)	-	-	.132	.9792	1.26	
	RFg	-1.00 (.28)	.75 (.04)	-	-	.154	.9726	1.06	
Di	C	-1.84 (.24)	.96 (.06)	.46 (.07)	5.51 (.24)	.066	.9961	2.21	15.48
	RLs	-1.86 (.28)	.98 (.07)	.43 (.09)	5.40 (.26)	.079	.9950	2.47	13.23
	RFs	-1.66 (.35)	.93 (.08)	.48 (.12)	5.42 (.44)	.105	.9913	2.94	5.21
	RLg	-1.81 (.23)	.95 (.05)	.52 (.06)	5.46 (.25)	.064	.9963	2.43	14.01
	RFg	-1.59 (.19)	.89 (.04)	.31 (.12)	6.14 (.19)	.071	.9936	2.56	15.59
		Growth of the gizzard as a function of FFEBM							
Mono	C	-1.60 (.31)	.70 (.05)	-	-	.171	.9617	.63	
	RLs	-1.44 (.32)	.68 (.05)	-	-	.200	.9530	.55	
	RFs	-1.42 (.30)	.67 (.05)	-	-	.188	.9578	.81	
	RLg	-1.64 (.31)	.71 (.05)	-	-	.171	.9613	.49	
	RFg	-1.53 (.40)	.69 (.06)	-	-	.220	.9358	.56	
Di	C	-2.11 (.07)	.81 (.01)	-.45 (.13)	6.55 (.04)	.029	.9992	2.04	135.60
	RLs	-2.05 (.23)	.82 (.04)	-.60 (.56)	6.48 (.15)	.104	.9903	1.59	11.60
	RFs	-1.97 (.20)	.80 (.04)	-.50 (.49)	6.50 (.14)	.097	.9916	2.26	12.05
	RLg	-2.21 (.09)	.83 (.02)	-.23 (.16)	6.48 (.06)	.039	.9985	2.77	73.26
	RFg	-2.20 (.15)	.84 (.03)	-.44 (.22)	6.49 (.08)	.064	.9959	2.61	44.23

[1-7] As in Table 1.

means that growth of the digestive tract is largely related to the early fat-free body growth (Lilja, 1983; Katanbaf *et al.*, 1988; Nir *et al.*, 1993).

The total digestive tract did not enter the mature phase within the observed range of FFEBM (up to 24 wk of age). The β_2 was, on average, .44, which suggests a prolonged growth path up to the mature state. To the contrary, the gizzard matured at a FFEBM of about 665 g, reflecting an age of 10 to 11 wk for C-pullets. Although the fact, that none of the β_2-values of the gizzard was different from zero, it is remarkable that all had a negative sign. Some reduction in weight due to a decreasing water content of the gizzard could be an explanation. Similar slope values for monophasic growth of the total

Table 3. Parameter estimates and SE (in parentheses) of growth of the gizzard and intestines[1], each as a function of the digestive tract of WL-pullets, fed different feeding regimens[2], described by a Multiphasic Allometric Function[3].

Mono/Di phasic	Trmt[2]	Parameter estimates Scale parameter	Allometric slope phase 1	Allometric slope phase 2	Breakpoint	Goodness of fit RSD[4]	CD[5]	DW[6]	F(2,18)[7]
		Gizzard growth as a function of the digestive tract							
Mono	C	-.79 (.14)	.98 (.04)	-	-	.106	.9734	.49	
	RLs	-.79 (.09)	.98 (.03)	-	-	.084	.9866	.76	
	RFs	-.76 (.10)	.96 (.03)	-	-	.088	.9846	.72	
	RLg	-.80 (.12)	.98 (.03)	-	-	.095	.9782	.92	
	RFg	-.70 (.12)	.94 (.03)	-	-	.099	.9764	.57	
Di	C	-.92 (.12)	1.03 (.04)	-.73 (.93)	3.99 (.08)	.085	.9845	1.06	6.40
	RLs	-.89 (.10)	1.01 (.03)	.13(1.35)	3.93 (.21)	.078	.9894	.97	2.38
	RFs	-.89 (.09)	1.01 (.03)	-.59(1.55)	4.00 (.12)	.070	.9911	1.18	6.63
	RLg	-.94 (.12)	1.03 (.03)	-.04 (.90)	3.97 (.13)	.082	.9856	1.50	4.55
	RFg	-.84 (.13)	.99 (.04)	.31 (.49)	3.91 (.16)	.088	.9830	.76	3.55
		Intestines growth as a function of the digestive tract							
Mono	C	-.60 (.09)	1.02 (.02)	-	-	.072	.9884	.53	
	RLs	-.60 (.06)	1.02 (.02)	-	-	.056	.9946	.84	
	RFs	-.62 (.06)	1.03 (.02)	-	-	.058	.9942	.80	
	RLg	-.60 (.08)	1.02 (.02)	-	-	.064	.9910	.87	
	RFg	-.66 (.08)	1.04 (.02)	-	-	.063	.9920	.69	
Di	C	-.52 (.09)	.98 (.03)	2.08 (.64)	3.99 (.09)	.061	.9926	1.03	5.19
	RLs	-.54 (.07)	.97 (.02)	1.54 (.80)	3.92 (.21)	.052	.9957	1.08	2.32
	RFs	-.54 (.06)	.99 (.02)	1.98 (.97)	4.00 (.12)	.047	.9965	1.21	6.13
	RLg	-.51 (.08)	.98 (.02)	1.62 (.51)	3.95 (.13)	.055	.9940	1.43	4.62
	RFg	-.57 (.08)	1.01 (.03)	1.44 (.32)	3.91 (.17)	.057	.9942	.90	3.31

[1] Intestines defined as: total digestive tract minus gizzard.

[2-3] As in Table 1.

[4] Residual SD: n in all fits was 22; Each observation is represented by 5 to 8 pullets.

[5-6] As in Table 1.

[7] *F*(2,18) represents the significancy of the addition of a second phase; Critical *F*(2,18)-values are 3.55 ($P<.05$) and 6.01 ($P<.01$).

digestive tract or the stomach relative to the fat-free body in pigs were reported by Doornenbal and Tong in 1981 (.71; gut), Davies in 1983 (.83; stomach), and Tess *et al.* in 1986 (.78; stomach), respectively.

'Intestines' were defined as 'the digestive tract minus the gizzard'. Data of gizzard and intestines were fitted relative to the total digestive tract (Table 3). The diphasic model was not always better than the monophasic one, due to large variations in the $ß_2$-estimate. Again, no differences in parameter estimates between treatments were demonstrated. The first allometric slope in the diphasic model is for both gizzard and intestines equal to 1 (unity): the total digestive tract grew proportionally in both segments at this early stage. At around a gut weight of 52 g (breakpoint 3.94; Table 3), gizzard growth is over. Mature gut weight (m_2) is approximately 60.47 ± .53 g: thus at 85% of mature gut weight, growth of the gizzard stopped. Adult weight of the gizzard at that stage (m_1) was 24.01 ± .38 g. Consequently, relative gain in the intestines increased enormously from that moment onwards ($ß_2$ = 1.7, on average).

Metabolic active organs, like the gut, are quite sensitive to nutrient restrictions (Elsley, 1964; Kyriazakis and Emmans, 1992). It was reported by Nir *et al.* (1987), that pullets might adapt morphologically to an intermittent (e.g. skip-a-day) feeding regime: hypertrophy of feed storage organs, a significant increase in the relative weight of liver, pancreas, jejunum and ileum of White Leghorn pullets at 61 days of age provided a double feed intake during the allowance days. Katanbaf *et al.* (1989) reported similar increases in relative weights of segments of the digestive tract, due to different methods of feed restriction.

It is suggested here, that an intermittent feeding programme (including starvation for one or two days) may affect growth of the gut, particularly induced by a continous physical pressure on the gut wall ('stretching' by over-eating). On the other hand, if a daily feed restriction is subjected to pullets, only small amounts of feed will be present in the gut during the day. In the latter situation, relative changes in gut weight may be not 'real' changes, and disappear if calculated relative to the fat-free body.

To demonstrate these phenomena, results of Katanbaf *et al.* (1989) were recalculated. They reported changes in weight of the duodenum relative to BW at 43 days of age in pullets restricted by three methods of feed restriction: .38% (*ad libitum* (AL)); .46% (daily feed restriction (ALR)); .48% (skip-one-day (SOD)); .53% (skip-two-days (STD)). The largest relative increase in duodenum weight was observed between AL and ALR. If we recalculate the data relative to the fat-free body, the values are .50% (AL), .53% (ALR), .56% (SOD), and .64% (STD). Differences between AL and ALR ('normal' daily restriction) become smaller, whereas the differences between ALR and STD are more pronounced. This suggests that the proposition made before could be a logical explanation for increased relative gut weights following intermittent feeding.

Growth of the heart, liver, and ovary

Whereas no treatment differences were observed for these three organs, the overall estimates were tabulated. During the first allometric phase, all three organs grew

Table 4. Overall parameter estimates and SE (in parentheses) of growth of plumage, the heart, liver, and ovary, each as a function of fat-free empty body mass of WL-pullets, described by a Multiphasic Allometric Function[1].

y	Mono/Di phasic	Parameter estimates Scale parameter	Allometric slope phase 1	Allometric slope phase 2	Breakpoint	Goodness of fit RSD[2]	CD[3]	DW[4]	F(.,.)[5]
Heart	Mono	-4.14 (.07)	.86 (.01)	-	-	.094	.9912	1.83	
(n=50)	Di	-4.66 (.11)	.98 (.03)	.73 (.03)	5.36 (.22)	.070	.9952	2.38	19.64
Liver	Mono	-2.01 (.37)	.83 (.06)	-	-	.095	.8693	1.77	
(n=35)	Di	No convergence reached							
Ovary	Mono	-15.55 (3.4)	3.48 (.51)	-	-	1.527	.5483	.68	
(n=40)	Di	-.53 (3.0)	.94 (.49)	18.58 (3.5)	6.69 (.05)	.885	.8563	1.75	38.59

[1] $\ln(y_x) = \ln(\alpha) + \beta_1 \ln(x)$ (monophasic, Mono)
$\ln(y_x) = \ln(\alpha) + \beta_1 \ln(x) - .05\,(\beta_1 - \beta_2)\,\ln[1 + e^{(\ln(x) - \ln(c))/.05}]$ (diphasic, Di)
where $\ln(\alpha)$ is the scale parameter, β_1 and β_2 are the allometric slope of phase 1, and 2, respectively, ln (c) is the breakpoint.

[2] Residual SD; Each observation is represented by 5 to 8 pullets.

[3] Coefficient of Determination.

[4] Durbin Watson statistic: a value around 2 indicates no autocorrelation.

[5] $F(.,.)$ represents the significancy of the addition of a second phase; Critical values ($P<.01$) were for $F(2,46)$ = 3.20, and for $F(2,36)$ = 3.26.

proportionally to one another (β_1's are .80 to .90). These values are of the same magnitude as the ones reported in monophasic allometric studies with pigs: .81 - .88 (heart) and .64 - .81 (liver), both relative to fat-free mass or 'muscle + bone' (Doornenbal and Tong, 1981; Davies, 1983; Tess *et al.*, 1986).

The heart still increased in weight during the second part of the rearing period (β_2 of .73). The individual treatment fits were monophasic (R.P. Kwakkel, Unpublished results), because in 3 of 5 times the diphasic fit per treatment failed to converge. Except for the overall situation ("simple" model, without a treatment effect), the fit resulted in a significant diphasic model. Adult heart weight was 6.01 g ± .07 g. Due to the large variation in the liver data, a second growth spurt at around onset of lay could not be detected. At 1000 g FFEBM, liver weight was 41.26 ± .36 g. The rapid increase in rate of growth of the ovary at around 800 g FFEBM (breakpoint 6.69; aging 14 to 15 wk) is in perfect agreement with the 'critical' amount of FFEBM found at the start of the very well pronounced body growth spurt (the 'maturity growth spurt'; Kwakkel *et al.*, 1993, Subm.A). Mature ovary weights have been discussed by Kwakkel *et al.* (Subm.A).

Concluding remarks

With respect to multiphasic allometric functions, an allometric slope below or above 1 does not directly indicate that the slope below 1 represents an 'early-maturing' body structure and the slope above 1 a 'late-maturing' body structure. The breakpoint in the allometric curve, in relation to a second (or third) slope that equals to zero, is a better marker to illustrate that the respective organ has reached its mature state. A slope far below 1 only indicates that 'growth rate is quite slow', however, it does not neccessarily intend that maturity is surely attained. There might start a next growth spurt after a period of 'zero-growth'.

In this study, comparisons between feeding regimens on organ growth relative to FFEBM were made. It can be derived from the results, that most of the organ growth patterns are strongly related to growth of the fat-free body. In a number of studies, growth of parts of the body affected by a restricted feeding regime, is presented in terms of 'as a percentage of BW'. The results then can be fully obscured by the variation in fat content as being the component most directly involved in lower planes of nutrition (Kwakkel *et al.*, Subm. B). It is assumed then that the variable fat part in the body is not related to functional growth and maturity of particular organs.

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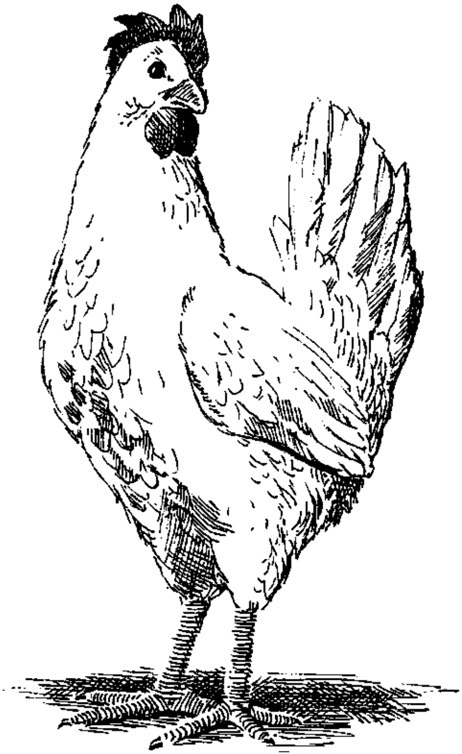

GENERAL DISCUSSION

GENERAL DISCUSSION

The layer pullet is reared to be prepared for subsequent egg production. The rearing period itself is a stage during hen's life in which it does not "produce" (Leeson, 1986), and, as a consequence, pullets are reared on a least-cost basis. 'Controlled' feeding regimens (feeding levels below *ad libitum* or imbalanced diets) are therefore regular practice for rearing hens. The potential ability of a young hen to produce a large number of saleable eggs during the 60 wk of a layer year is normally judged by her BW at the end of rearing. Since the beginning of the 1980's, poultry nutritionists became aware of factors that may interfere in the 'quality' of the young hen. Patterns of body growth in relation to the rate of deposition as well as the chemical and morphological composition of the accrued body mass have been studied by many workers in this field (a.o., Wells, 1980; Plavnik and Hurwitz, 1982; Johnson *et al.*, 1985; Leeson and Summers, 1984; Burgess, 1986; Leeson, 1986).

In the General Introduction three objectives were formulated. These objectives were (1) to investigate the effects of method, period and severity of nutrient restriction on pullet growth and subsequent egg performance, (2) to assess the role of body composition as being more relevant than body weight in determining the onset of lay and adult layer performance, and (3) to study the relationships between body constituents that may provide a biological framework of pullet growth.

The results of the three studies described in this thesis, will be discussed in this chapter in relation to these objectives. It was aimed in this study to identify phases of development during the growth period in which nutritional deficiencies would become 'critical'. These aspects were studied by means of analyzing growth in a multiphasic way.

A proposal for a practical pullet feeding strategy that establishes a clear link between the nutritional status during particular stages of rearing on the one hand and the requirements (demands) for growth and adult layer performance on the other is presented. As a final part of the thesis, prospects for further research are given.

DESCRIPTIONS OF GROWTH

There is no such term within the animal sciences as growth whose sense is familiar but whose meaning is often not very well understood (Batt, 1980). Growth, for example body growth, has been defined in several ways, as 'the sum of increases in size of its various body components' (Lilja, 1983), 'a highly complex physiological phenomenon under close hormonal regulation (Leung, 1986), 'a complex process whereby various body parts increase at different rates' (Katanbaf et *al.*, 1988), or as 'the increase in size and changes in functional capabilities of the various tissues and organs that occurs from conception through maturity' (Grant and Helferich, 1989), and so on. In each definition, the authors' own discipline of research is part of the phrase. In general, the growth process ('how complex it may be') includes increases in cell number (*hyperplasia*) during early stages of animals' life and increases in cell size (*hypertrophy*) during later stages of life.

Temporal growth is a description of growth in which an increase in weight or length is related to age (time). Relative growth, on the other hand, relates changes in gain of one body constituent (organ or tissue) to changes in gain (or, more generally: size) of another body constituent (groups of organs or tissues or the whole body). In principal, the term 'growth' is more related to temporal studies, whereas the term 'development' is connected to relative growth studies.

Temporal growth

Since the beginning of this century, the increase in weight as a function of age has attracted growth modellers in an attempt to quantify growth. The most familiar mathematical growth functions that have been developed are the Logistic, the Von Bertalanffy, and the Gompertz growth curves (see Zoons et *al.*, 1991). The essential feature of these growth curves is that they provide parameter estimates that are biologically interpretable, such as point of inflection, asymptotic weight, and overall growth rate (Maurice et *al.*, 1982). A marked difference between these three growth functions is that their point of inflection occur when 50, 30, and 37% of the asymptotic weight is, respectively, attained (Ricklefs, 1968).

In a study on growth of birds, Ricklefs (1968) examined the growth patterns of 105 species by fitting the growth curve of each species to one of these three growth functions. He concluded that in the larger and slower growing species, as is the domestic hen, there is a preponderance for the Gompertz growth curve. Todays poultry nutritionists fit their growth data most frequently to the Gompertz curve (Ricklefs, 1985; Bowmaker and Gous, 1989; Emmans, 1989; Gous, 1992) or, in some occasions, to the Logistic function (Maurice et *al.*, 1982).

It has been demonstrated in this thesis (Chapter 4) that growth of the pullet body is characterized by more than one single inflection point, *in casu* three points of inflection. As a consequence, the monophasic growth equation might have been a too simple model in a lot more occasions. One of the most accurate criteria to judge if a multiphasic pattern is present in the data structure, is the sign (autocorrelation) and specific pattern of the residuals (observed minus estimated value). The Durbin-Watson test can be used to help in statistical choices. The Coefficient of Determination is of minor value. It showed that although the relationship was clearly diphasic, this fit criterion gave already a value above .95 in the monophasic situation.

It must be noticed that, at some levels of aggregation (e.g. organs), growth can often be described satisfactorily by monophasic functions (Bowmaker and Gous, 1989; R.P. Kwakkel, Unpublished results). If, however, data are recorded frequently and the measured structure is, on behalf of the anatomical conformation, expected to be a sum of different components with differential growth rates, a multiphasic approach is preferentially used.

The Gompertz curve has its single inflection point at 37% of mature weight, as mentioned earlier, resulting in a quick ascending phase (up to maximum growth) and a prolonged descending phase. Data that are fitted well to the Gompertz curve may sometimes be better fitted to a diphasic logistic growth curve. Types of the diphasic logistic growth curve have been presented in this thesis (Chapter 4).

In general, mathematical models tend to smooth observed variation and might obscure real biological phenomena (Belt *et al.*, 1992). On the other hand, some mathematical models (like polynomials) may provide an excellent fit to the collected data, accommodating every small irregularity and obscuring real underlying trends (Hunt (1982), quoted by Burgess, 1986). Therefore models should be used with care, but they can assist the scientist towards a better understanding of the growth concept (Belt *et al.*, 1992).

In this study no comparison was made to studies in which data were fitted to functions including higher degree polynomials. It is the authors' belief to use a growth model that is in any case meaningful in its parameters.

Relative growth

To study 'age-effects' during and after periods of undernutrition, it is usual to follow a relative growth approach (Chapter 6). The simple (= monophasic) allometric equation of Huxley (1932), which is of the form $y = \alpha x^{\beta}$, relates a body constituent y (dependent variable) to another body constituent x (independent variable) by a scaling factor α (to scale differences in absolute weights) and the allometric growth coefficient ß (the slope in the log-log form of the function). If the allometric slope ß is 1, then the weight of y is over the entire growth interval proportional to the weight of x. If ß does not equals 1, than weights are no longer proportional, but the ratio between the relative growth rates is still constant over the specific growth interval. A distinction between 'early' ($\beta < 1$) and 'late' maturing ($\beta > 1$) body constituents can be made on the basis of the monophasic allometric slope values.

The original Huxley equation or its log-log form is used frequently in relative growth studies by workers in animal sciences (see Gould, 1966). Until recently (Lilja, 1983; Lilja *et al.*, 1985; Katanbaf *et al.*, 1988), the allometric principle has not been used very often in poultry research: relative growth values were performed by presenting organ or tissue weights as a percentage of total BW. In other species (like pigs and cattle) the frequent use of the allometric equation is more related to its straightforward calculations than to the relationship with any biological principle (Fowler and Livingstone, 1972). Although a physiological basis of the allometric approach has been discussed by many authors (For example: Michael Katz in 1980), the equation is used in literature in its most effective way: as an empirical tool in describing relative growth processes.

The simple allometric function has been critisized by many authors because (1) of its applicability to a very limited time interval (Von Bertalanffy, 1960; Belt *et al.*, 1992), (2) the non-biological background of the equation, (3) as well as the fact that summation of the parts do not give always the total (Gould, 1966; Seebeck, 1968). The third criticism directly refers to the situation in which 'life-time' animals are being under study. In that case, a monophasic allometric equation does not hold for the whole traject. The use of the allometric equation over wider age-intervals, without getting erroneous fits, was made possible by the application of more than one simple function to fit different segments with different allometric slopes. Transitions or 'breaks' between the different allometric levels were chosen by visual inspection (Lilja *et al.*, 1985). This has the disadvantage that by subjective judgement, a group of observations is allocated 'randomly' to a linear segment. The mathematical estimate of the breakpoint in the multiphasic allometric function exclude this kind of subjectivism.

In the design of multiphasic allometric studies, an equal distribution along the log(x)-axis is a prerequisite. In our study, observations were based on regular 2- and 3-wk age-intervals, and therefore discontinously distributed along the log (x)-axis. This may be especially a problem in estimating the smoothness of the bend in the transition area. It was the main reason that we set this parameter to a fixed value.

In relative growth studies, one of the major assumptions is that a purely gravimetric relationship between body structures represents the index of functioning of the body structures involved. Changes in weights or lengths of organs, however, may not always give a real indication of the temporal changes in functional properties of these respective organs (Fowler, 1980; Widdowson, 1980).

A major problem in allometric studies is the choice of the independent variable. The relevance to choose plucked fat-free empty body mass (FFEBM) as the independent variable, to study growth of body constituents of pullets that have been subjected to different feeding strategies, has been discussed extensively in the Chapters 6 and 7. However, if the fat-free body is chosen as the independent variable, an important assumption is made instantaneously: fatty tissue does not affect organ function. A related point of discussion may be whether the entire chemically derived amount of fat need to be excluded or not to obtain the independent variate. Theoretically, only the depot or storage part of lipid tissue acts independently from the rest of the metabolic active body. If parts of total gain in fat, however, grow proportionally to the fat-free body during particular stages of juvenile life (see Chapter 6), there would be no difference in choosing total fat-free or 'depot' fat-free as the independent variable. Two phases of fat growth were revealed in this study (Chapters 4 and 6). The first fat growth spurt seems, in situations in which energy is not limiting, highly related to growth of the fat-free body mass (Chapter 6). Whereas the second fat growth spurt acts more as an energy store to buffer nutritional fluctuations to maintain body energy supply, independently from the rest of the metabolic active body. This division into two phases seems relevant for poultry, whose largest part of the storage energy is deposited in one particular organ: the abdominal fat pad. In other farm animal species like ruminants, several fat depots may play an important role in the storage of energy for subsequent poor seasonal situations. In that case, the diphasic distinction between 'functional' and 'depot' fat will be not sufficient distinguishable. Fatty tissue in different anatomical regions within the animal may have dissimilar patterns of development and therefore will respond differently to varying planes of nutrition (McMeekan, 1940; Leat and Cox, 1980). The early-maturing sites of fatty tissue (intermuscular, perirenal, mesenteric) may be less readily affected by undernutrition than later-maturing sites (subcutaneous, intramuscular, abdominal).

Modelling growth from nutrient intake

It is obvious that in the above mentioned growth functions the required nutritional input is not included. Parks (1982) therefore, combined both aspects (growth and input) by generating a function that described growth as a function of feed intake, and cumulative feed intake as a function of age.

Generally, there are two growth - nutrient approaches:

The first approach is to predict growth on the basis of units of ingested nutrients ('metabolic models'), that can be purely empiric or quite mechanistic. It is build around the approximation that growth follows from ingested feed. Some examples in pig modelling are the concepts presented by Whittemore and Fawcett (1976) and Moughan and Verstegen (1988). The central issues in these models are the 'marginal protein efficiencies' and the 'maximum protein retention'. These models are build around 'dose-response' relationships, mainly applied to meat-type animals (fattening pigs or broilers) over a short period of life. The important question is whether or not a broiler has amounts of abdominal fat at slaughter age that are considered to be undesirable and how to reduce this.

The second approach is to predict growth on the basis of inherent, biological growth patterns, characterized by physiological changes, modified along the physiological age axis by nutritional alterations. It follows the approximation that feed is needed to accomplish a 'desired' growth rate and composition. These models are applicable to animals that are kept in husbandry for a longer term (E.g., sows, hens, including their rearing stages). The 'day-to-day' calculation of growth responses, which is of particular interest in the first approach, is of minor interest in the second: the main aim here is that the animal is not being retarded in such a way that irreversible damages to the development of vital body structures occur. The multiphasic model is an example of the second approach.

Multiphasic growth modelling

An empirical model is defined as a mathematical function in which 'input is related to output', without any description of the working of the system (Fisher, 1989). Both multiphasic functions, described in this thesis, are empirical models. The functions relate measurements of growth of a body structure to measurements of time (age) or another body structure.

The multiphasic growth approach, however, is based on the biological phenomenon of "growth cycles or waves". The existence of more than one 'growth cycle' was already perceived at the beginning of this century. The 'real' acceptance of the theory of a discontinous, sequential growth, however, came when Hammond and his colleagues of the Cambridge School of Agriculture introduced it in the early thirties (Hammond, 1932; Palsson, 1955).

It is stated here that the multiphasic approach may give some 'mechanistic' information: it describes a relationship between a dependent and independent variable(s) by a pathway that represents a theory of how growth is biologically generated (Zoons et *al.*, 1991). In this thesis it was stressed that there might be some possible limits in changing growth characteristics by dietary manipulations, due to the existence of biologically related 'fixed' patterns of tissue growth. This may support the proposition that multiphasic growth modelling is somehow mechanistic modelling.

THE FUNCTIONAL GROWTH PLAN

If there is a situation of non-limited growth, the growing pullet will ingest nutrients to form a particular quantity of tissue, dictated by mechanisms that govern the basic organization of the body plan during aging (Ricklefs, 1967). The Domestic Fowl (*Gallus Domesticus*) is precocial. It receives relatively little parental care (Visser, 1991). From a biological point of view, the layer pullet need to walk from day-old for fouraging purposes. This type of early post-hatch behaviour implicates that these birds need to have a relatively well developed locomotory system (i.e., early growth of leg bones and leg muscles). On the other hand, altricial birds are being fed by their parents. In order to leave the nest 'as soon as possible' (bad times may come) they must be able to ingest the offered feed quite fast. A well-developed gastro-intestinal tract at an early stage in life is a necessity. Hence, functional priorities may affect relative growth rates of the several tissues in the body. Thompson (1942) stated in this respect that 'form is related to function'. These phenomena illustrate the biological laws of 'functional maturity' (Thompson, 1942; Ricklefs, 1975). These 'laws' have been adopted and incorporated in biomedical research since a long time, but unfortunately, not yet in farm animal sciences. According to Ricklefs (Pers. comm), the joint effort to cooperate in biological and animal sciences is lacking. Research in both field has consequently developed divergently. There is, however, an urgent need to integrate research programmes in order to maintain a high quality of science. An animal is not a 'thing' that only deposits specific quantities of protein and lipids in the body that can be manipulated to any extent (i.e., the animal sciences point of view). Of course, it grows by gaining in these tissues, but the deposition of these substrates takes place in specified organs and organ systems, governed by some kind of hierarchy in developmental order (i.e., the biological sciences point of view).

There have been some initiatives towards an integrated approach in poultry research recently (Ricklefs, 1985; Lilja, 1983; Lilja *et al.*, 1985; Katanbaf *et al.*, 1988). The biological idea of differences in growth rate of specific organs, due to the hierarchical order based in the inherent growth plan, was adopted by workers in the field of poultry science (Lilja *et al.*, 1985, Katanbaf *et al.*, 1988). Lilja (1983) hypothesized that interspecific growth rates in birds after hatch is determined by the distribution of growth over 'supply' (digestive tract) and 'demand' (pectorals, feathers) organs: higher growth rates related to a larger part of early growth allocated to growth of the gastro intestinal tract (gut). Based on a growth study with different lines of Japanese quail, Lilja *et al.* (1985) suggested that initial growth rates could have been restricted by the capacity of the gut (a 'supply' organ) to convert feed. These investigations on growth patterns of individual organs and the subsequent division into two 'functional' groups gained knowledge on the 'functional growth plan' in birds.

Non-limited pullet growth was presented in the present investigations by a fourphasic model on BW gain in *ad libitum* fed pullets (Chapter 4). The phases in this model were not induced by dietary characteristics. Voluntary feed intake did not decrease after maximum gain in each of the four phases had been reached. This may illustrate the inherent changes in rates of protein and fat deposition.

The sequential nature of growth of body constituents is assumed to be represented by individual organ growth spurts that are underlying the multiphasic body growth curve.

Monophasic logistic growth functions were therefore separately fitted to data of skeletal bones (shank, tibia, and keel) and metabolic active organs (gut, gizzard, liver, and heart) in these *ad libitum* fed pullets (R.P. Kwakkel, Unpublished results).

The first body growth spurt in the fourphasic model had a total duration of about 22 wk, with its maximum growth rate at around 7 wk, and accounted for a total of 1150 g BW gain (Chapter 4). The skeletal bones, shank and tibia, had their maximum growth rate at around 4 wk of age. The digestive tract succeeded the body frame with growth spurts at around 6 wk of age. Both body structures (skeleton and gut) accounted for about 32% of total body growth within the first main body growth spurt (R.P. Kwakkel, Unpublished results). The remaining largest part was attributed towards non-dissected muscle tissue. The small second phase that was fitted separately from the first one, was not related directly to growth of particular body organs.

Growth of the sexual organs and concomitant fat growth, which is underneath the third body growth spurt, has been discussed in Chapter 5 in detail. The fourth growth phase is likely to represent a combination of increases in abdominal fat deposition *per se* or increases in BW due to initial egg formation.

MANIPULATION OF BODY GROWTH AND BODY COMPOSITION

The practical relevance of .40% digestible lysine

In our experiments, low-lysine levels in the diets were used as a method of qualitative restriction in order to cause a marked growth retardation. Singsen and coworkers reported already in the sixties that deficient lysine levels were an adequate method to retard body growth of pullets and alter subsequent egg performance (Singsen *et al.*, 1964, 1965). Other researchers confirmed these possibilities in a way of manipulating growth without the labour-intensive practices of a traditional daily feed restriction (a.o., Couch and Trammell, 1970; Connor *et al.*, 1977). It was found by Couch *et al.* (1972), that low-lysine levels did not have a clear effect on amino acid profiles of body organs, except for the bone marrow.

It should be mentioned that the lowest level of lysine in our diets (approx. .40% digestible lysine in the experiments 2 and 3; recommended level by the Dutch CVB was .85%) was below the levels commonly reported in literature. It is about 70 to 80% of recommended levels. It need to be stressed, however, that the low-lysine levels applied in our experiments were **not** designed for a direct use in practical pullet diets, as suggested recently by Hocking and Maxwell (1992) in relation to welfare implications. These feeding strategies were designed to manipulate growth to such an extent, that contrasts in growth and production could be expected.

Effects of method of restriction

Two methods of nutrient restriction were applied in our studies: (1) a qualitative, low-lysine diet, supplied *ad libitum*, and (2) a quantitative, a restricted allowance of daily feed intake. The advantage of the present study as compared to other studies on restricted feeding, was the design of the trial, in which pullets were fed in one of two rearing phases such a restricted amount of an adequate diet, that they matched body weights with their lysine-restricted counterparts throughout that restrictive period (Chapter 3). This 'pair-gain' method resulted in marked differences in body composition between the two groups (Chapter 6).

Compared to the group of pullets that received *ad libitum* the control diet, pullets that were fed the low-lysine starter diet reduced their feed intake voluntarily by 44% (Chapter 3). BW was retarded by 57% at 6 wk of age. RLs-pullets showed similar percentages losses of Cfat (59%), CP (59%) and ash (56%) up to 6 wk of age (end of restrictive period; R.P. Kwakkel, Unpublished results). To understand the feeding behaviour of these pullets, it may be useful to compare their energy intakes with those of the *ad libitum* fed controls. Table 1 shows the calculated energy intakes over the 42 days period as ME (metabolizable energy) intake per MBW (metabolic BW: BW raised to the power of .75) per day. The RLs-pullets consumed somewhat lower amounts of ME per MBW.d^{-1} than the C-pullets (769 and 857 kJ, respectively). Obviously, some mechanism forced the RLs-pullet to stop eating. The partitioning of energy towards maintenance, protein and fat growth (Table 1) do, of course, entirely account for the ME-intake observed in the C-group. The somewhat smaller amount of calculated ME-requirement compared to the observed ME-intake for the RLs-pullets may be due to an incorrect assumption of the ME-requirement for maintenance and/or a lower effective ME-intake than observed, due to the deamination of large amounts of imbalanced protein. As already concluded from the results in Chapter 6, the ratio between protein and fat accretion in the RLs-pullets is equal to the one of the C-pullets (ratio is 2.3). It is interesting to know why the low-lysine pullets did not consume more of the diet (provided *ad libitum*). If these pullets would have eaten more, a larger part of the ME had been partitioned towards growth, in particular fat growth. It may be argued that at early ages the layer pullet is not stimulated to consume more feed for additional fat accretion ('functional' fat growth; see Chapters 4 and 6).

The ideas of Gous *et al.* (1990) for broiler growth fit to some extent quite well to the multiphasic nature of juvenile pullet growth. They conducted studies with broilers to test the propositions of the Edinburgh Growth Model (Emmans and Fisher, 1986). Gous *et al.* (1990) slightly modified the assumptions of the Edinburgh Growth Model by stating that

Table 1. Calculated energy intake levels and partitioning of metabolizable energy for maintenance and growth of the different treatment in the respective phases of restriction (R.P. Kwakkel, Unpublished results).

Traits	0 - 6 wk of age			7 - 12 wk of age		
	C	RLs	RFs	C	RLg	RFg
Mean MBW[1] ($kg^{.75}$)	.345	.213	.206	.831	.725	.723
ME-intake[2] (kJ)	296	164	136	696	589	533
ME-intake per mean MBW ($kJ.kg^{-.75}$)	857	769	662	837	813	737
Metabolizable energy requirement[3] ($kJ.d^{-1}$)						
Maintenance	147	84	84	328	294	294
Protein growth[4]	100	36	40	130	74	97
Fat growth[4]	40	15	5	93	67	29
total	**287**	**135**	**129**	**551**	**435**	**420**
Protein to fat ratio (g/g)	2.3	2.3	7.0	1.3	1.0	3.1

[1] Mean metabolic body weight was calculated as $[(BW_{wk1})^{.75} + (BW_{wk2})^{.75} + .. + (BW_{wk6})^{.75} / 6]$

[2] Daily intake of metabolizable energy: ME of the starter and grower diet was 11.7 and 11.8 $MJ.kg^{-1}$, respectively (Chapter 3).

[3] Assumptions for energy requirements were:
ME_m : 420 $kJ.kg^{-.75}.d^{-1}$ (Chwalibog *et al.*, 1984)
$ME_{protein}$: 60.3 kJ per g retained protein (Emmans and Fisher, 1986)
ME_{fat} : 56.0 kJ per g retained fat (Emmans and Fisher, 1986).

[4] Based on body compositions of pullets, aging 0, 6 and 12 wk of age.

broilers at a given stage of maturity and fed a balanced diet would 'seek' for a particular lipid-to-protein ratio. Gous *et al.* (1990), however, suggested that birds on imbalanced diets (diets in which energy is not first limiting) would deposit excessive amounts of fat. In our case, RLs-pullets clearly were fed an imbalanced diet.

The proposition that birds 'seek' for a particular body composition may be valid if we assume that fat growth can be distinguished into a 'functional' fat growth phase (the bird 'seeks' a particular protein to fat ratio, based on biological constrains in accretion; Chapter 4) and a 'non-functional' fat growth phase (the bird is able to deposit excessive amounts of additional fat). Broilers do probably attain their second fat growth spurt (accretion of abdominal fat) at much younger chronological ages than do layer pullets, as a result of divergent selection procedures.

This can explain the results of the RLg-pullets when compared to the C-pullets in the respective phase of restriction (Table 1). If the low-lysine diet was applied from 7 to 18 wk of age, the largest BW reduction (20%) was achieved at wk 12. Feed intake of the RLg-pullets was 15% below that of the control pullets at that age. Cfat, CP, and ash losses were 19, 24, and 22%, respectively (R.P. Kwakkel, Unpublished results). Fat growth has been depleted to a lower extent than protein and ash growth, which would fit to the theory of excess lipid deposition on a low-lysine diet. Both the C- and RLg-pullets consumed similar amounts of ME per MBW.d^{-1} (837 and 813 kJ, respectively; Table 1). The summation of energy required by the different processes reveals that the calculated value is largely underestimated for both groups of pullets (for C: 551 vs. 696 kJ and for RLg: 435 vs. 590 kJ). This is possibly due to an underestimated ME-requirement for maintenance. The protein to fat ratio, however, is lower for the RLg-pullets (1.0) when compared to the C-pullets (1.3). This suggests that some extra fat deposition occurs in the RLg-birds. Obviously, the RLg-pullets had attained the non-functional fat growth phase, in which the intake of extra energy up to an 'ordinairy' level of ME per MBW.d^{-1} could be accrued as abdominal fat.

RFs-pullets that were restricted in total amount of feed from 0 to 6 wk of age reduced BW by 57% ('pair-gained'; Chapter 3). They showed the largest percentages of losses in the fat part (83%), followed by protein (55%) and ash (52%) at 6 wk of age. If the feed restriction had been applied from wk 7 onwards (RFg), the largest BW reduction (19%) was achieved at wk 12. At that age fat, protein, and ash losses were 48, 15, and 16%, respectively (R.P. Kwakkel, Unpublished results).

The RF-pullets consumed somewhat lower amounts of ME per MBW.d^{-1} than the C-pullets in the respective period (Table 1: C vs. RFs: 857 and 662 kJ; C vs. RFg: 837 and 737 kJ). For the RFg-pullets, the calculated total ME from assumed maintenance and growth is somewhat less than observed underestimated (Table 1). It seems that the assumed ME-requirement for maintenance in the grower period of rearing (Chwalibog *et al.*, 1984) is too low.

The ratio between protein and fat accretion in the RFs- and RFg-pullets (7.0 and 3.1) is much larger than those of the C-pullets in the respective phases (2.3 and 1.3). It is generally known that if one reduces all nutrients by means of applying a low feeding level, a situation will happen in which energy is the first limiting resource.

Thus, mostly fat will be mobilized or not accrued to the 'normal' levels (Chapter 6). Fat, however, is not depleted first totally, and then followed by muscle and bone: it occurs simultaneously, but the impact on fat depletion is much greater (Leat and Cox, 1980).

The low amount of fat that is accrued on a low plane of feeding suggests that, although the young pullet 'seeks' some kind of optimal protein to fat ratio, a large part of this functional fat is still not truly essential for body functioning, and still mobilizable. This suggests that within the first fat growth spurt different sites of more or less essential, and thus mobilizable fat structures exist. The lower cumulative feed consumption from day zero up to onset of lay by the RF-groups compared to the RL-counterparts is obviously due to the smaller amounts of 'expensive' fat accretion in these groups.

At 24 wk of age, assumed to be the start of the adult phase, average amounts (Means ± SEM) of Cfat, CP, water, and ash in the pullet body of all groups were 252.3 g ± 4.0, 229.3 g ± 1.6, 49.5 g ± 1.2, and 741.0 g ± 5.4. In conclusion, body composition was indifferently between treatments at that age.

THE 'BASIC' PULLET BODY IN RELATION TO ONSET OF LAY

One of the objectives formulated in the present study was to investigate the role of body composition in determining onset of lay. It was postulated that the attainment of a particular body composition could play a major role in the initial development of the reproductive body (Chapters 2 and 3).

The delay in achieving sexual maturity was highly correlated with BW at end of rearing, as was reported by Lee *et al.* (1971) and Gous and Stielau (1976). They regressed the delay in onset of lay as a difference to *ad libitum* fed controls (Y in days) towards BW at 18 or 20 wk of age relative to the control group (X in %). In both studies, similar significant equations were found ($Y = 54.1 - .53X$ ($P<.01$), and $Y = 58.3 - .59X$ ($P<.01$), respectively). These equations, however, were based on data from quantitative feed restriction experiments. Gous (1978) illustrated in his own experiments that the relationship was less clear when both qualitative and quantitative restriction methods were incorporated in the data set. In their first trial (Gous and Stielau, 1976: only feed restriction) the regression equation explained 98.4% of the total variance (coefficient of determination: CD), whereas in their second trial (Gous, 1978: feed and low protein restrictions) the CD was lowered to a value of 87.4%. Gous (1978) explained this phenomenon by stating that a low protein or low lysine diets might have a greater impact on delaying sexual maturity than the method of quantitative restriction.

Thus, the relationship between BW at the end of the rearing period and the delay in sexual maturity holds, whenever nutrient restrictions are compared *within method of restriction*. In Figure 1, this relationship is presented for the experiments from this thesis, including the work of Wells (1980), who also compared qualitative and quantitative methods of restriction within the same experiment. The regression equation ($n=23$; $Y = 24.8 - .23X$ ($P<.03$)) did not fit very well (CD was 21.4%). This illustrates the relative unimportant value of BW at end of rearing for predictive purposes concerning initial egg performance (Chapters 2 and 3).

In Chapter 5, it was concluded that a critical amount of fat-free tissue is required for initiating growth processes of the reproductive organs around 14 to 15 wk of age. It is clear that a lysine restriction prior to this age delays the point at which this 'basic' body

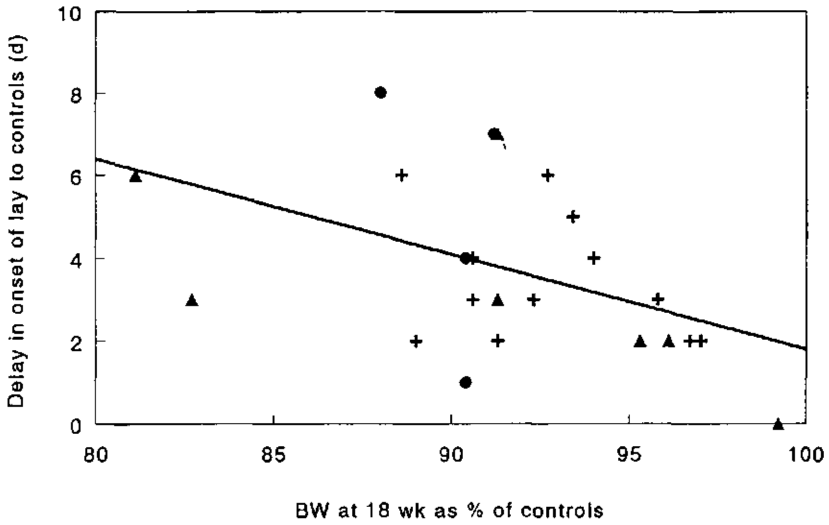

Figure 1. The relationship between the delay in onset of lay and BW at end of rearing (▲ : Chapter 2; ● : Chapter 3; + : Wells, 1980).

is achieved in terms of chronological age. Whereas a feed restriction is limiting energy gain primarily, a similar severity of the restriction on the basis of BW will result in a quicker attainment of this critical amount of fat-free tissue. This was evident in our experiments: when the pure effect of BW was eliminated, then lysine restricted birds delayed onset of lay by up to 5 days compared to feed restricted birds. The literature on determinants for onset of lay has been reported extensively in Chapter 5. In Chapter 6 it was shown that the composition within the fat-free body can hardly be manipulated by nutritive decisions.

Fowler (1968) summarized the principles of 'functional' growth into a single basic proposition: "the animal tends to adjust to nutritional changes in such a way that the vital functional relationships between essential body components are preserved, or modified to a form which gives the animal its best chance of survival and successful reproduction".

INTERACTION BETWEEN EGG SIZES AND ADULT BODY GROWTH

Hens restricted during the grower period of rearing laid a 1.5 g heavier egg than those restricted during the starter period of rearing, despite similar BW at end of rearing.

This effect was nor influenced by the method of restriction (Chapter 3). So, egg sizes were related neither to chronological age, as postulated by Williams and Sharp (1978) in Johnson *et al.* (1984), nor to BW, as suggested by Summers (1983) and Summers and Leeson (1983) or skeletal size, mentioned by Burgess (1986). Some workers, however, reported findings similar to ours: nutrient restrictions early in pullet life, for example by means of a step-up protein programme, could reduce subsequent egg sizes (Connor *et al.*, 1977; Leeson and Summers, 1980; Doran *et al.*, 1983; Chi, 1985).

Larger egg sizes of grower-restricted birds may be related to BW gain during laying. From Chapter 2 (experiment 2), it was concluded that lysine-restricted pullets during the starter period of rearing clearly compensated growth losses during laying. Feed-restricted pullets during the grower period did not fully compensate a lower BW at end of rearing and the birds remained lighter throughout laying (Chapter 2). It is proposed here that 'catch-up' growth during lay is more related to the period (early vs. late) than to the method of restriction. Early-restricted pullets showed some more 'catch-up' growth than late-restricted pullets in the third experiment (Chapter 3).

An explanation for this could be the theory on compensatory growth as suggested by McCance and coworkers (McCance, 1977). McCance and Widdowson (1974) hypothesized the existence of 'critical' moments in body and organ growth during immature stages in both men and animals. In their theory a critical moment meant that the hypothalamus sets its mature size and mass. A period of undernourishment below this 'critical setting' will be followed by a period of compensatory growth till 'normal' mature sizes have reached. On the contrary, restrictions which have taken place prior to this critical moment might be followed by an incomplete compensatory growth spurt, i.e. ending up in a smaller mature weight and/or size of the respective structure (McCance, 1977). It may be argued that for the 'rate of gain' of body growth during lay (which is almost fat deposition), the 'critical setting' by the hypothalamus will occur during the period of increased abdominal fat deposition. If pullets were under restrictive nutritional conditions during that period of rearing (the grower phase !), a slower 'rate of gain' following the period of limitation (i.e., during lay) may prevent the bird from excessive fat accretion and concomitant BW gain. If this is true, than the differences in egg sizes may be the result of allocating ingested nutrients for synthesis of egg mass where body mass increase is less needed. If energy intake is calculated for the 19 to 32 wk period of laying in the early and late restricted groups, the latter group consumed about 21 $kJ.d^{-1}$ more during 19 to 32 wk of age than the other group (R.P. Kwakkel, Unpublished results). This excess could be attributed entirely to the synthesis of the extra 1.7 g egg mass, which costs about 17 kJ ME (egg synthesis costs 10 kJ per g egg: Emmans and Fisher, 1986). Thus the extra feed is mostly used for extra egg energy.

External egg quality (broken, soft-shelled, double yolks) was not clearly affected by the feeding regimens in this study. According to Hocking (1987), the occurrence of 'multiple ovulation' during the first few wk of lay differs between strains and feeding levels prior to egg production. In that way, variation in the incidence of 'defective' egg mass can occur. Whereas most authors do not state explicitly the various 'classes' of egg mass production, part of the variation within feeding strategies might be explained by this (Johnson *et al.*, 1984; Hocking, 1987).

A SCHEMATIC MODEL OF PULLET GROWTH

A schematic model for critical pullet growth and subsequent egg performance in response to feeding strategies is presented below.

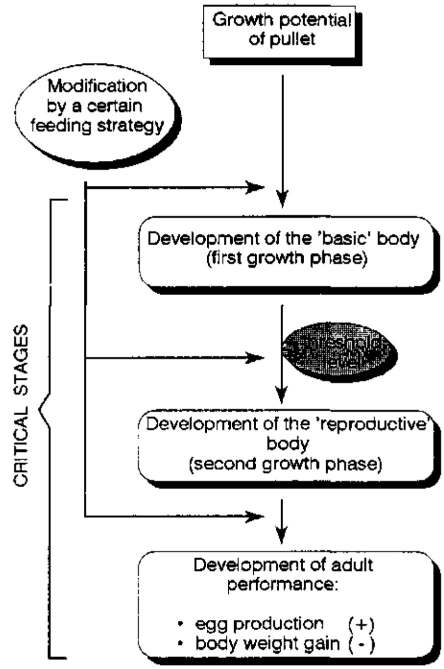

Figure 2. A schematic outline of critical phases of pullet development and layer performance as affected by feeding strategies.

SCIENTIFIC AND PRACTICAL IMPLICATIONS

A practical feeding strategy based on biological considerations

Nutritional decisions made by the pullet farmer should be timed around physiological changes during important stages in pullet's life. The main point is that we need to locate such 'critical' changes. This is what the multiphasic growth approach is aiming for. In this section, some recommendations are made towards the formulation of practical rearing diets and suggested restriction levels, based on our results.

An early lysine – or protein – restriction affected adult performance negatively by a delay in onset of lay (Chapter 5) and by decreased egg weights (Chapters 2 and 3). This treatment retarded muscle development and consequently the development of the frame size, as was illustrated by the strong relationships within the fat-free body (Chapter 6). In an *ad libitum* feeding situation, shank and tibia matured before 13 wk of age (Chapters 6 and 7), and restrictions may cause problems at onset of lay while birds are still 'catching up' their body frame size. A permanent smaller body frame due to a severe feed restriction is unlikely to occur (Chapter 7; Leeson and Summers, 1984).

Three important stages during pullet's life arose from this study: (1) The fat-free body (in this study: FFEBM) seems to be a major determinant in initiating processes of puberty (Chapter 5). A FFEBM of about 800 g is needed before sexual development starts (approx. 1150 g BW for this WL-strain (15 wk of age); *see* Chapter 4). High protein starter diets (or in other words: adequate levels of essential amino acids) from hatch up to 500 g FFEBM (around 8 wk of age) are critical to sustain this type of 'functional growth' (Chapter 6); (2) From that stage of development, the bird will gain more slowly, although feed intake in the *ad libitum* situation will still increase: the pullet predestinates nutrients for increased fat growth (Chapter 6). A 'mid-term' feed restriction, as proposed by Wells (1980), is then advisable; (3) A gradual cessation of the feed restriction from 14 wk of age onward might increase daily feed intake (appetite) slowly and will support the 'critical' development of the reproductive organs, which will affect performance positively (Chapter 3; Johnson *et al.*, 1985; Bowmaker and Gous, 1989; Hurwitz and Plavnik, 1989). In contrast to a direct switch to *ad libitum*, this gradual transition might prevent too many irregular eggs at the onset of lay (Hocking, 1987), but it will be sufficient to deposit some energy reserves to cope with negative balances during early lay. A uniform flock at point of lay enables the farmer to adjust feed quantities properly, in this way avoiding to hinder some birds in desired feed intake (Balnave, 1984; Hocking, 1987).

A factorial approach based on these data and considerations can easily be translated into a feeding strategy on the farm. In literature, an example of such a factorial model has been presented recently (Bowmaker and Gous, 1989). They included data of amino acid compositions of the major reproductive organs, in order to quantify requirements (with assumptions for efficiencies) for the individual stages (based on weekly calculations) of reproductive growth. Amino acids were not analyzed in the present thesis.

Welfare implications

Though there is no simple measure of welfare (Hill, 1983), it has been related to concepts of 'stress' and 'animal adaptability'. Restricted feeding may induce stress and thus may be considered as undesirable for the young fowl (Freeman, 1985). The experimental tool for an ethologist to assess the animal's adaptability (how to cope with a given set of circumstances) is the introduction of a stressor in combination with the measurement of the stress response (Scott *et al.*, 1983). Stress indicators that are frequently used are plasma corticosterone to assess short term effects and adrenal weights and, more recently, heterophil to lymphocyte ratios to assess long term effects. In respons to restricted feeding, young pullets elevated plasma corticosterone concentrations by 73 (feeding level: 75% of *ad libitum*; Freeman *et al.*, 1981) to 300% (starvation; Nir *et al.*, 1975) as compared to *ad libitum* fed birds. In the study of Freeman *et al.* (1981), these levels returned within 5 wk to 'normal' values, indicating some kind of adaptative abilities. In both studies adrenals responded to feed deprivation by increasing relative weights. Karunajeewa (1987) judged 'controlled' feeding systems for pullets on their impact for animal welfare. Karunajeewa (1987) and Savory (1992) concluded that the renewed interest for the application of qualitative restriction methods (diluting diets) rather than quantitative methods could be due to the less negative influences on welfare. Hocking and Maxwell (1992) reported stereotypic behaviour, higher heterophil to lymphocyte ratios, and increased corticosterone levels as a result of a 50% feed reduction in broiler breeders. Before 7 wk of age, however, they found no evidence of stress .

In recent years, public concern for welfare in farm animals turned out to be a key issue in the bio-industry. On the longer term, the scale of this issue as being a welfare problem depends on what level of feed restriction is considered to be acceptable (Savory, 1992). Hocking *et al.* (1989) suggested for broiler breeders that it may not be necessary to restrict feed intake throughout the entire rearing period. Indeed, the results of the present study (on layer pullets) indicate that a restricted feed supply throughout rearing, which is common practice now, should be replaced by a set of alternating non-restrictive and restrictive periods. The non-restrictive periods of critical tissue growth will clearly enhance pullet's well-being. If such management steps should prove to be inadequate for improving pullet welfare to an acceptable level, then a reduction in economic efficiency may be the only alternative (Savory, 1992). Automized feeding equipment may provide the possibility to increase the feeding frequency with a concomitant reduced meal size. This situation is only useful if sufficient feeder space is available.

Economic returns

Fine tuning of the birds' nutritional requirements during rearing may lead to improved egg yields over feed costs during the laying period which in turn will be beneficial for the egg producer's income. Verbij and Van Horn (1991) calculated the effects of a better performance on the 'net balance' per hen (mainly egg yields minus feed costs) under Dutch circumstances. They reported that an increase in overall rate of lay by 1% would increase the net balance per hen by 15%. The net balance per hen will increase by 20%, when the bird produces a 1 g heavier egg, and by 7% when she consumes 1 g less feed per day.

In the second experiment (Chapter 3), pullets of treatment RFg consumed 12% less feed during rearing than did the *ad libitum* fed control group. Moreover, they laid throughout the entire laying period a 1.7 g larger egg and had a 1.7% increased rate of lay. On the other hand, they consumed 1.8 g more feed during the laying period. This would result in an extra profit of more than 40% per hen housed. No adjustments were made in these calculations for larger eggs entering lower price categories.

The environmental burden caused by the excess of manure is important in intensive livestock in the Netherlands. An excess of mineral excretion on farm level may be charged by legislation in the near future. Thus, reducing the manure output by means of a reduced feed intake during rearing may be beneficial. The effect of an altered feeding strategy, however, on manure output will be marginal, because the proposed feeding regime will rather imply a re-distribution of similar amounts of feed than a reduction of the amounts which are fed currently to layer pullets.

Endocrine control of multiphasic growth processes

Hormones exert their effects on growth by means of influencing the flow of nutrients through various metabolic pathways (Oddy and Lindsay, 1986). For example, the inherent fat growth spurt around puberty, due to an increased rate of fat deposition rate, is a direct effect of circulating sex hormones (Leat and Cox, 1980; Short, 1980; Ford and Klindt, 1989). Estrogens promote the replication of cultured adipocyte precursors (Leat and Cox, 1980).

In addition, hormone profiles may be affected by nutritive changes. Several workers showed that plasma levels of growth hormone (GH) and the tyroid hormones triiodothyronine (T3) and thyroxine (T4) can be influenced by applying a particular feeding programme. Nir *et al.* (1987a) fed chickens intermittently and found that on days when pullets where not fed, plasma levels of GH and T4 increased, while T3 decreased. GH and T4 levels returned to the level of full fed pullets, while T3 concentration was either identical to or higher than the T3 level of full fed pullets.

In the light of the multiphasic growth theory as presented in this thesis, it would be interesting to know whether transitions from one growth phase into another are accompanied or even preceded by hormonal changes (Belt *et al.*, 1992). If this happens, which is most likely, hormone analyses may be a valuable tool in the *in vivo* assessment of critical growth spurts of underlying body structures.

Alternatives for studying body composition

One of the major problems when studying body composition in farm animals is the large variation within data of serial-slaughter experiments. This implied in our experiments, that a large number of birds was needed to be killed to obtain a representative sample. The use of an *in vivo*, non-destructive technique for detecting body tissues in maturing pullets would provide a major improvement in this field of research. The total number of birds per experiment would decrease tremendously.

Nuclear Magnetic Resonance Imaging (NMR-i) is such a technique. NMR-imaging is currently being used in human medicine as a non-invasive, continous method of providing *in vivo* information on anatomical and morphological structures in living animals (Liu *et al.*, 1992). In future research, the NMR-i technique can be used to study abdominal fat deposition and mobilization during the process of maturation and onset of lay.

SUMMARIZING CONCLUSIONS

The results described in this thesis clearly demonstrated that:

1. Body weight at end of rearing is of minor importance in determining egg production features such as onset of lay and egg weight. Method of restriction (nutrients that are offered) and phase of restriction (growth curve) is more relevant in this respect (Chapters 2 and 3).
2. The 'maturity growth spurt' is related to both the moment of rapid sexual development, and onset of lay in the layer. The assessment of the maturity growth spurt in a flock by weekly random weighing may therefore be a valuable tool to the producer for making nutritional decisions (Chapter 5).
3. The fat-free empty body of pullets is the major determinant in initiating physiological processes that will result in reproductive development (Chapter 5).
4. Early fat growth is biologically related to growth of the fat-free body. Fat growth in the body of layer pullets can be distinguished in at least two phases: a 'functional' phase and a phase during which fat is stored as an energy buffer. Moreover, pullets on a low-lysine diet did not increase their fat-to-protein ratio in the body at very

young ages (Chapters 4 and 6).

5. The composition of the fat-free body was not affected by any of the dietary treatments. However, the composition suddenly changed at a particular physiological age towards a body with an increased DM-content (Chapter 6).
6. To assess any effect of a nutritional deprivation on organ and tissue weights, growth figures should be expressed as a percentage of, or relative to, the fat-free body mass instead of BW or EBM. This conclusion, that originates from the ideas of the Cambridge School (1930 - 1960) has been scarcely used in nutritional poultry growth studies (Chapters 6 and 7).
7. Multiphasic analyses of pullet growth quantified some growth relationships between body constituents that had never been revealed if the monophasic approach had been used (Chapter 6 and 7).

The existence of critical growth phases (development of the 'basic' and the 'reproductive' body) may not be neglected in evaluating and improving modern rearing programs for layers. Nutrient partitioning processes are directed by the competing demands of particular body structures, that are in a growth spurt. Adjusting feeding programmes to such processes of multiphasic growth must be the clear focus of todays nutritionists in appealing towards future poultry production systems.

A multiphasic analysis of growth is an indispensable tool in studies in which the effects of feeding strategies on body development and subsequent performance need to be assessed.

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SUMMARY

Layer pullets are reared in preparation for subsequent laying performance. The rearing stage itself is a period in hen's life in which it does not yet "produce", and, as a consequence, pullets are reared on a least-cost basis. For decades, controlled feeding programmes (feeding levels below *ad libitum* or low-protein diets) have been designed from an economic perspective; to control the growth of young pullets towards a specific target weight and age.

Recent information, however, has stressed that the pattern of body growth in relation to the rate of development of particular organs during rearing may interfere with the 'quality' of the young hen.

The pattern of body growth depends on the growth pattern of the individual body constituents. Each body constituent (organs or tissues), however, will follow its own pattern of growth and functional maturation, and thus a variation will exist in its nutritional demand over time. This implies that the supply of nutrients for some organs or tissues may be critical at certain rearing stages. Ignoring these 'critical periods' by only focussing on target weights at the end of rearing may negatively affect future development and adult performance.

The aim of this study was to evaluate the growth of body constituents in the layer pullet in relation to egg performance, in order to provide a framework for a biological feeding strategy in which poor nutritional situations could be avoided during critical rearing stages.

Three experiments are reported, in which White Leghorn pullets were subjected to a daily restricted amount of feed (quantitative restriction) or a low-lysine diet (qualitative restriction) during one of two rearing phases (starter: 0-6 wk of age or grower: 7-18 wk of age). The feeding regimens were imposed in order to achieve sufficient contrasts in the pullets' responses, rather than to present 'ready-to-use' feeding regimens. Egg performance was recorded in two of the three experiments.

In the first experiment, the effects of three methods of nutrient restriction on BW gain and feed intake were investigated during rearing (0-18 wk of age) (Chapter 1). A conventional step-down lysine regime (.85 to .57% digestible lysine in 3 steps) was compared to a step-up lysine regime (.65 to .77% digestible lysine) and a 'mid-term' (7-15 wk of age) feed restriction (85% of *ad libitum*). Between feeding regimens, feed consumption differed to a maximum of 7%, as a result of a changed pattern of BW development during rearing. However, there were no differences in BW at 18 wk of age between the treatments (Chapter 1).

In the second experiment, different low-lysine levels in a starter diet (RL: .40 to .85% digestible lysine) were compared to daily feed restrictions during the grower

period (RF: 80% of *ad libitum*; 7 to 15 or 7 to 18 wk of age). The experiment was conducted to define critical levels of restriction in relation to the ability of the pullets to compensate for growth retardation afterwards and to perform adequately (Chapter 2). In general, the onset of lay was related to the severity of the restriction for both RL and RF. A longer period of feed restriction during the grower phase, gradually decreased BW at the onset of lay, and cumulative feed intake up to the onset of lay (in terms of 50% rate of lay). On the other hand, all RL-pullets commenced egg production at similar BW and cumulative feed intake. Consequently, RF-pullets required less feed up to the onset of lay than RL-pullets. Moreover, RF-pullets (grower phase) maintained a lower BW throughout the entire laying period, whereas RL-pullets (starter phase) compensated for BW retardation during the laying period. No differences in rearing and laying performance were found between pullets that received .70% and .85% (the latter is the Dutch recommended level) digestible lysine in the starter diet. In this experiment, the effects of method and phase of restriction were confounded, because the lysine restriction had only been applied during the starter phase, while feed had been restricted only during the grower phase of rearing.

A third experiment was therefore designed to study method and phase of restriction as independent effects. The results of this factorial experiment are reported in Chapter 3. In each rearing phase (starter or grower), a lysine restriction (RL) was compared to a daily feed restriction (RF), in which the RF-pullets received a quantity of feed sufficient to pair-gain with their RL-counterparts. Hence, both groups followed identical patterns of BW gain. This design enabled an interpretation of the experimental results without the confounding effect of BW *per se*, whose variation within experiments is one of the major problems in evaluating results reported in the literature. Despite the different feeding regimens, all four restricted groups had similar BW at 18 wk of age. Even with similar patterns of BW gain in the restrictive phases, RL-pullets delayed the onset of lay by about 5 days compared to the 'pair-gained' RF-pullets. Hens restricted during the grower phase of rearing laid a heavier egg (by 1.5 g) than those restricted during the starter phase, regardless of which method of restriction had been applied. The grower-restricted pullets gained less BW during lay than the starter-restricted pullets, thereby probably allocating nutrients towards egg production instead of body growth. Similar to the results of the second experiment, as reported in Chapter 2, RF-pullets needed less feed up to the onset of lay than RL-pullets.

It was emphasized that different patterns of body growth or different methods of restriction could lead to similar BW at the end of rearing (Chapters 1, 2, and 3), but resulted in differences in subsequent performance (Chapters 2 and 3). On the basis of these findings, it was concluded that BW at the end of rearing is of minor importance in determining subsequent layer performance than is the type (which nutrient?) and phase (what age?) of restriction.

To evaluate the effects of the feeding regimens in more detail, pullet growth and development was studied by multiphasic growth analyses (Chapters 4, 5, 6, and 7). A number of birds of the third experiment was therefore sacrificed by serial slaughtering throughout the rearing and early laying period, to determine chemical body composition and organ growth in each treatment. Two multiphasic models were fitted to these data:

1. The Multiphasic Growth Function (MGF) describes growth as a function of age, based on a summation of 'n' logistic functions. It allows the study of distinguishable growth spurts;
2. The Multiphasic Allometric Function (MAF) describes the growth relationship between two body constituents. The MAF is an extension of the simple allometric model ($Y = \alpha X^{\beta}$), and allows for changes in the relationship between the two body structures.

The usefulness of both multiphasic functions was shown based on data of the *ad libitum* fed birds (Chapter 4). Data of all treatments were used to describe changes in absolute growth around puberty (Chapter 5), and the relative growth of body components (Chapters 6) and body organs (Chapter 7), as a consequence of the feeding regimens during rearing.

A fourphasic growth function was fitted to body growth data of the *ad libitum* fed pullets during the rearing and early laying period (Chapter 4). It was revealed that growth in the first two phases consisted of the development of 'maintenance' structures like bones and metabolic organs (Chapter 7). The third growth phase at around 19 wk of age was called the 'maturity growth spurt'; 40 to 70 % of total growth within the maturity growth spurt consisted of growth of the reproductive organs (Chapter 5). The fourth growth phase presumably consisted of body fat deposition.

Temporal growth patterns of the body components dry matter, crude protein, crude fat, and ash, were all diphasic. The allometric relationships between crude protein, crude fat, and ash, each as a function of dry matter, were also diphasic (Chapter 4). It was speculated that the early growth of protein, fat, and ash could be functionally related to each other, presumably consisting of muscle growth, intramuscular fat deposition, and skeletal growth, respectively. Both protein and fat growth at later stages of rearing seemed to be related to sexual development and abdominal fat deposition, respectively (Chapter 4).

Patterns of growth around puberty were studied in Chapter 5. The aim was to investigate mechanisms that control the onset of lay. It is generally believed that a hen must attain a minimum BW in combination with a 'particular' body composition before she initiates egg production. Differences between the RL- and RF-pullets in onset of lay (about 5 days), were clearly reflected by differences in the occurrence of the maturity growth spurt.

If one assumes that the maturity growth spurt acts as an indicator of sexual development, it is interesting to know if there were differences in body composition at the beginning of the maturity growth spurt. The fat content of the body varied considerably between the feeding regimens (112 to 179 g Cfat). Protein and fat-free body mass, showed much less variation between the treatments at that stage of sexual development (187 to 201 g protein and 806 to 870 g fat-free body mass, respectively). It was concluded that a certain amount of protein or fat-free tissue, deposited in essential - maintenance - body structures (= development of the 'basic' body), is critical for the initiation of sexual growth (= development of the 'reproductive' body; Chapter 5).

Another essential consideration in pullet growth may be whether the development of certain body constituents is *more* affected by an applied restriction than others. Hence, a description of relative growth is relevant (Chapters 6 and 7).

In Chapter 6, diphasic allometric relationships between each chemical body component and the plucked empty body mass (EBM), were observed for all treatments, with different slopes for the first and second phases. These diphasic relationships, however, were, to a large extent due to the diphasic growth pattern of fat *per se*. If fat-free EBM (FFEBM) instead of EBM was chosen as the independent variable in the allometric relationship between each fat-free body component and total FFEBM, differences between the allometric slope of the first and second phase became smaller. Up to approximately 300 to 400 g FFEBM (the first allometric phase), a slope of 1 (proportional growth) between each fat-free component and total FFEBM was found, regardless of the applied feeding regime. This indicated a constant composition of the fat-free body in the first growth phase. Between 350 and 1000 g FFEBM (the latter amount present at around 24 wk of age), protein grew relatively faster than ash and water. Again, no differences between the feeding regimens were observed. The results suggested that relative gain within the FFEBM was *not* influenced by feeding regime, but only affected by aging of the fat-free body (Chapter 6).

During the first growth phase, proportional growth between Cfat and FFEBM was observed in pullets fed *ad libitum* and in pullets fed the low-lysine diet (RLs-pullets). It was suggested, that lipid growth at early ages mainly represents functional fat deposition, related to growth of the fat-free body at that stage (Chapters 4 and 6). The low-lysine pullets voluntarily decreased their energy intake to avoid some extra fat deposition. At a later stage, lipid growth represents mainly storage fat.

Fat growth was affected too a large extent by a low feeding level (RF-pullets). Fat seemed to serve as an independent energy buffer: its deposition rate dependent on the plane of nutrition. It was concluded that probably most of the effects on organ weight reported in the literature (e.g., as a weight per 100 g BW) are simply a consequence of the mobilization of fat *per se*. FFEBM was proposed to be a better independent variable than EBM or BW in relative growth studies including nutritional factors.

This was illustrated by the assessment of organ growth as a function of FFEBM (Chapter 7). Growth of three skeletal bones (shank, tibia, and keel), the digestive tract (gizzard separately), the heart, the liver, and the ovary, each relative to FFEBM were described by a diphasic model (Chapter 7). Allometric slopes of the first growth phase were almost identical for all treatments for each constituent, suggesting that the variation in organ growth rate, due to the applied regime, followed the variation in growth rate of FFEBM. An allometric slope of the second phase around zero was related to the attainment of maturity for the respective constituent. Similar curves for the different treatments suggested that the growth pattern and attainment of maturity for most organs depended upon a certain amount of FFEBM. The keel, however, grew relatively faster (high priority) if an early nutrient restriction was applied. No differences in mature organ weights between the feeding regimens were observed.

The results confirmed the 'classical' observation that an inhibited growth of the skeleton and the digestive tract, as a consequence of a nutritionally induced growth retardation, is related to the decrease in growth rate of the fat-free body.

Finally, in the General Discussion some scientific and practical implications are discussed.

Summarizing, the results described in this thesis clearly demonstrated that:

1. Body weight at the end of rearing is of minor importance in determining egg production features such as the onset of lay and egg weight. The method of restriction (nutrients that are offered) and the phase of restriction (growth curve) is more relevant in this respect.
2. The 'maturity growth spurt' is related to both the moment of rapid sexual development and the onset of lay in the young hen. The assessment of the maturity growth spurt in a flock by weekly random weighing may therefore be a valuable tool to the producer for making nutritional decisions.
3. The fat-free empty body of pullets is the major determinant in initiating

physiological processes that result in reproductive development.

4. Early fat growth is biologically related to growth of the fat-free body. Fat growth in the body of layer pullets can be distinguished into at least two phases: a 'functional' phase, and a phase during which fat is stored as an energy buffer. Moreover, pullets on a low-lysine diet did not increase their fat-to-protein ratio in the body at very young ages.
5. The composition of the fat-free body was not affected by any of the dietary treatments. However, the composition suddenly changed at a particular physiological age towards a body with an increased DM-content.
6. To assess any effect of a nutrient restriction on organ and tissue weights, growth figures should be expressed as a percentage of, or relative to, the fat-free body mass instead of BW or EBM. This conclusion, which originated from the ideas of the Cambridge School (1930 - 1960) has been scarcely used in nutritional poultry growth studies.
7. Multiphasic analyses of pullet growth quantified some growth relationships between body constituents which would never have been revealed if the monophasic approach had been used.

The existence of critical growth phases (development of the 'basic' and the 'reproductive' body) may not be neglected in the evaluation and improvement of modern rearing programs for layers. Nutrient partitioning processes are directed by the competing demands of particular body structures, that are in a growth spurt. Adjusting feeding programmes to such processes of multiphasic growth must be the clear focus of today's nutritionists in the development of future poultry production systems.

The multiphasic analysis of growth is an indispensable tool for studies in which there needs to be an assessment of the effects of feeding strategies on body development and subsequent performance.

SAMENVATTING

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Vitale, jonge hennen met een goede produktieverwachting tegen het einde van de opfokperiode zijn belangrijk voor het rendement in de legpluimveehouderij op de lange termijn. Het lichaamsgewicht aan het eind van de opfokperiode (het zogenaamde 'streefgewicht') wordt gezien als een goede maat voor de kwaliteit van de jonge leghen. Uit economische overwegingen worden 'gecontroleerde' voerschema's (beperkt voerniveau of een laag-eiwit voer) tijdens de opfokperiode gehanteerd om dit streefgewicht bij een juiste leeftijd te bereiken.

Sinds het begin van de jaren tachtig zijn er echter aanwijzingen dat de gewichtsontwikkeling in relatie tot het moment waarop en de snelheid waarmee bepaalde organen zich ontwikkelen mede bepalend is voor de kwaliteit van de jonge leghen.

Het groeiverloop van het (totale) kuiken, dat leidt tot het 'gewenste' lichaamsgewicht, hangt af van het groeiverloop van de afzonderlijke lichaamscomponenten (bv. organen). Elk orgaan of weefsel volgt een uniek groeiverloop tot het moment dat het dier volwassen is. Dit betekent dat het aanbod van nutriënten bij toepassing van de huidige 'gecontroleerde' voerschema's mogelijk beperkend is voor een specifiek orgaan wanneer deze in een fase van maximale groei verkeert.

De in dit proefschrift beschreven experimenten hebben zich geconcentreerd rond de vraag: zijn er kritieke groeifasen in de ontwikkeling van een jonge leghen? En zo ja, heeft de onthouding van nutriënten in zo'n fase onomkeerbare gevolgen voor de latere eiproduktie? Anders gezegd: kan het kiezen voor een bepaalde voerstrategie tijdens de jeugdfase blijvende gevolgen hebben voor de ontwikkeling van bepaalde organen en orgaansystemen en kan dat op die manier de volwassen eiproduktie negatief beïnvloeden ?

Het doel van deze studie was te onderzoeken in welke mate de groei van diverse lichaamscomponenten kan worden beïnvloed, en welke effecten dit heeft op de uiteindelijke eiproduktie. Deze informatie zou dan als basis kunnen dienen voor voerstrategieën die op gecontroleerde wijze de nutriëntenvoorziening afstemmen op de gewenste ontwikkeling van lichaamscomponenten.

In drie experimenten werden Witte Leghorn kuikens onderworpen aan een voerbeperking (kwantitatief) of een lysinebeperking (kwalitatief) tijdens de start- (0-6 weken) of groeifase (7-18 weken) van de opfokperiode. Men dient zich te realiseren dat géén van deze beperkingen een directe weerspiegeling is van een in de praktijk gehanteerde voerstrategie. De restrictieniveaus waren niet direct ontleend aan praktijkstrategieën maar hadden tot doel zodanige groeiverschuivingen te realiseren dat de sturingsmechanismen die hiervoor verantwoordelijk zijn bestudeerd konden worden. Produktieresultaten tijdens de legperiode werden geregistreerd in het tweede en derde experiment (Hoofdstuk 2 en 3).

In het eerste experiment werden de effecten bestudeerd van drie voerstrategieën op lichaamsgroei en voeropname tijdens de opfokperiode (0-18 weken) (Hoofdstuk 1). Een normaal 'step-down' lysine-regime (van .85% verteerbaar lysine in drie stappen naar .57%) werd vergeleken met een 'step-up' lysine-regime (van .65% naar .77% verteerbaar lysine) en een 'mid-term' voerbeperking (voerniveau: 85% van *ad libitum* van

7-15 weken). Ofschoon sommige voerstrategieën, als gevolg van een veranderend groeiverloop tijdens de opfok, een verschil in voeropname van maximaal 7% lieten zien, werden er geen verschillen geconstateerd in het lichaamsgewicht op 18 weken.

In een tweede experiment werden de effecten bestudeerd van grotere verschillen in lysine-aanbod (RL: .40 tot .85% verteerbaar lysine) tijdens de startfase. Daarnaast werd een voerbeperking tijdens de groeifase (RF: 80% van *ad libitum* op een leeftijd van 7-15 weken danwel 7-18 weken) toegepast. Dit om te onderzoeken of opfokkuikens, bij een zwaardere beperking, verschillen in groei laten zien die mogelijk later in de opfok worden gecompenseerd, en of deze verschillen van invloed zijn op de uiteindelijke produktie (Hoofdstuk 2). De groeiachterstand en een vertraagde legrijpheid (legpercentage van 50%) bleken, zowel bij de RL- als RF-kuikens, direct gerelateerd aan de mate van beperking. Een langdurige voerbeperking tijdens de groeifase (RF: 7-18 weken) resulteerde in vergelijking met een korter durende voerbeperking (RF: 7-15 weken) in een lager lichaamsgewicht en een lager cumulatief voerverbruik bij aanvang van de leg. De RL-kuikens echter kwamen, ondanks een verschillend lysinegehalte in het startvoer, op een gelijk lichaamsgewicht en bij een gelijke cumulatieve voeropname aan de leg. Dit betekende dat de RF-kuikens minder voer nodig hadden tot legrijpheid dan de RL-kuikens. RF-kuikens bleken daarnaast een lager lichaamsgewicht te handhaven gedurende de legperiode. Er werden geen aantoonbare verschillen gevonden in produktieresultaten tijdens zowel de opfok als de leg tussen kuikens die via het startvoer .70% verteerbaar lysine danwel .85% verteerbaar lysine (= norm) verstrekt hadden gekregen. In dit experiment werd de lysinebeperking *alleen* toegepast tijdens de startfase en de voerbeperking *alleen* tijdens de groeifase. Daarom was het in dit experiment niet mogelijk beide effecten afzonderlijk te toetsen: methode en periode van beperken waren immers verstrengeld.

Een derde experiment werd opgezet om methode en periode van beperken te kunnen bestuderen als onafhankelijke factoren. De resultaten van dit experiment staan beschreven in Hoofdstuk 3. In elk van de beide opfokfasen (start of groei) werd een lysinebeperking (RL) vergeleken met een voerbeperking (RF). De RF-kuikens kregen dagelijks zoveel voer dat ze een gelijke groei lieten zien als de RL-kuikens. Aldus hadden beide groepen een identiek gewichtsverloop. In de proefopzet werd dus voor het effect van lichaamsgewicht als zodanig gecorrigeerd. Ongeacht de verschillen in voerstrategie, bereikten de vier beperkte proefgroepen een gelijk lichaamsgewicht op 18 weken. RL-kuikens kwamen 5 dagen later aan de leg dan RF-kuikens, ondanks een identieke gewichtscurve. Opfokhennen die tijdens de groeifase beperkt waren geweest, legden gedurende de gehele legperiode gemiddeld een 1.5 g zwaarder ei dan de hennen die in de startperiode beperkt waren geweest, ongeacht de methode van beperken. Uit dit experiment bleek, evenals uit experiment 2, dat de RF-kuikens minder voer nodig hadden tot legrijpheid dan de RL-kuikens (Hoofdstuk 2).

Dieren met een gelijk lichaamsgewicht op 18 weken gaven dus verschillen in eiproduktie te zien, afhankelijk van het groeipatroon (vroeg/laat beperken) of de methode van nutriëntbeperking (voer/lysine) tijdens de opfokperiode (Hoofdstuk 2 en 3). Op basis van deze bevindingen werd geconcludeerd dat het lichaamsgewicht aan het einde van de opfokperiode ('streefgewicht') geen juiste voorspeller is van de produktie tijdens de legperiode.

In de hoofdstukken 4, 5, 6, en 7 zijn de effecten van de verschillende voerstrategieën uit experiment 3 nader bestudeerd. Een groot aantal kuikens werd daartoe op gezette tijden tijdens de opfok- en de vroege legperiode uit de proef genomen. Van deze dieren werden de orgaangewichten en de chemische samenstelling bepaald. Orgaan- en weefselgroei werd bestudeerd met behulp van twee meerfase groeifuncties:

1. de Meerfase Groei Functie (MGF) beschrijft groei als een functie van leeftijd. De MGF is gebaseerd op een sommatie van een aantal logistische functies, die elk een te onderscheiden groeispurt (groeigolf) weergeven;
2. de Meerfase Allometrische Functie (MAF) beschrijft de groeirelatie tussen twee lichaamscomponenten. De MAF is een uitbreiding van het eenvoudige allometrische concept ($Y = \alpha X^{\beta}$). De MAF veronderstelt dat er gedurende de groei veranderingen kunnen optreden in de allometrische relatie tussen twee componenten (Hoofdstuk 4).

De resultaten van de *ad libitum* gevoerde controle-groep zijn gebruikt ter illustratie van het gebruik van beide meerfase functies in groeistudies bij kuikens (Hoofdstuk 4). Groeiveranderingen tijdens sexuele rijping (Hoofdstuk 5) en relatieve groei van chemische componenten (Hoofdstuk 6) en organen (Hoofdstuk 7) werd beschreven voor alle proefgroepen.

Lichaamsgroei van een opfokkuiken tot een leeftijd van 32 weken kon beschreven worden met een vierfase groeifunctie (Hoofdstuk 4). Groei in de eerste twee fasen bestond voornamelijk uit de ontwikkeling van organen ten behoeve van zogenaamde onderhoudsprocessen, zoals het skelet en enkele metabole organen (Hoofdstuk 7). De derde groeifase, rond een leeftijd van 19 weken, werd de 'puberteitsgroeispurt' genoemd: zo'n 40 to 70% van de totale groei binnen de puberteitsgroeispurt bestond uit groei van het reproduktie-apparaat (Hoofdstuk 5). De vierde fase bleek voornamelijk te bestaan uit buikvetaanzet.

De groeicurven van de chemische componenten droge stof, ruw eiwit, ruw vet en as, elk als een functie van de leeftijd, waren allen tweefasisch. De allometrische relaties tussen eiwit, vet en as, elk als een functie van droge stof, waren eveneens tweefasisch. De hypothese werd geformuleerd dat tijdens de eerste groeispurt (vroege opfokperiode) de aanzet van eiwit (spieren), vet (intramusculair vet) en as (skeletgroei) wel eens functioneel aan elkaar gerelateerd zouden kunnen zijn. Tijdens de late opfokperiode lijkt eiwitaanzet direct gerelateerd aan sexuele ontwikkeling, terwijl vetgroei op dat moment louter bestaat uit buikvetgroei (Hoofdstuk 4).

Groei rondom de puberteit werd bestudeerd in Hoofdstuk 5. Het doel was enig inzicht te krijgen in de mechanismen die de legrijpheid bepalen. Algemeen wordt aangenomen, dat een bepaald minimum lichaamsgewicht in combinatie met een 'specifieke' lichaamssamenstelling vereist is, alvorens de jonge hen haar eerste ei zal produceren.

Verschillen tussen RL- en RF-kuikens in moment van legrijpheid (5 dagen) bleken gerelateerd te zijn aan de verschillen in het moment waarop de puberteitsgroeispurt optrad.

Aannemende dat de puberteitsgroeispurt een goede indicator is voor de sexuele ontwikkeling, was de vraag naar de lichaamssamenstelling aan het begin van deze groeispurt, uiterst relevant. Vethoeveelheden in het kuikenlichaam varieerden sterk (112 - 179 g vet) ten gevolge van de toegepaste voerstrategie. Daarentegen varieerden eiwit en de totale vetvrije massa veel minder (187 - 201 g eiwit en 806 - 870 g vetvrije massa, respectievelijk). Er werd geconcludeerd dat een zekere hoeveelheid eiwit of vetvrij weefsel, als onderdeel van 'onderhouds'-organen, aangezet moet zijn voordat de sexuele ontwikkeling op gang komt (Hoofdstuk 5).

Op de vraag, of de ontwikkeling van bepaalde lichaamscomponenten ten gevolge van een deficiënte voeding nu *meer of minder* achterblijft bij dat van andere lichaamscomponenten, kan een antwoord gegeven worden met behulp van een relatieve groeistudie (Hoofdstukken 6 en 7).

Tweefase allometrische relaties werden berekend tussen elke chemische component en EBM (lichaamsgewicht zonder maagdarminhoud en veren). De allometrische groeicoëfficiënten van de eerste en tweede fase verschilden nogal van elkaar (Hoofdstuk 6). Het tweefase patroon werd echter voor een groot deel bepaald door het tweefasisch groeiverloop van vet als zodanig. De verschillen tussen beide groeicoëfficiënten werden duidelijk kleiner wanneer FFEBM (vetvrije EBM) in plaats van EBM werd gekozen als de x-variabele in de allometrische relatie. Gedurende de eerste allometrische groeifase (tot ± 350 g FFEBM) was de groeicoëfficiënt tussen elke vetvrije component en FFEBM ongeveer 1, ongeacht de voerstrategie. Dit duidde op een constante samenstelling van het vetvrije lichaam in die groeifase. Vanaf 350 g FFEBM groeide eiwit relatief iets sneller dan water en as, hetgeen duidt op een leeftijdseffect. Wederom waren er geen behandelingsverschillen.

De vetfractie van *ad libitum* gevoerde en die van lysinebeperkte kuikens groeide proportioneel met FFEBM tijdens de eerste groeifase. Het lijkt er dus op dat vetgroei op jonge leeftijd hoofdzakelijk functionele eigenschappen vertegenwoordigt en nauw gerelateerd is aan de groei van het vetvrije lichaam (Hoofdstukken 4 en 6). RL-kuikens beperkten zichzelf vrijwillig in de voeropname om daarmee een te hoge energieopname en daaraan gerelateerde vetaanzet te vermijden. Op latere leeftijd (vanaf zo'n 10 weken) is er depotvetvorming mogelijk en bestaat de vetaanzet voornamelijk uit buikvet.

Bij een laag voerniveau (RF-kuikens) daalde de vetaanzet sterk. Vet fungeert daarbij vooral als energievoorraad in het lichaam, waarbij de mate van aanzet en afbraak (mobilisatie) varieert met het voerniveau.

Er werd geconcludeerd dat waarschijnlijk de meeste effecten van een voerstrategie op orgaan- of weefselgroei (als percentage van het lichaamsgewicht of EBM), zoals deze in de literatuur beschreven zijn, voor het overgrote deel het gevolg zijn van een mobilisatie van vet als zodanig. In overeenstemming met de 'klassieke' publikaties van de "Cambridge School of Agriculture" (1930-1960), is het gebruik van een vetvrije maat (bv. FFEBM) als onafhankelijke (x-) variabele te verkiezen boven een vethoudende maat in relatieve groeistudies vanuit nutritioneel perspectief.

Het bovenstaande werd geïllustreerd aan de hand van de allometrische relaties tussen enkele organen en FFEBM (Hoofdstuk 7). Twee botmaten (loopbeen en tibia), het maagdarmkanaal, de spiermaag afzonderlijk, de lever, het hart en het ovarium werden uitgezet tegen FFEBM in een tweefase model. De allometrische groeicoëfficiënt van de eerste fase was gelijk voor alle behandelingen. Dit suggereert dat het groeivertragend effect op deze organen gelijk is aan dat van het FFEBM. Daar waar de tweede groeicoëfficiënt gelijk was aan nul, werd het bereiken van de volwassen status verondersteld. Een identiek breekpunt (overgang tussen twee fasen) voor de proefgroepen binnen één lichaamsstructuur, duidde erop dat de volwassen status afhankelijk is van een bepaalde hoeveelheid FFEBM. Het borstbeen (derde botmaat) evenwel, groeide sneller dan het FFEBM bij een vroege beperking (hoge groei-prioriteit). Er werden geen verschillen in volwassen gewicht binnen organen tussen proefgroepen waargenomen. Deze resultaten onderschrijven de theorie, dat een vertraagde orgaangroei, als gevolg van een voerbeperking, veelal direct gerelateerd is aan de vertraging van de FFEBM.

In de afsluitende Discussie worden deze bevindingen in een biologisch kader geplaatst. Enkele toepassingen van het "meerfase groeimodel" worden beschreven.

Samengevat hebben de resultaten, zoals beschreven in dit proefschrift, het volgende duidelijk aangetoond:

1. Het streefgewicht is van geringe betekenis als voorspeller van de eiproduktie. De methode van beperking en de fase waarin dit plaatsvindt zijn in dit verband duidelijk belangrijker.
2. De 'puberteitsgroeispurt' is zowel gerelateerd aan de ontwikkeling van de reproduktie-organen, als aan het moment van legrijpheid. Het bepalen van de 'puberteitsgroeispurt' in een koppel kan voor de legpluimveehouder een waardevol hulpmiddel zijn bij het nemen van een juiste beslissing met betrekking tot de te volgen voerstrategie.
3. Het op gang komen van biologische processen die leiden tot legrijpheid is sterk gerelateerd aan de ontwikkeling van het vetvrije kuikenlichaam.
4. Vetgroei tijdens de vroege jeugdfase van opfokkuikens is biologisch gerelateerd aan de groei van het vetvrije lichaam. Tijdens de groei van het vetweefsel in het kuiken worden twee fasen onderscheiden: de fase van functionele vetgroei en die van depotvetgroei.

   Kuikens op een lysinedeficiënt voer verlaagden de voeropname, om op deze manier extra vetaanzet te vermijden.
5. De samenstelling van het vetvrije kuikenlichaam werd niet beïnvloed door de voerstrategie, echter wel door de leeftijd van het kuiken.
6. Het gebruik van een vetvrije maat als onafhankelijke variabele (x) in een relatieve groeistudie met dieren op een verschillend voerniveau is te verkiezen boven een vethoudende maat, vanwege de verstorende invloed van het sterk fluctuerende depotvet. Het vetvrije lichaam is een betere maat voor de fysiologische leeftijd van het dier dan het lichaamsgewicht.

   Deze 'klassieke' gedachte van de Cambridge School of Agriculture, om groei van organen en weefsels uit te drukken ten opzichte van de vetvrije massa, is ten onrechte nauwelijks overgenomen door onderzoekers in de pluimveevoeding.
7. Een meerfase analyse van groei geeft inzicht in de biologische principes met betrekking tot de groei van jonge kuikens.

De meerfase analyse van groei is een onmisbaar hulpmiddel in onderzoek waarbij effecten van voerstrategieën op lichaamsontwikkeling en eiproduktie worden bestudeerd.

Laatst hoorde ik weer iemand zeggen
dat ze nog altijd eieren leggen.

(Uit: "Het allermooiste ei", een prentenboek
van Helme Heine met tekst van Willem Wilmink.)

Curriculum Vitae

René Peter Kwakkel werd geboren op 19 februari 1958 te Zaandam. Na de lagere school werden twee jaren MAVO doorlopen, waarna de overstap naar het VWO werd gemaakt. In 1978 behaalde hij zijn Atheneum-B diploma aan de Chr. Scholengemeenschap Jan Arentsz te Alkmaar. In datzelfde jaar begon hij met de studie Zoötechniek aan de toenmalige Landbouwhogeschool te Wageningen. De praktijktijd bracht hij door aan de Tierärtzliche Hochschule in Wenen in 1984. De studie werd in juni 1986 afgesloten met een verzwaard hoofdvak Dierfysiologie gecombineerd met Veevoeding, en als bijvak Vruchtbaarheid en Voortplanting. Aansluitend werd hij voor een periode van drie jaar als toegevoegd docent/onderzoeker bij de vakgroep Veevoeding aangesteld, met als belangrijkste taken het coördineren en uitvoeren van onderwijs en onderzoek op het terrein van de pluimveevoeding. Het onderzoek dat toen is gestart staat beschreven in dit proefschrift. Na een jaar verlenging werd in juni 1990 de tijdelijke aanstelling omgezet in een vast dienstverband. Een leerzame onderbreking van de dagelijkse werkzaamheden vormde het voorzitterschap van het Youth Programme Committee tijdens het 19e Wereld Pluimvee Congres (Amsterdam, 1992).