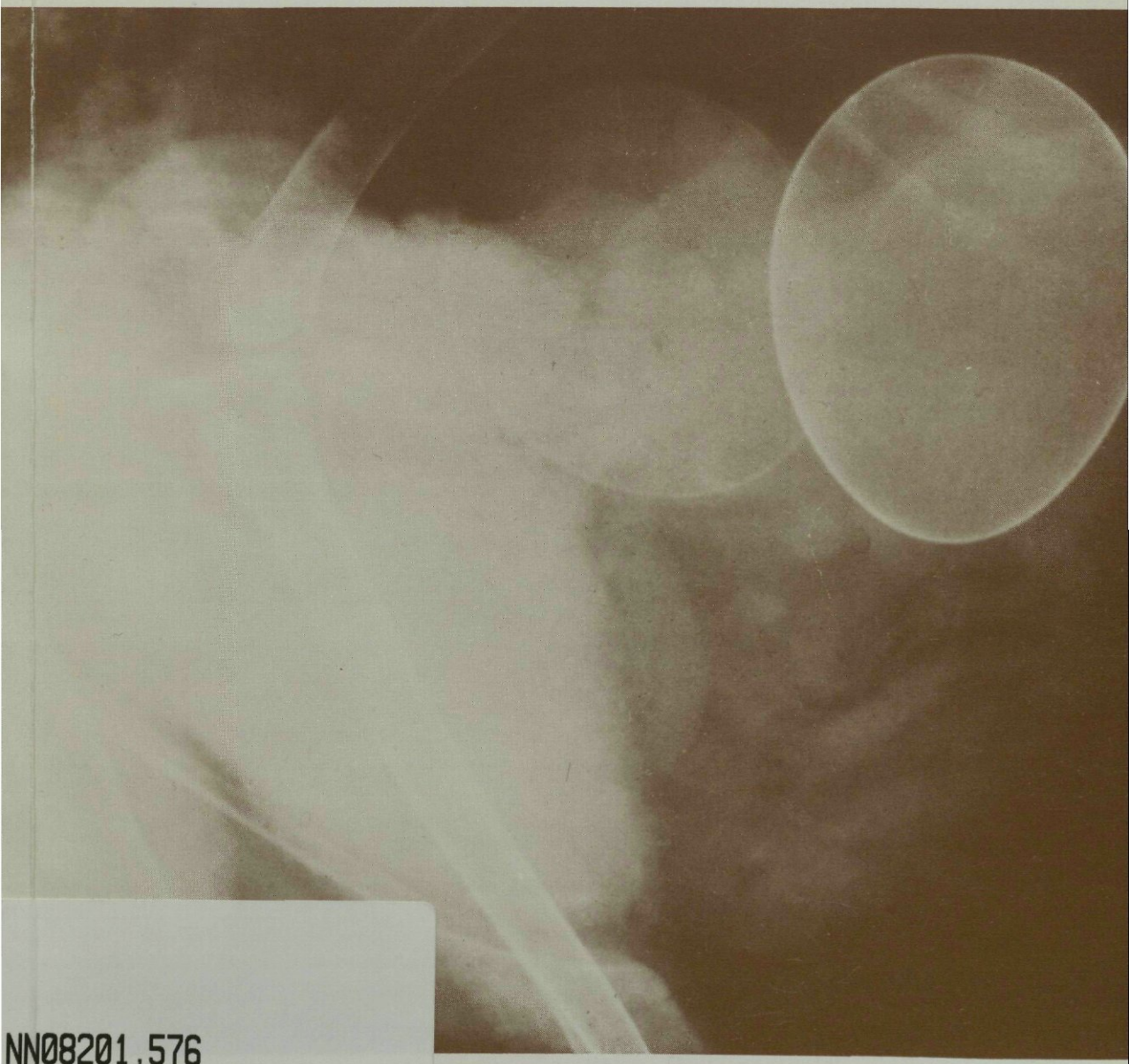
Physiological and genetical aspects of egg production in White Plymouth Rock pullets



J. H. van Middelkoop

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Physiological and genetical aspects of egg production in White Plymouth Rock pullets

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus, prof. dr. ir. H. A. Leniger,
hoogleraar in de technologie,
in het openbaar te verdedigen
op vrijdag 18 januari 1974 des namiddags te vier uur
in de aula van de Landbouwhogeschool te Wageningen

Centre for Agricultural Publishing and Documentation

Wageningen – 1973

Stellingen

1. Noch de aanwezigheid van een ei in het oviduct, noch het tijdstip van leggen is van invloed op de regulatie van een volgende ovulatie.

dit proefschrift

2. Vermindering van de dooierproduktie kan zowel leiden tot een verhoging als tot een verlaging van de broedeiproduktie.

dit proefschrift

3. De regulatie van de ontwikkeling van de follikels op het ovarium is bij vogels nog onvoldoende onderzocht.

dit proefschrift

4. Een langer verblijf van het ei in het oviduct bij een regiem van 14 uur licht en 13 uur donker kan slechts indirekt worden toegeschreven aan de invloed van het afwijkend schema.

O. Melek et al., 1973. Br. Poult. Sci. 14: 493–498

5. De aanbeveling van Carter om het optreden van kneuseieren langs genetische weg te verminderen, geldt evenzeer voor huisvesting op de grond als voor die in kooien.

T. C. Carter, 1971. Br. Poult. Sci. 12: 259–278

6. Bij het zoeken naar de oorzaak van beengebreken bij landbouwhuisdieren moet behalve aan de voeding ook aandacht worden besteed aan de erfelijke aanleg en de huisvesting.

7. De inspanning ten behoeve van het genetisch onderzoek dient afhankelijk te zijn van het belang van de problemen en niet van het aantal fokbedrijven.

8. Het is niet juist om octrooi te verlenen op de methode om normale kuikens te fokken van dwergmoederdieren en normale hanen.

Brevet d'Invention 1.576.407; 23 juni 1969, Frankrijk

9. De bestrijding van hart- en vaatziekten is geen argument om ongenuanceerd aan te dringen op een laag eiergebruik.

10. Naast de aandacht die al aan de voeding van het paard wordt besteed in de bekende paardengezondheidskalender, zou meer voorlichting van rijkswege gewenst zijn.

11. Een goede registratie van alle sportrassen kan voorkomen dat sommige rassen of typen ongemerkt verdwijnen.

12. De verkoop van een praktijk, zoals dit in de veterinaire en in de medische wereld gebruikelijk is, is niet in overeenstemming met het principe van de vrije artsenkeuze.

13. In dezelfde tijd, waarin men de natuur steeds meer wil beschermen, dreigt het kraaien van een haan het effect te krijgen van een semi-lethale faktor.

Proefschrift van J. H. van Middelkoop
Wageningen, 18 januari 1974

Abstract

Middelkoop, J. H. van (1973) Physiological and genetical aspects of egg production in White Plymouth Rock pullets. Doctoral thesis, Wageningen (available also as Commun. Spelderholt Inst. Poultry Res. 205), ISBN 90 220 0499 6. (xvi) + 76 p., 27 tables, 12 figs, Dutch and Eng. summaries.
Also: Agric. Res. Rep. (Versl. landbouwk. Onderz.) 813.

White Plymouth Rock pullets selected for a high 8–week bodyweight have an unsatisfactory production of hatching eggs, but this is not the only problem. In addition a great proportion of ovulated yolks are lost for the formation of normal eggs, because they are laid in abnormal eggs. In a study on the relationship between yolk production and egg formation, abnormal eggs were found to be laid when ovulation rate exceeded the limit of one ovulation per egg formation period.

Research on the genetic background showed that laying of double-yolked eggs, of two eggs a day, and of normal eggs only is genetically controlled to a large extent. Correlations were calculated between these laying traits and 8-week bodyweight, but those estimates did not provide a conclusive answer.

Economically, first eggs of a pair have also to be seen as abnormal, because they proved almost unhatchable.

With the help of the sex-linked dwarfing gene *dw*, it was shown that a reduction of yolk production in the ovary can result in an increase in normal egg laying in hens whose ovulation rate is too high. There seems therefore to be the relationship between total yolk production and the laying of abnormal or normal eggs.

Voorwoord

Dit proefschrift is het resultaat van een onderzoek op het Instituut voor Pluimveeonderzoek 'Het Spelderholt' te Beekbergen, waarvan ik de direktie en het bestuur wil danken voor de gelegenheid die zij mij hebben geboden om dit proefschrift voor te bereiden en uit te werken. De manier waarop dit proefschrift tot stand kwam, wordt het beste weergegeven met een uitspraak van koningin Juliana: 'Doen kunnen we alles, maar dan ook alleen allen samen.' De vele mensen, die mij met raad en daad hebben geholpen, zal ik niet alle met name noemen, omdat dat veel weg zou hebben van het overschrijven van de personeelslijst van ons instituut. Ik hoop overigens, dat mijn waardering voor alle verkregen hulp reeds in de dagelijkse omgang tot uiting is gekomen.

Pit, u heeft zich altijd volledig ingezet voor de betrouwbaarheid en volledigheid van de verzamelde gegevens. Dank zij de oplettendheid en de belangstelling voor het werk van u en van al degenen die u weer hebben geholpen, is het mogelijk geworden om dit resultaat te bereiken.

Waanders, ik wil u bedanken voor uw inbreng bij het verwerken van de gegevens. Behalve het geven van adviezen heeft u tesamen met vele anderen vrijwel alle berekeningen uitgevoerd.

Kuit, u heeft een belangrijke rol gespeeld bij het tot stand komen van dit proefschrift door er op aan te dringen hier aan te beginnen en door uw begeleiding tijdens het verloop van het onderzoek. Veel steun heb ik ook ondervonden van Simons, waar ik hem dan ook zeer dankbaar voor ben. Ook Van Tijen wil ik graag bedanken voor alle vrije tijd die hij voor mij heeft willen opofferen.

Professor Van Albada, het heeft mij verheugd dat u mij de gelegenheid heeft geboden om mijn studie op deze wijze te kunnen afronden, omdat u meer voor mij heeft betekend, dan alleen mijn promotor. Ook na de voltooiing van het proefschrift hoop ik nog vele discussies met u te mogen hebben.

Curriculum vitae

De auteur ontving in 1960 het einddiploma HBS-B aan het Christelijk Lyceum te Arnhem en begon in datzelfde jaar met zijn studie aan de Landbouwhogeschool te Wageningen. In 1967 behaalde hij het ingenieursdiploma, richting veeteelt. Omdat uitstel niet altijd afstel geeft, werd daarna de militaire dienstplicht vervuld bij de geneeskundige troepen. Na dat intermezzo is de auteur in december 1968 in dienst getreden bij het Instituut voor Pluimveeonderzoek 'Het Spelderholt' te Beekbergen.

Samenvatting

Fysiologische aspekten

Hoofdstuk 3

Bij zware moederdieren kan gedurende de gehele legperiode regelmatig worden waargenomen dat een hen twee eieren per dag legt. Bij een experimentele stam White Plymouth Rocks van ons instituut werd onderzocht wat de oorzaak hiervan is. In die gevallen dat een hen twee eieren per dag legt, zijn meestal beide eieren abnormaal; over een deel van het eerste ei is een extra laagje kalk afgezet, terwijl het tweede, dat een afgeplatte vorm heeft, gewoonlijk een onvolledig gevormde schaal bezit.

De hypothese was, dat het leggen van twee abnormale eieren per dag het gevolg is van een tijdelijk verblijf van die twee eieren tegelijk in de schaalklier. Om deze hypothese te bewijzen, werd bij deze eieren de tijd van leggen geregistreerd en de schaaldikte op verschillende plaatsen bepaald. Verder werden röntgenfoto's gemaakt van hennen die twee eieren tegelijk in de schaalklier hadden. Daarnaast werd op dergelijke hennen nog sektie verricht. De resultaten van dit onderzoek bevestigden de hypothese. Bovendien kon een verklaring gegeven worden voor het ontstaan van de afwijkingen. Als het tweede ei in de schaalklier komt, wordt het tegen het eerste ei, waarvan de schaal gewoonlijk al volledig is gevormd, aangedrukt en wordt misvormd doordat het onder dergelijke omstandigheden nog groter wordt door het opnemen van 'plumping'-vloeistof. Als gevolg van de ligging van die twee eieren kan dan op een deel van het tweede ei geen schaalmateriaal worden afgezet, maar komt dit terecht op het eerste ei.

Het voorkomen van twee eieren tegelijk in de schaalklier is mogelijk, doordat het leggen van het eerste ei om de een of andere reden een aantal uren wordt uitgesteld. Het tweede ei daarentegen wordt voortijdig uitgedreven of gewoon op de normale tijd gelegd. Het gevolg van een en ander is, dat beide eieren binnen 24 uur na elkaar gelegd worden; meestal zelfs binnen 20 uur. Hierdoor staat dit verschijnsel bekend als het 'leggen van twee eieren per dag' of als een 'eierpaar'.

Op basis van dit onderzoek kon worden gesteld, dat een tweede dooier van het ovarium vrijkomt, vóórdat het voorgaande als een normaal ei gelegd zou worden. Aangenomen mag worden dat het interval tussen beide betrokken ovulaties korter is dan de tijd die voor het oviduct nodig is om een normaal ei te vormen.

Hoofdstuk 4

Eieren, die op grond van de karakteristieke extra kalkafzetting gemakkelijk herkend kunnen worden als het eerste van een eierpaar, zijn niet geschikt als broedei. Het bleek namelijk dat van de bevruchte eieren niet meer dan 13–26 % uitkwamen. De invloed van

het langere verblijf in de eileider werd nagegaan door normale eieren gedurende 10–11 uur na het leggen op lichaamstemperatuur te houden en/of door in dezelfde tijd te voorkomen dat koolzuurgas uit het ei zou ontwijken. De broedresultaten van eieren die op een dergelijke manier waren behandeld, weken echter niet af van die van de onbehandelde eieren.

Vervolgens werd nagegaan of de slechte broedresultaten soms het gevolg konden zijn van een gestoorde gasuitwisseling via de eischaal. Hiertoe werden de poriën in de schaal van 'eerste' eieren geteld en werd de poreusheid gemeten. Het bleek dat de poreusheid van deze eieren minder was dan de helft van die van normale eieren afkomstig van dezelfde hennen. Deze lagere poreusheid moet toegeschreven worden aan het afsluiten van een groot aantal poriën door de extra kalkafzetting. Het effekt van het afsluiten van poriën in de schaal werd onderzocht door normale eieren geheel of voor de helft te bedekken met een laagje paraffine. Bij een deel gebeurde dit direkt na het leggen en bij een ander deel net voordat de eieren in de broedmachine werden geplaatst. De broedresultaten van de eieren waarvan het halve oppervlak bedekt was, kwamen overeen met die van echte 'eerste' eieren. Hierdoor werd bevestigd, dat het afsluiten van poriën in de schaal door de extra kalkafzetting bij 'eerste' eieren als de voornaamste oorzaak gezien kan worden voor de hoge embryonale sterfte tijdens het broeden.

Hoofdstuk 5

Het onderzoek naar het leggen van twee eieren per dag werd uitgebreid tot de oorzaak van het ontstaan van de meest belangrijke vormen van afwijkende eieren. Tijdens deze proef werd de eiproduktie ieder uur gecontroleerd, waarbij tevens werd genoteerd welke typen eieren er gelegd werden. Deze controle werd uitgevoerd gedurende een aaneengesloten periode van viereneenhalf etmaal. Aan de hand van de verkregen gegevens werd nagegaan of de normale eieren inderdaad alleen op dat deel van de dag werden gelegd als werd verwacht op grond van de theoretisch vastgestelde ovulatieperiode en de tijd die nodig is om een normaal ei te vormen. Op deze manier werd nagegaan of de theoretisch vastgestelde ovulatieperiode overeen kwam met de feiten.

Uitgaande van de aldus vastgestelde ovulatieperiode, werd hierna bepaald wanneer het leggen van een windei of van een zwakschalig ei verwacht kan worden op basis van de tijd die nodig is voor het bereiken van de verschillende stadia tijdens de eivorming. Door dit te vergelijken met de tijd dat dergelijke eieren in werkelijkheid gelegd werden, bleek dat het leggen van eieren met een onvolledige schaal het gevolg was van een voortijdig uitdrijven, alhoewel niet gezegd kan worden dat dit de enige oorzaak is. Nadat was vastgesteld hoe lang windeieren, zwakschalige en normale eieren in de eileider verbleven, werd aan de hand hiervan de tijd van ovuleren van de dooiers in de gelegde eieren verkregen uit de legtijden.

Op deze manier werd bepaald welke eieren afkomstig waren van verschillende ovulaties, die in dezelfde ovulatieperiode plaatsvonden en welke eieren verband hielden met ovulaties die in verschillende perioden plaatsvonden. Hierbij bleek dat een groot deel van de dooiers, die betrokken waren bij het leggen van abnormale eieren, afkomstig waren van verschillende ovulaties die in dezelfde periode plaatsvonden. Van een ander deel van deze dooiers kon worden gezegd dat de betrokken ovulaties inderdaad in verschillende perioden plaatsvonden, maar dat het interval toch nog korter was dan de tijd die nodig is

voor de vorming van een normaal ei. Normale eieren worden gelegd als de ovulaties minstens 21 uur uit elkaar liggen.

Hoewel het leggen van de meeste vormen van afwijkende eieren in verband gebracht kan worden met een relatief kort ovulatie-interval, lijkt het er steeds meer op dat bij het leggen van twee eieren per dag het oviduct eveneens een belangrijke rol speelt. Vermoed wordt namelijk, dat bij hennen die regelmatig twee eieren per dag leggen, het oviduct meer tijd nodig heeft voor de vorming van een normaal ei dan bij de hennen die alleen normale eieren produceren. Tot op heden kan de juiste duur van de eivorming niet worden vastgesteld, omdat het nog niet gelukt is om het tijdstip van de ovulaties bij een normaal leggende hen te meten. Hierdoor was het ook niet mogelijk om te bepalen of er verschillen bestaan tussen de diverse hennen voor wat betreft de duur van de eivorming.

Genetische aspekten

Hoofdstuk 6

In 1969 werd begonnen met het ontwikkelen van twee lijnen uit de experimentele stam White Plymouth Rocks door in het ene geval te selecteren op het leggen van dubbeldooiers en in het andere geval op het leggen van normale eieren. Naast de selectielijnen werd tevens een controlestam aangehouden. In 1970 werd een derde lijn geïntroduceerd, waarbij werd geselecteerd op het leggen van twee eieren per dag.

De eiproduktie werd voor iedere hen bepaald over een vaste periode van respectievelijk 80 en 200 dagen na het leggen van het eerste ei. Van de kenmerken waarop geselecteerd werd, werd de effectieve erfelijkheidsgraad geschat uit de resultaten van de selectielijnen. Om een nauwkeurige schatting te krijgen, werden de selectieresultaten eerst gecorrigeerd voor verschillen in jaarinvloed op basis van de gegevens van de controlestam. De erfelijkheidsgraad van de totale dooierproduktie en die van het leggen van normale eieren werd zowel geschat uit de dochter-moeder regressie, als uit de variantie-analyse. Daar enerzijds voor het leggen van dubbeldooiers en van twee eieren per dag in beide gevallen een hoge erfelijkheidsgraad werd gevonden en terwijl anderzijds de schattingen voor de erfelijkheidsgraad van de totale dooierproduktie niet duidelijk afweek van die van het leggen van normale eieren, mag worden aangenomen dat speciale genen een belangrijke rol spelen bij het leggen van genoemde vormen van afwijkende eieren.

Deze aanname wordt bevestigd door het feit, dat het mogelijk bleek om afzonderlijk te selecteren op het leggen van dubbeldooiers en op het leggen van twee eieren per dag.

Als er inderdaad belangrijke verschillen bestaan in de duur van de eivorming bij de verschillende hennen, dan zou dat kunnen inhouden dat selectie op het leggen van twee eieren per dag in feite neer komt op een wat trager werkend oviduct, terwijl selectie op normale eieren een gecorreleerd effekt zou hebben op een snelle eivorming. Op die manier zou dan verklaard kunnen worden waarom het mogelijk is om apart te selecteren op het leggen van twee eieren per dag en op het leggen van dubbeldooiers, terwijl zij beide in verband staan met een hoge dooierproduktie. Nader onderzoek zal moeten uitwijzen in hoeverre bovengenoemde veronderstelling juist is.

Tussen de kenmerken normale eiproduktie, dubbeldooiers, leggen van twee eieren per dag, totale dooierproduktie en het lichaamsgewicht op een leeftijd van acht weken werden de phenotypische (r_p) en de genotypische (r_g) correlatie-coëfficiënten berekend.

Het bleek dat zowel r_g als r_p tussen de totale dooierproduktie en de andere eiproduktie-kenmerken positief waren, waarbij de correlaties met het leggen van twee eieren per dag de laagste waarden hadden. Het berekende verband tussen het lichaamsgewicht op een leeftijd van acht weken met de onderscheiden eiproduktie-kenmerken laat geen duidelijk beeld zien, omdat zowel diverse negatieve als positieve waarden zijn gevonden. De verkregen selectie-resultaten tonen aan dat het mogelijk is om de normale eiproduktie te verhogen, zonder dat de groeisnelheid t.o.v. de controlestam direkt achteruit gaat. Het selecteren op het leggen van dubbeldooiers en op twee eieren per dag leidde in beide gevallen echter tot een hoger gewicht op acht weken. Het semi-lethale karakter van het leggen van twee eieren per dag is een reden te meer om te onderzoeken of er misschien een positieve correlatie bestaat tussen de duur van de eivorming en de groeisnelheid van het dier.

Hoofdstuk 7

De invloed van het geslachtsgebonden gen *dw* op de eiproduktie werd bestudeerd door de gegevens over aantal en type eieren van dwerghennen te vergelijken met die van hun normale zusters. Deze hennen werden verkregen door heterozygote *Dwdw* hanen te paren met hennen van de drie selectielijnen van broed 1971. Uit dit onderzoek kwam naar voren, dat de normale hennen eerder aan de leg kwamen en meer dooiers produceerden dan hun dwergzusters.

Behalve bij de hennen van de lijn, die geselecteerd was op het leggen van normale eieren, leidde de verminderde dooierproduktie bij de dwerghennen tot het leggen van meer normale eieren. Deze resultaten bevestigen de veronderstelling, dat het mogelijk is om bij hennen met een overmatige dooierproduktie door het infokken van de dwerg-faktor een groter aantal normale eieren te krijgen.

Contents

Glossary

1 Introduction 1

2 Foundation stock 2

Physiological aspects

3 Shell abnormalities due to the presence of two eggs in the shell gland 3
Archiv für Geflügelkunde 35:122–127 (1971)

4 Hatching problems of hard-shelled eggs with additional shell deposition produced by broiler mothers 13
Archiv für Geflügelkunde 36:63–70 (1972)

5 The relationship between ovulation interval of White Plymouth Rock pullets and the laying of abnormal eggs 25
Archiv für Geflügelkunde 36:223–230 (1972)

Genetical aspects

6 Selection trial on the laying of normal and abnormal eggs of White Plymouth Rock pullets and estimation of genetic parameters 37

7 Influence of the dwarfing gene on yolk production and its consequences for normal egg laying of White Plymouth Rock pullets 64
Archiv für Geflügelkunde 37: 192–196 (1973)

8 Summary of chapters 3–7 71

9 General discussion and conclusions 74

Glossary

A number of expressions are used in another meaning or in a more restricted meaning than normally. This glossary includes only a few terms necessary to avoid misunderstanding or ambiguity. It also includes some technical terms and abbreviations.

Compressed-sided egg Egg with flattened area thinner than the rest of the shell and usually surrounded by a ring of wrinkled shell. In other papers, this phenomenon is also termed 'truncated egg' (Romanoff & Romanoff, 1949) or 'slab-sided egg' (Nestor & Bacon, 1972).

Dwarfing gene Recessive sex-linked dwarfing gene.

Egg-pair See 'two eggs a day'.

'First' egg Hard-shelled egg with characteristic additional shell deposition as observed on the first of an egg-pair. In Chapter 5 (p. 32) the expression is used in another meaning.

Laying pattern (1) Number of different types of eggs laid by one hen in a fixed period. (2) Sequence in which the different types of eggs are laid.

Membraneous egg Egg being in a membraneous or membraneous-like stage, being still soft and in a flexible state.

Oviposition period Part of the day during which eggs of a specified type are generally laid. 'Laying period' is sometimes used in this sense.

Ovulation period Part of the day in which ovulations can occur.

Ovulation rate Speed of successions of ovulations.

Plumping Growing of the egg in the shell gland by uptake of secretion fluid through the shell membranes (Sturkie, 1965).

'Second' egg Egg laid as the second in an egg-pair. 'Second' egg is also used to indicate eggs recognized as such through a compressed side. In Chapter 5 (p. 32) the term is used in a broader sense.

Shell gland The term uterus is often used by other authors, but has been avoided to distinguish it from the mammalian uterus.

Soft-shelled egg Egg with a rigid but still incompletely formed shell.

True cuticle Outer organic layer of the eggshell (Simons, 1971).

Two eggs a day Two eggs laid by the same hen, of which the first laid is hard-shelled with a layer of additional shell deposition and is followed by another abnormal egg within about 20 hours. This concept does not include other cases when two eggs are laid on the same day, unless indicated otherwise.

Yolk formation See 'yolk production'.

Yolk production Number of follicles in the ovary in the rapid growing stage that reach maturity. Normally follicles in the ovary with a diameter greater than 0.3 cm are in the rapid growing stage.

dw See 'dwarfing gene'.
HDB Hen day basis.
ME Metabolizable energy.

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'Two roads diverged in a wood, and I–
I took the one less traveled by,
And that has made all the difference.'

(Robert Frost: The road not taken)

1 Introduction

When selecting for fast-growing broiler mothers one is continually faced with the problem of an unsatisfactory production of hatching eggs. Besides, since the research of Jaap and his coworkers (Jaap & Muir, 1968; Jaap & Clancy, 1968) it became clear that broiler mothers lay many more abnormal eggs than has so far been supposed. In other words these research workers have shown that it is not only the fact that those heavy birds lay at a moderate rate, but in addition that something is going wrong in the process of egg formation. It is quite possible that more insight into the cause of abnormal egg laying may provide a key to the improvement of the production of hatching eggs. For this reason, research was started in 1969 to study physiological and genetical aspects of the egg production of broiler mothers. The experiments were so arranged that the link between the different types of abnormal eggs was stressed, and the relation to the production of normal eggs was studied.

The importance of an improvement in the hatching egg production can easily be demonstrated. Compared with typical laying hens and with the number of yolks lost in abnormal egg production, it should be possible to increase the egg production by at least 10 %. This increase would reduce the cost price of a hatching egg by almost 10 %. For a total of 350 million eggs that are incubated yearly for broiler production in the Netherlands, this amounts to a saving of about seven million guilders. A higher hatching egg production is not only advantageous for a lower cost price but also for higher returns from feedstuffs for the broiler production, meaning for the Netherlands a saving of about 15 million kg of feed without reducing the production of broilers.

This thesis is based both on the author's unpublished work and on his research that has already been reported elsewhere. These publications are incorporated word for word in Chapter 3, 4, 5 and 7. To give a clear survey of my research so far, a general discussion together with the main conclusions is included.

References

Jaap, R. G. & J. A. Clancy, 1968. Reproductive idiosyncrasies of the broiler pullet. In: Proc. 3rd Europ. Poult. Conf.: 74–79.

Jaap, R. G. & F. V. Muir, 1968. Erratic oviposition and egg defects in broiler-type pullets. Poult. Sci. 47 : 417–423.

2 Foundation stock

In 1962 and again in 1963 pedigree hatching eggs of a commercial White Plymouth Rock strain were bought from a Dutch breeder. After hatching, rearing and breeding, the flock consisted of 680 pullets and 162 cocks in 1964, originated from 13 sires. Every year from 1964 to 1969, a new generation was bred with mild selection for a higher 8–week bodyweight. The cocks used for every replacement descended from at least 10 different sires, which were neither full nor half sibs. The degree of inbreeding was restricted to 1.0–1.5 % or less per generation.

The best way to characterize the strain used for this research is by giving a summary of their mean performance before the trial.

Character	Hatch				
	1964	1965	1966	1967	1968
8-week bodyweight					
♂♂	1000	1120	1220	1170	1220
♀♀	900	990	1070	1040	1060
Total number of eggs (HDB) to 64 weeks	139	122	128	145	162

From this table it can be seen that we are dealing with a normal and current strain of White Plymouth Rocks.

3 Shell abnormalities due to the presence of two eggs in the shell gland

Already published in Archiv für Geflügelkunde 35:122–127 (1971)

Introduction

The mothers of broiler chicks not only lay fewer eggs than layers but also a higher percentage of abnormal eggs. Jaap & Muir (1968) found that during the first six months of the laying period broiler-type pullets lay more abnormal eggs than egg-type pullets. They found that membranous or soft-shelled eggs varied from 1.5 % to 5.9 % in the broiler-type as compared with 0.7 % to 1.9 % in the egg-type pullets in different populations reared and housed under similar conditions. Double-yolked eggs were laid twice as frequently during this period by the hens producing broiler chicks. We recently observed in our own experiments that these heavy birds also laid more eggs with yolk material on a part of the outer surface than did layers during the same period.

Mothers of broiler chicks may lay two eggs a day. Two eggs laid on the same day accounted for 0.8 % to 6.2 % of yolks from broiler-type as compared with 0.1 % to 2.0 % from egg-type pullets in the different strains (Jaap & Muir, 1968). Some of these eggs were membranous. The shell eggs of these pullets recorded as two ovipositions on one day have peculiar abnormal shells (van Middelkoop & Simons, 1970; Foster, 1970b). The fact that two eggs are laid by the same bird in close succession does not necessarily imply that they are recorded as two eggs a day. This depends on (1) the time of laying, and (2) the times of collection. Thus it is also possible to find these typical abnormal shell eggs on two succeeding days. For clearness of understanding it should therefore be noted that in this paper 'two eggs a day' and 'two eggs laid within 24 hours' means two eggs with the peculiar abnormalities observed in shell eggs laid by the same bird in close succession.

Literature

The laying of two shell eggs a day by the same bird has already been described by Drew (1907) and Curtis (1914). It should be noted that the authors did not mention shell abnormalities and that the eggs were laid on the trap-nest. Other workers who later described the phenomenon of the laying of two eggs per day were especially struck by the fact that the second egg was misshapen (Scott, 1940; Grau & Kamei, 1949; Weiss & Sturkie, 1952; Foster, 1970a). The abnormality of the first egg laid is not as evident as that of the second, as shown by the fact that an egg with the characteristic symptoms of the second egg was described by Klein as early as 1750. Grau & Kamei (1949) noted that the first egg had thick 'sandy' shells, and a thicker shell was also found by Foster (1970a, b). Eggs with a rough sandpaper-like shell to the touch were also reported by Jaap & Muir (1968), but they failed to state whether this concerned the first or second egg. Scott (1940) noted that a membranous egg with a flattened area is caused by the contact with another egg in the oviduct.

So far as we know, the relationship between the shell abnormality of the first and second egg has not yet been clearly defined. Several workers believe the abnormalities to be caused by the contact of two eggs in the oviduct. In explanation of the malformation of the second shell egg they emphasize the distortion of the shell membranes of the second due to unusual pressure before a hard shell is obtained (Scott, 1940; Weiss & Sturkie, 1952; Foster, 1970a). Grau & Kamei (1949) only note that the malformed egg 'evidently had entered the uterus while the first egg was still present'. Foster's hypothesis (1970b) about the correlation between the laying of two shell eggs a day and the peculiar abnormalities met with in these eggs tallies with our own hypothesis on the subject. In that hypothesis is stated that the first egg is held in the shell gland past its normal time of oviposition. The next egg to pass down the oviduct arrives at the shell gland and presses against the retained egg. As the second egg is in the unplumped state this pressure causes the characteristic malformation.

Materials and methods

White Plymouth Rock pullets of a pure strain hatched in 1969 were investigated. This generation consisted of three batches hatched on 21st January and 4th and 18th February. The chickens were sexed when one day old; males and females were reared separately. Up to the age of 18 weeks the birds were kept on the floor in natural daylight. At this age the hens were housed in separate 30 × 46 cm laying cages, the flock consisting of 624 birds. By means of artificial light the birds were given a minimum 14-hour day from 5 a.m. to 7 p.m. The adult hens were always fed ad libitum a ration of 2540 kcal ME/kg, 14.1 % crude protein, 2.55 % Ca and 0.5 % P. Their mean bodyweight was 1020 g at eight weeks and 2662 g at 18 weeks. The age at the first egg was 151 days. During the first 80 days of a hen's lay the average production was 48 eggs, 41 of which had hard shells and a normal shape.

Shell thickness was measured at different points of all shell eggs noted as having been laid at the rate of two eggs a day during the last fortnight of August. Neither of these paired eggs showed the normal pattern of shell thickness. The shell thickness of 33 pairs of shell eggs was measured with a micrometer in the parts equidistant from the waist.

On 25th September ten birds which had regularly laid two eggs in a 24-hour period were transferred to batteries enabling the time of laying to be automatically recorded. The eggs were recorded as they rolled down and passed a threshold after having been laid. Although not entirely accurate, this time was taken as the time of oviposition. In 41 cases it was possible to measure the thickness of the thin and thick points of the shell of both eggs.

Results and discussion

The first of a pair shell eggs had a hard shell with additional rough shell calcification (Fig. 1), sometimes over the entire surface of the egg but usually in the form of a band over a part of it. This band is more or less longitudinal. The true cuticle (outer organic layer of the egg) is present under the rough calcified layer, as shown in the photograph (Fig. 2). The normal situation has been described by Simons (1971). The presence of the true cuticle under the extra shell deposition means that the first egg was fully formed. It

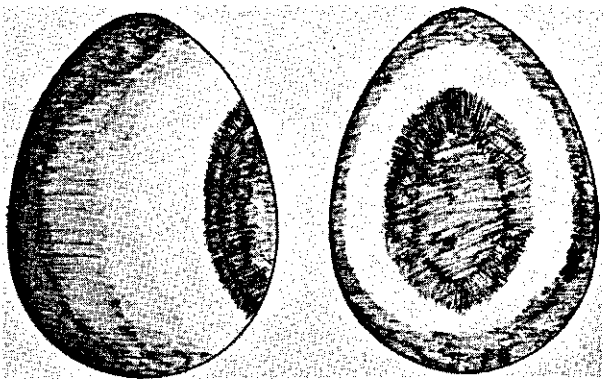

Fig. 1. Diagrammatic representation of the additional calcification shown as a light band over the surface of the egg.

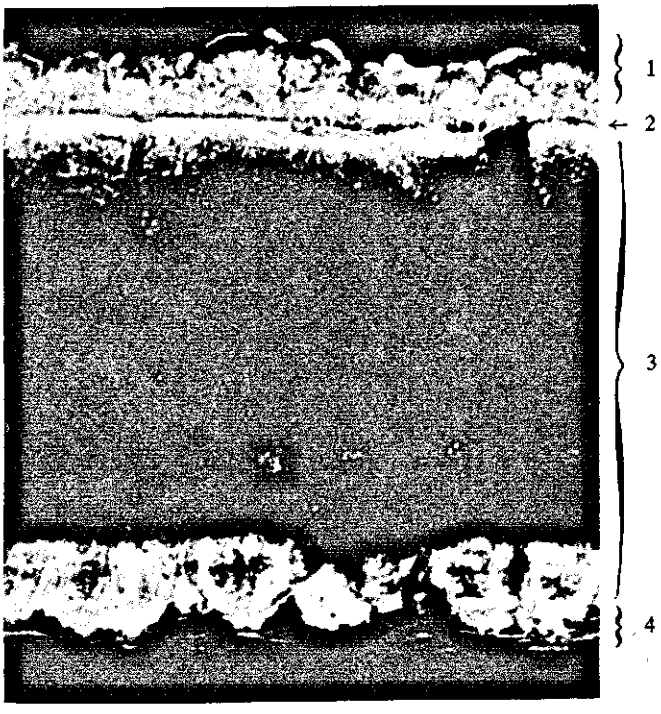

Fig. 2. Radical section of the shell of the first egg of a pair at a point with additional shell deposition. 1 = extra shell deposition, 2 = true cuticle, 3 = 'normal' shell, 4 = shell membranes.

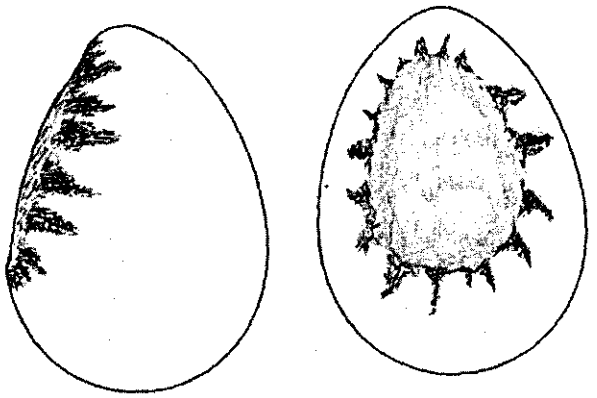

Fig. 3. Diagrammatic representation of an egg with a compressed-sided shell.

also means that the calcification had been interrupted for at least sufficient time for the true cuticle to be deposited. Since the local extra shell deposition on brown eggs is white, this would also imply that shell formation stopped and then restarted.

The second egg is often more or less soft-shelled. It usually has a flattened area (Fig. 3) which is thinner than the rest of the shell and surrounded by a ring of wrinkled shell. The flattened area is closer to the pointed than to the blunt end. This phenomenon will hereafter be termed 'compressed-sided shell', not 'truncated egg' – the term employed by Romanoff & Romanoff (1949) – because our own expression designates both the shape of the egg and the cause of the malformation.

In August 1969 the shell thickness of 33 pairs of eggs and in October 1969 the shell thickness of 41 pairs of eggs were measured at the thicker and thinner parts of the shell at the same distance from the waist. As shown in the table, the first egg differed by about 0.04–0.05 mm in thickness between the places where the shell appeared to be normal and the places with extra shell deposition. The same result had been obtained from measurements of the shell thickness of the second egg at places where the shell had a normal shape and where it had been compressed, the shell being thinner at those places (see table).

The thickness of the additional calcified layer corresponds to the difference in thickness of the compressed-sided shell and the rest of the shell of the second egg. The sign test ($P > 0.10$) did not show that the slight difference between the thickness of the extra shell deposition on the first egg and the extra thinness of the compressed part of the shell of the second was significant.

It is noticeable that the difference in shell thickness between the thicker and thinner places of the first and second egg is limited to a certain value. A century ago von Nathusius (1869) found in his measurements of shell thickness of an egg with a

Laid in	Number of pairs	Mean difference in thickness and standard deviation (mm)	
		first egg	second egg
August	33	0.043 ± 0.018	0.042 ± 0.018
October	41	0.044 ± 0.019	0.048 ± 0.023

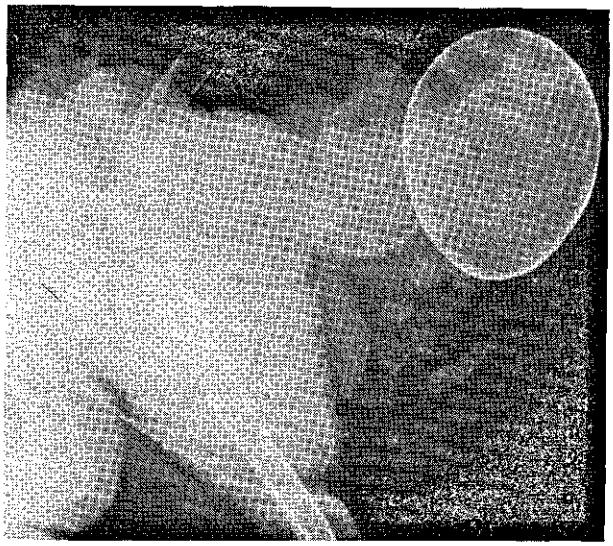

Fig. 4. Radiograph taken at 11.30 p.m. showing two eggs together in the oviduct.

compressed-sided shell a difference of 0.05 mm between the thicker and thinner parts of the shell, which was of the same order of magnitude.

It was observed that hens which often lay two eggs a day show this habit fairly regularly. It usually appears at the beginning of a clutch. With the help of the automatic recorder it was shown that the times of oviposition of the first egg were usually the beginning of the night until past midnight. On an average the second shell egg is laid 15 hours later but with a wide range, as shown by the standard deviation of about five and a half hours (n = 41).

The regularity with which this phenomenon occurred enabled us to predict with some certainty when certain hens would lay two eggs a day. Hens expected to lay two eggs in brief succession were selected at certain times. Ten to fifteen hours before the bird was expected to lay the first egg, radiographs of the hen were taken and this was repeated at regular intervals. Of one bird these radiographs were taken from some time before the first egg was expected to be laid until after the oviposition of the second. They confirmed our assumption that the additional calcification of the first egg and the compressed egg shell of the second are caused by the two eggs lying close to each other in the shell gland. Fig. 4 shows these eggs lying with their longitudinal axis more or less at right-angles to the direction of movement through the oviduct. The eggs would not be in this position if the first egg was in the shell gland and the second still in the isthmus. After the first egg had been laid radiographs showed that the second occupied the normal position of an egg in the oviduct.

According to Jaap & Muir (1968) it is highly improbable for two eggs to be in the shell gland at the same time. Our post-mortem examination of these pullets of which X-rays had shown two eggs situated near each other in the shell gland revealed that a part of the first egg was in the vagina in a more or less longitudinal direction (Fig. 5). Previously we had already assumed this to be due to the distribution of the extra calcification of the first egg. The part of the egg in the shell gland obtained additional calcification with the

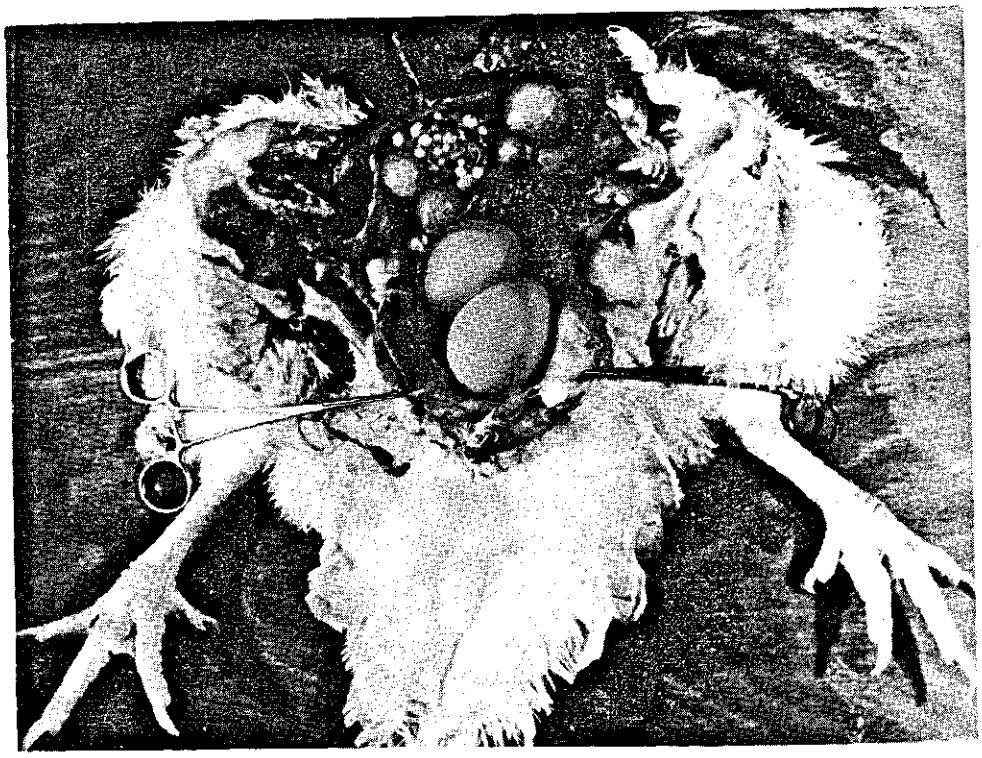

Fig. 5. Photograph of two eggs near each other in the shell gland, revealed by post-mortem examination.

exception of the part lying against the second egg and the part in the vagina. The second egg is pushed against the first egg, resulting in a compressed-sided shell nearer the pointed than the blunt end. The first eggs sometimes have an additional calcification over the entire surface, which is probably due to the first egg turning during this stage. As may be expected this occurs more easily when the eggs are smaller, especially when they are so small that the shell gland contains the two whole eggs.

Occasionally the second egg had a flattened area at the pointed end instead of near of it. These eggs are often abnormally rounded on this side of the egg. This can be explained by assuming that in this case the pointed end of the second egg pushed against the first egg while in the shell gland. While the second egg stays in the shell gland and the first one is expelled, the latter will be laid or may remain in the vagina. Jaap & Muir (1968) cite the case of a membraneous egg in the shell gland and a normal egg in the vagina of the same hen.

It has been observed that sometimes the second egg occasionally has both a compressed-sided shell and the symptoms of a body checked egg as had been described by Romanoff & Romanoff (1949), however, the authors were unaware of the cause. The origin of the body checked eggs we found, can be explained by the bumping against each other of the two eggs in the shell gland. The fact that usually it is only the second egg which is affected in this way is in agreement with the ratio of the shell thickness of the two

eggs at this time. In addition the second had the disadvantage of a compressed-sided shape.

In some instances we also found that the second egg had both an abnormal shape and partly extra calcification on the surface. In these cases the third egg showed the characteristic symptoms of a second. The extra calcification besides its abnormal shape of the second egg might be explained by assuming that the third egg approaches the second in the shell gland after the first has been laid.

The so-called first egg stays far longer in the shell gland than is normal. Although at present we cannot state exactly how long it remains in the oviduct, and in the shell gland in particular, we are convinced of this longer stay for the following reasons:

a. the time of laying the first egg in relation to a subsequent oviposition within 24 hours, when the latter was a shell egg it had been laid on a 'normal' time;

b. the true cuticle is beneath the additional calcification of the first egg, while the rest of the shell seems to be of normal thickness (0.35 mm; n = 41);

c. birds were observed to go to the trap-nest at the normal time of laying but without result. During the following evening or night a first egg was laid on the floor. According to Wood-Gush (1963) this means that the first egg had been in the oviduct far longer than normal.

After the first egg has been laid the second tries to conform its compressed part to the original egg shape while in the shell gland. The following explanation may be preferred for the fact that the egg will not be of normal shape throughout. The second egg arrives in the shell gland in a membranous and unplumped state. Since the first hard-shelled egg is still present in the shell gland, it presses on the second. Under such abnormal conditions the membranous egg grows by plumping. It is suggested that the fibres of the shell membranes are pulled apart during the plumping process (Simons, 1971). As for the impressed part, it is conceivable that the fibres are less pulled apart during plumping. It is also possible that the transition from convex into concave at the outline of the point of contact causes an abnormal pulling apart of the fibres. This would explain the flattened area in a membranous egg. After the first egg has disappeared from the shell gland the second tends to conform as far as possible to the normal egg shape. This tendency will probably be hindered by the suggested distortion of the normal configuration of the shell membranes at the compressed part during plumping. More rapid shell formation has then already begun so that this part of the membranes is covered by a hard shell more rapidly than the rest of the egg. By this time plumping may also have diminished. The degree of malformation of the second shell egg is probably affected by the rate of shell formation with respect to the enlargement of the egg due to further water intake.

The characteristic extra calcification over a part of the egg, found in the first of a pair of shell eggs, also occurred in pairs of eggs when the second was more or less membranous. The latter showed not only a flattened area (Scott, 1940; Foster, 1970b) but also had still less calcification on the compressed side than on the rest of the egg. In these cases time of laying of the first egg was as much delayed as when the second had a hard shell. Foster (1970b) made the same observation. This is readily understandable, since the second egg will only acquire a hard shell if it is not laid soon after the first as these eggs did, but is retained in the shell gland. All this supports the hypothesis that the malformation of the second egg in the shell gland is due to a disproportionate pulling apart of the fibres of the shell membranes during plumping while the egg is compressed by the retained hard-shelled egg.

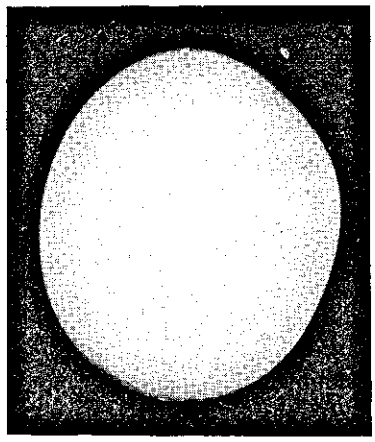

Fig. 6. Photograph of an egg with a characteristic bulge.

Especially at the beginning of the laying period, when the eggs are small, a further type of misshapen shell egg was observed. Both eggs had a bulge near the waist with a diameter of 1–2 cm (Fig. 6). The shell of this bulge was thinner than in the rest of the egg. It is presumed that these eggs arrived shortly after each other in the shell gland so that both were still membranous and unplumped as they pushed against each other. It is therefore assumed that both eggs are compressed to the same extent. As these eggs are fairly small they can grow by plumping while still in the shell gland. Since no shell is deposited on the points of contact, the shell membranes can here continue to stretch. The continued moisture intake of the egg causes a bulge at this point. As a result of the stretching of the membranes, the point of contact between the two eggs is getting smaller and still more surface can be covered with shell. This would explain the formation of two shell eggs with a bulge. This type of egg was usually laid in pairs or in quick succession.

To obtain some idea of the extent to which abnormal eggs were laid, the occurrence of several categories of abnormalities were calculated in our strain from the third to the sixth month of lay. During this period 7.2 % of the total egg production of these heavy hens was, recorded as membranous or soft-shelled eggs. About half these eggs were not laid at the rate of one egg per day per bird. Hard-shelled eggs included, 8.3 % of the total egg production was laid with another egg on one day by one bird. Assuming one shell egg to be the normal rate of laying, 13 % of all eggs were abnormal. Viewed in this light hens producing chicks for broiler production lay a far greater number of abnormal eggs than they would if the laying of two eggs a day were not taken into account.

Conclusions

Broiler-type pullets lay many abnormal eggs. With a few exceptions they can be classified as follows:

a. double-yolked eggs;
b. membranous eggs of normal shape;
c. soft-shelled eggs of normal shape;
d. membranous eggs with a flattened area;
e. more or less soft-shelled eggs with a flattened area;

f. shell eggs which have partly an additional calcification;
g. more or less soft-shelled eggs with a bulge;
h. eggs of normal shape with yolk material on the outside.

Abnormalities d, e, f and g are due to the presence of two eggs near each other in the shell gland. The most common pattern of two shell eggs laid on one day by the same bird is an egg of type f followed by an egg of type e. The interval between the two ovipositions was estimated to be 15 ± 5.5 hours.

When two eggs are together in the shell gland they usually exhibit the same pattern. They lie with their longitudinal axis more or less at right-angles to the direction of movement through the oviduct. The second egg is misshapen soon after it arrives in the shell gland where it is compressed by the first. The first egg, of which the shell is usually completely formed, is very often partly pushed into the vagina. Owing to the position of these two eggs part of the second egg is deprived of calcification during this period. The first egg shows an additional shell calcification, sometimes on the whole surface of the egg but usually as a band over a part of it. This band covers the egg in a more or less longitudinal direction. When the egg in the shell gland is still membranous when approached by the second, it is assumed that both eggs will acquire a bulge.

Summary

The phenomenon of the laying of two eggs a day was studied in a White Plymouth Rock experimental strain. The laying of two abnormal shell eggs a day can be attributed to the presence of two eggs in the shell gland. The time of oviposition was noted and the shell thickness measured at different points of eggs laid by the same hen within 24 hours. Radiographs were taken of hens with two eggs together in the shell gland. A post-mortem examination was also performed on a bird with two eggs in the shell gland. The cause of the formation of these abnormal shells was investigated.

Acknowledgement

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4 Hatching problems of hard-shelled eggs with additional shell deposition produced by broiler mothers

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Introduction

Broiler mothers produce more abnormal eggs than might be expected. This phenomenon is associated with the fact, that the birds have hitherto been kept on the floor with a view to fertilization. With the traditional method of housing most of the abnormal eggs are lost in the litter or dropping pit as a result of the nesting behaviour (Wood-Gush, 1963). In most cases all the abnormal eggs will only be collected when the hens are placed in cages. This is why such an important abnormality as the laying of two shell eggs per day has so far attracted little attention. Pairs of shell eggs are recognizable by their characteristic appearance as well as the time of laying (van Middelkoop & Simons, 1970; Foster, 1970) owing to the temporary simultaneous stay of the two eggs in the shell gland (van Middelkoop, 1971). The first egg of a pair, which is apparently the more normal one may be followed either by a membranous or a shell egg.

During the hatching of the new generation of our Institute's experimental strains of White Plymouth Rocks it was noted that hardly any chicks were hatched from the first eggs of a pair. Since the shell eggs with the characteristics of pairs of eggs may amount to some per cents of the total production of broiler strains, it is important to know to what extent these eggs are suitable as hatching eggs. For instance in 1970 the control strain of White Plymouth Rocks at our Institute produced an average of six hard-shelled eggs per bird identified as first eggs of a pair throughout the 10-month laying period. The following experiments were used to check whether these eggs were fertilized, and when this was the case, to identify the factors influencing the embryonic mortality in these eggs.

Experiment 1: Hatching results

Materials and methods

Of the experimental strain of White Plymouth Rocks 62 hens were selected which were noted for laying many pairs of shell eggs. These hens were kept in individual cages and fertilized by artificial insemination. The eggs were collected daily, stored at 15 °C and placed in the incubator once a week. Before doing this, their external appearance was visually examined and they were divided into the following four categories:

EC = a hard-shelled egg on which the characteristic local Extra Calcification, as found on the first egg of a pair, is clearly visible.

EC? = a hard-shelled egg on which the extra shell deposition could be established by means of a magnifying glass.

EC?? = a hard-shelled egg on which despite the use of a magnifying glass the presence of

the characteristic calcified layer is still doubtful.

N = an apparently normal hard-shelled egg.

Eggs with a compressed-sided shell, i.e. those laid as the second of a pair are not considered in this experiment.

The eggs were incubated at 37.4–37.8 °C and a relative humidity of 52–54 %. During the last three days of incubation the relative humidity was raised to 75–80 %. The eggs were candled on the 7th and 18th day of incubation. All eggs culled by candling were opened to see whether they were fertilized; it was established from the dead embryos on which day they had died. The criterion for fertilization was the diameter of the blastodisc (Kaltofen, 1961). When this diameter was 10 mm or less, the egg was considered to be unfertilized. The Hamburger & Hamilton standard (1951), was used for determining the day on which the embryo died. Although this standard relates to specific laying birds, the error due to this cause was considered acceptable because the establishment of the day of death must be regarded as an approximation. What is important is that the same standard was used throughout. After 21 days of hatching the chicks were removed from the incubator, the chicks in the pipped eggs were considered to have died on the 21st hatching day.

Results and discussion

For this experiment a total of 617 hard-shelled eggs were placed in the incubator, 554 of which proved to be fertilized. The distribution of this number of eggs over the different classes and the hatching results per group are shown in Table 1.

Of the eggs which were certainly laid as the first of a pair, it may be expected with 95 % confidence that 88–98 % of the embryos will die during incubation. Even when the EC? eggs are included, the chance of dying is still 74–87 %.

The percentage of EC eggs classified as unfertilized was significantly higher than the normal (N) eggs ($P < 0.01$). The author knows no reason why these eggs should be more difficult to fertilize. It might be due to an error in the examination of the embryos which died at a very early stage. If this is the case, it would mean that the chance of hatching is in fact lower than indicated here.

The distribution of embryonic death over the different hatching days for the EC and the normal eggs is shown in Fig. 1.

Table 1. Hatching results of different types of hard-shelled eggs.

Class	Incubated eggs number	Fertilized		Hatched	
		number	percentage	number	percentage of fertilized eggs
EC	106	85	80.2	6	7.1**
EC?	57	50	87.7	20	40.0**
EC??	111	106	95.5	68	64.2**
N	343	313	91.3	256	81.8

** Different from N at $P < 0.01$ level (χ^2-test).

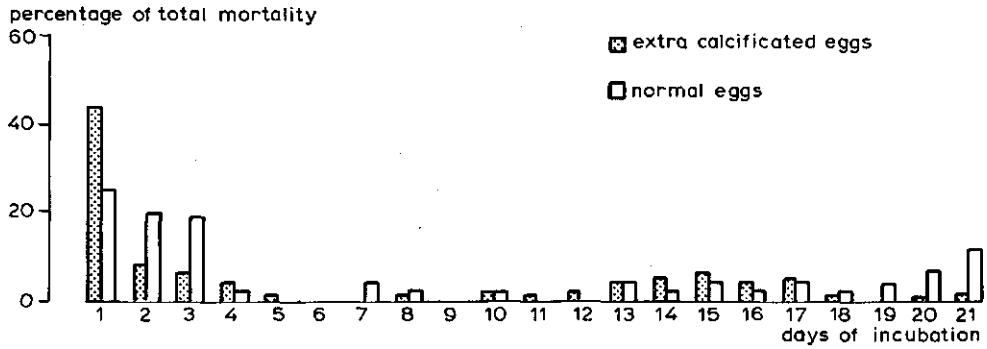

Fig. 1. Distribution of embryonic death.

Although it seems that relatively more embryos in EC eggs die at the beginning of the hatching period than in the normal eggs, this could not be proved statistically ($P > 0.1$). Since the distribution of embryonic death over the hatching period gives no clear indication of the cause of the high percentage of mortality, two factors in which the EC eggs are distinguishable from normal ones (longer stay in the shell gland and extra shell deposition) will be considered separately.

Experiment 2: Longer stay in the shell gland

The first egg of a pair of shell eggs remains in the oviduct far longer than usual (van Middelkoop, 1971). It has since been found by regular palpation of the oviduct through the colon in seven hens, that the first egg is kept in the shell gland and vagina for at least 30 hours, although large differences were found between individual animals. Compared with the 20–22 hours found in normal cases, i.e. White Leghorns (Warren & Scott, 1935), the first shell-egg remains an estimated 8–10 hours longer than usual in this part of the oviduct. This estimate is in agreement with the calculation based on the difference in time interval between two succeeding normal eggs in a clutch and between the members of a pair of hard-shelled eggs. As far as the germ development is concerned, this means (1) that during this period the egg is preincubated at a higher temperature than usual, (2) that the embryonic development is interrupted at a stage which differs from the usual one, and (3) that this development takes place while the carbon dioxide tension in the egg remains the same. The question is how far these factors have an adverse effect on the development of the embryo.

Literature

When the egg is laid the blastodisc has normally reached a diameter of about 3.5 mm. Usually the process of gastrulation has then started which means that the separation of epi- and hypoblast has begun. The exact stage of the blastoderm during oviposition may vary somewhat, but in any case it has not proceeded beyond halfway the formation of the entoblast (Hamilton, 1952). Research workers have long been seeking a correlation between stage of development at oviposition and hatching results. In eggs laid in the

afternoon, Hutt & Pilkey (1930) found about twice as much mortality during incubation as in those laid in the morning. They attempted to explain this by assuming that the germs in the 'morning' eggs were more developed.

Later on Hays & Nicolaides (1934) came to the conclusion that in eggs of hens with good hatching results, the germs were developed beyond the pre- and early gastrula stage. Wellplanned experiments by Taylor & Gunns (1935 and 1939) showed that the stage of development of the germ is characteristic of the animal and that the germ in the first egg of a clutch had a significantly greater size than in the other eggs of the same clutch (1935). However no correlation could be demonstrated between the stage of development and hatching results. The fact that Mérat & Lacassagne (1961) were unable to demonstrate a correlation between the place of an egg in a clutch and the hatching results can be seen as an indirect confirmation of Taylor & Gunns' results. Although within the range to which germ development is restricted no correlation appears to exist in normal eggs between the stage of this development at the time of oviposition and the succeeding hatching results, this need not be true of the first eggs of a pair, which as we found remain in the oviduct about 8–10 hours longer than is usual. Since the primitive streak begins to form after 7–9 hours incubation (Spratt, 1942), the germ may be expected to have reached this development stage in the 'first egg'.

Provided the eggs do not cool down too abruptly (Kaestner, 1895) the embryonic development can be interrupted without harm for 5–7 days after 6–18 hours of incubation. This period decreases with decreasing storage temperature. According to Kaestner a horizontal position of the eggs during storage stimulates the origin of an aberrant germ development after resumption of the incubation. Alsop (1919) reported on the influence of the incubation at body temperature of the hen on the course of the germ development. She found that 20 of the 25 embryos incubated during 22–24 hours at 105–107.5 °F showed aberrations. It has been found that a carbon dioxide content of 4 % in the air surrounding the eggs during the first four days of incubation is not usually detrimental to the hatching results (Taylor et al., 1956). It is estimated that during this period the pH of the albumen does not drop below 8 (Sadler et al., 1954). The value of 7.4 found in fresh eggs (Sauveur & Mongin, 1970) is nowhere near reached.

Thus the literature gives no decisive answer about the influence of longer stay in the oviduct on the succeeding hatching results. With the help of the following experimental design two factors which might have a detrimental effect on the embryonic development were further investigated, viz. remaining at body temperature for a longer period and the impossibility of carbon dioxide escaping, as a result of which the pH of the white remains unchanged.

Material and methods

The experiment was divided in three treatment groups and a control. The treatments consisted of

a. eggs kept at body temperature for 10½ hours directly after lay (T);
b. prevention of the escape of carbon dioxide for 10½ hours at a normal storage temperature of 15 °C (G);
c. eggs kept at body temperature for 10½ hours directly after lay and during the same time prevention of the escape of carbon dioxide (TG).

To prevent the temperature from rising too much in the T and TG groups a temperature of 41 °C was aimed at instead of 41.3 °C as given by Sturkie (1965). The escape of carbon dioxide was prevented by storing the eggs at a given carbon dioxide pressure of the ambient air. It was assumed that when there was no change in the pH no carbon dioxide escaped. This criterion was used because the effect of carbon dioxide is chiefly revealed by the pH (Sadler, 1954). Moreover this feature is readily determined. We found empirically that the atmosphere should contain 15 % carbon dioxide gas at 15 °C and 25 % at 41 °C. The desired carbon dioxide tension outside the eggs was reached by replacing a part of the normal air by carbon dioxide. 16 eggs per day were included in each group. With a few exceptions these had been laid not longer than 30 minutes before the beginning of the experimental treatment. Directly after lay these eggs were wrapped in aluminium foil and placed in a thermocontainer in order to prevent escape of carbon dioxide and cooling down during collection and removal to the treatment room. The eggs were produced by 20–29 week-old White Leghorns. To keep the experiment under control, the pH of an egg was determined daily just before each treatment and just after. After the experimental treatment the eggs were stored at 15 °C together with those of the control group and placed weekly in the incubator for determination of the hatching results. In all, four batches of eggs were set. On the fourth occasion the eggs of the G and TG groups were subjected to a somewhat different treatment. To ensure that any detrimental effect of the carbon dioxide percentage was demonstrated as clearly as possible this percentage was raised to 45 % in these groups; in this experimental design a higher percentage appeared to be unobtainable.

During the first 18 days the eggs were incubated at 37.5–37.7 °C and a relative humidity of 52–53%; during the last three days of incubation the relative humidity was raised to 75 %. The hatching results were judged in the same way as in the first experiment in this series.

Results and discussion

During collection of the eggs and removal to the treatment room the temperature in the thermocontainer did not fall below 37.5–38 °C. The temperature in the room where the T and TG groups were being treated varied from 40.5 to 41.0 °C. This is on the average about half a degree centigrade lower than the deep-body temperature (Sturkie, 1965).

The pH in the albumen of an egg before and after the experimental treatment is an index of the success of the gas treatment. Table 2 summarizes the averages, standard error of the various pH determinations, and the exact duration of the experimental treatments. The time reported for the control group relates to the interval between the two pH determinations. The pH determinations of the albumen in 60 eggs immediately after they were laid gave a value of 7.5 ± 0.1. This result is close to the value of 7.4 given by Sauveur & Mongin (1970). The pH values observed at the start of the treatment and the data of the eggs after the gas treatment show that the pH in the white was about the same as that in a fresh egg. This is a satisfactory result, because the acidity in the albumen of fresh first eggs of a pair agrees with that of fresh normal eggs: 7.4 ± 0.1 (n = 21).

Table 3 shows the results of the second part of the experiment in which the carbon dioxide content outside the eggs in the groups G and TG was raised to 45 %. In this case

Table 2. Summary of the different pH determinations.

	CO_2 content during treatment (%)	Temp. during treatment (°C)	pH albumen before	No.	pH albumen after	No.	Duration treatment
Group T	± 0.03	41	7.5 ± 0.1	13	8.3 ± 0.3	14	10 h 40 ± 11 min
Group G	15	15	7.6 ± 0.1	14	7.5 ± 0.1	14	10 h 58 ± 15 min
Group TG	25	41	7.5 ± 0.1	11	7.4 ± 0.1	12	11 h ± 8 min
Control	± 0.03	15	7.6 ± 0.1	14	8.1 ± 0.1	14	10 h 49 ± 20 min

Table 3. pH determinations after modified carbon dioxide treatment.

	CO_2 content during treatment (%)	Temp. during treatment (°C)	pH albumen before	No.	pH albumen after	No.	Duration treatment
Group T	± 0.03	41	7.5 ± 0.1	5	8.3 ± 0.2	5	10 h 42 ± 12 min
Group G	45	15	7.6 ± 0.1	5	7.1 ± 0.1	5	10 h 56 ± 4 min
Group TG	45	41	7.5 ± 0.1	5	7.2 ± 0.1	5	10 h 55 ± 6 min
Control	± 0.03	15	7.6 ± 0.1	4	8.2 ± 0.1	5	10 h 44 ± 15 min

the pH fell about 0.4 and was therefore no longer in agreement with that of the fresh first eggs. Table 3 suggests a greater decline in pH in group G than in group TG. This result agrees with the experience that solubility of gases decreases with increasing temperature of the solvent.

No difference in hatching percentage of the fertilized eggs was found between the experimental groups and control in either the first or second part of the experiment:

Table. 4. Hatching results of the treated eggs.

	Incubated	Fertilized	Hatched	Percentage hatch of fertilized eggs
Carbon dioxide pressure in equilibrium				
Group T	224	157	134	85.4
Group G	209	148	126	85.1
Group TG	180	141	113	80.1
Control	225	167	139	83.2
Increased carbon dioxide pressure				
Group T	74	51	42	82.4
Group G	74	61	53	86.9
Group TG	74	55	46	83.6
Control	74	66	55	83.3

The experimental results show that neither the delayed escape of carbon dioxide, nor the prolonged stay at body temperature, nor both have any adverse effect on hatchability. It might be possible to conclude that the high percentage of embryonic mortality in the first eggs of a pair is not directly due to the abnormally long stay of the eggs in the oviduct. But the long stay may be detrimental, despite the fact that this could not be demonstrated. In the interval between oviposition and the start of the experimental treatment the eggs cooled down a few °C and some carbon dioxide escaped at the same time. But it seems unlikely that these deviations from the experimental design were so considerable that a potentially adverse effect of an abnormally long stay could not be demonstrated. A more important factor is that we do not know exactly how long the treatment should last. Moreover the more or less anaerobic atmosphere in the oviduct has not been investigated, and this, together with the high carbon dioxide content, might play an important part. It is known that the hatching results decrease when the oxygen content during the first four days remains below 18 % (Taylor et al., 1956). Nothing is known about the relationship when the oxygen content is lower during the first day only. In any case the hatching results of the second part of the experiment when ambient air during the treatment contained 45 % carbon dioxide and 10–11 % oxygen did not clearly differ from the controls. Further research is needed on the effect of the practically anaerobic atmosphere in which the germ develops during its abnormally long stay in the shell gland.

Experiment 3: Extra shell deposition

One consequence of the abnormally long stay of the first shell egg of a pair in the oviduct is extra shell deposition over a part of the egg. As we are concerned here with a calcium secretion which started after the completion of the shell formation and which was intended for another egg (van Middelkoop, 1971) it is to be expected that the pores at the site of this extra deposit are likely to be blocked. As this is fatal to the permeability of the shell and hence also to the embryonic development during incubation, a closer investigation was made of the possibilities of pore blocking and the effect on embryo development of the area of the shell surface on which the pores are blocked.

Material and methods

To gain an impression of the distribution of the open pores over the shell surface of a first egg of a pair as compared to a normal one, they were made visible by staining. The shell of eight first eggs of a pair and of three normal eggs were sawn lengthwise, as well as possible into two equal parts, so as to include the pattern of the extra shell deposit. The shell membranes, or at any rate the inner one, were removed and the halves washed and dried. After drying, 1/3 of a half of each egg was filled with methylene blue and 1/3 of the other half with eosine, since our impression was that methylene blue penetrates better in the pores than eosine, however, it also flows away sooner on the outside of the shell. Staining was continued for 10 minutes to give the dye ample time to penetrate into the smallest pores. After staining four pieces of shell of about 1 cm^2 were sawn out of the waist area. The number of pores per sq. cm was counted by means of a stereo microscope (X 12). As in Tyler's method (1953), the pieces were then treated with 2.5 % sodium hydroxide and concentrated nitric acid. After this treatment extra shell deposit was

usually dissolved and the pores were counted by means of an ordinary microscope (X 35).

Shell porosity was determined by measuring the decrease of egg weight per sq. cm of shell surface of unfertilized eggs under normal hatching conditions. The advantage of keeping the eggs in the incubator is the relatively large decrease of weight, and temperature and relative humidity are easy to control.

The actual escape of carbon dioxide from the egg was not measured. An impression was gained, however, by determining the time-dependent trend of the albumen pH. The pH was determined in eggs which were stored at 15 °C and a relative humidity of 70 %.

In order to simulate the effect of the pore blockage on the hatching results, normal eggs were coated with paraffin. Eggs of Rhode Island Red hens were wholly or half immersed horizontally in Paraplast with a melting point of 56–57 °C which was heated to about 60 °C. In all, two treatments were applied, each being carried out in two ways so as to constitute four experimental groups, viz.:

– one group (W^1) of which the eggs were being coated over the Whole surface immediately after laying;

– a second group (H^1) of which the eggs were being coated over Half of the surface immediately after laying;

– a third group (W^i) of which the eggs were being coated over the Whole surface just before incubation;

– a fourth group (H^i) of which the eggs were coated over Half of the surface just before incubation.

A group of untreated eggs was used as control. After two or three days of storage at 15 °C the eggs were set in order to determine the hatching results.

Results and discussion

The result of the staining was fairly surprising, not because far fewer pores became visible of the first eggs of a pair than is usually the case, but because of the surface distribution. In some eggs few if any pores could be counted on the part of the shell on which apparently no extra shell material had been deposited, whereas it had been expected that pore blockage would be restricted to the site of the extra shell deposition. No satisfactory explanation could be given for this unexpected result. It was necessary to establish whether the pores in this part of the shell were blocked or whether fewer of them were present. To this end the pores were recounted in the same pieces of shell after being treated with Tyler's (1953) hydroxide acid method. The result of both counts is presented in Table 5. As was expected, both in the first egg of a pair, and in the normal eggs, Tyler's method gave a higher count of pores than the staining method. The

Table 5. The average number of open pores per cm^2 before and after removal of the extra shell deposit.

	'First egg of a pair' (No.)								Normal eggs (No.)		
	1	2	3	4	5	6	7	8	9	10	11
Staining method	13	11	6	1	3	10	59	7	123	106	86
Tyler's method	106	128	126	172	110	121	105	90	153	115	102

Table 6. The porosity of first eggs of a pair and of normal eggs stored at a temperature of 37.8 °C and a relative humidity of ± 55%.

	Number	Average and standard error
First eggs of a pair	90	1.76 ± 0.77
Normal eggs	91	4.24 ± 0.90

abnormal difference between the two counts found in the first eggs of a pair, cannot be chiefly due to the more exact count of the open pores. The difference must also be attributed to the dissolving the additional shell deposit after application of the Tyler method. Even when the greatest difference between the two counts found in the normal eggs was taken into account for the influence of the counting method and this is substracted from the difference in the first eggs of a pair, the number of pores per piece of shell after the hydroxide acid treatment has still clearly increased. It can be seen from the bottom row in Table 5 that the number of pores per sq. cm of the original shell of a first egg of a pair lies within the normal range. But it has not been shown that all these pores extend to the true cuticle and that most of them do not end prematurely as some pores do in shells of normal eggs (Bryant & Sharp, 1934). To solve this question the pores in the shells of nine first eggs of a pair were stained in the manner described above. The parts of these shells were then boiled for an hour in sodium hydroxide (10 %) to remove the true cuticle (Simons et al., 1966). The shells were then dried and the additional shell deposit could be readily scraped off. Next the pores were stained again and a normal number of pores became visible even on the three shells of which only a few pores had first been seen. It may be assumed that most of the pores in the shell of the first egg of a pair actually extend to the true cuticle as in normal egg shells, and that they do not end blindly.

Following Mueller & Scott (1940), the permeability of the egg shell was expressed as the porosity, viz. the weight loss in mg/cm^2 of the shell surface per day (24 hours). In the first eggs a much lower porosity was found on storage in an incubator than in normal eggs of the same strain of broiler mothers (Table 6).

The decrease in porosity was also found by Jaap & Muir (1968). They reported that 'the incubation weight loss of the eggs with rough surfaces of a pair was below that of the controls'. This lower porosity of the first eggs of a pair tallies with what might be expected from the number of open pores (Almquist & Holst, 1931; Bryant & Sharp, 1934; Schoorl & Mos, 1968). The difference in porosity as compared with the normal eggs is too noticeable to be explained by the fact that the first egg of a pair is usually the first egg of a clutch (van Middelkoop, 1971) and that the first egg of a clutch has a significantly lower porosity than the succeeding eggs of the same clutch (Black & Tyler, 1944).

The weight loss per se is no index of the amount of carbon dioxide given off by the egg (Mueller, 1958), although the difference in water evaporation between eggs does provide some idea of the total pore area. Hence differences in porosity between eggs reflect the possibility of carbon dioxide escaping via the egg shell, since it partly depends on the number and size of the pores (Mueller, 1958). As the gas exchange via the egg shell is an important factor in hatching, the pH in the albumen of the stored first eggs of a pair

was compared with that of normal eggs of the same hens. When eggs are stored at 15 °C, the pH in the albumen of normal eggs rises to over 9 within a few days, as was also found by Sharp & Powell (1931) and others, while in the first eggs this level is not reached even after a week. In these eggs it is no exception for the pH in the albumen to be less than 8.5 even after a week of storage.

To simulate the effect of pore blockage on hatching results, two treatments were applied, each being carried out in two different ways. The complete coating served as the most extreme counterpart of the untreated controls. The treatment in which half the egg surface was covered with paraffin was based on the observation that the extra calcified layer is usually formed as band extending longitudinally over the egg. Only half the surface of these eggs were coated as the average porosity of the first egg is practically half of that of the normal ones, and it is also easier to carry out. By carrying out these treatments in two different ways it was possible to check how far pore blockage immediately after lay or during incubation only has a bearing on hatching results. As can be seen from Table 7 the coating of only half the surface is practically as detrimental to embryonic development as coating the entire surface.

Embryonic mortality in group W^i occurred at a somewhat later age than in group W^l. Although this seems obvious, it may be questioned whether this difference is entirely due to the small difference in the supply of oxygen. An air cell with a normal gas content was able to form in group W^i. It was shown that in the eggs of the group W^l small bubbles had formed under the paraffin layer. The fact that these bubbles probably contained carbon dioxide only would indicate that a certain overpressure occurred in these eggs and it cannot be assumed that this is conducive to embryonic development. No bubbles were found in the eggs of group W^i. The difference in pH in the albumen between these two groups cannot have been an important factor in this respect. According to Sadler et al. (1954) a low pH would have been favourable during the early stages of embryonic development.

The data of Table 7 clearly show that the longitudinal coating of half the surface of the shell is also unfavourable to the embryonic development. It was also shown that with regard to hatching percentage it makes no difference whether this treatment is applied directly after lay or immediately before placing in the incubator. As the paraffin layer hampers breakage of the egg shell, this mechanic cause may also contribute to the low hatching percentage in groups H^i and H^l. When the pipped eggs are considered as embryos which died during the hatching process, the chance of obtaining a viable chick from the fertile eggs set is 12–23 % with a confidence level of 95 %. This chance is about equal to

Table 7. Hatching results and distribution of embryonic mortality over the incubation period.

Treatment	Set	Fertilized	Died	Hatched	Distribution over incubation period (days)								
					1	2	3	4–6	7–9	10–12	13–14	15–18	19–21
Whole laying	115	95	95	0	76	19	0	0	0	0	0	0	0
Whole incubation	115	96	96	0	16	60	18	2	0	0	0	0	0
Half laying	116	100	87	13	4	8	3	7	0	5	9	19	32
Half incubation	117	95	73	22	3	5	1	6	3	1	8	13	33
Control	120	104	15	89	2	1	1	0	0	1	0	4	6

that in the first eggs of a pair (EC + EC?) of experiment 1. Even when the pipped eggs in these groups were reckoned as hatched chicks the chance was still only 33–40 %. Summarizing it may be concluded that the amount of pores blocked by the extra shell deposit is the main limiting factor determining embryonic development in the fertilized first shell eggs of a pair.

In this experiment it was noticeable that the air cell was unvariably located at the blunt end of the egg. The air cell was formed at the normal place even in eggs which had been coated over half the surface directly after being laid.

Conclusions

Shell eggs with the characteristics of the first of a pair laid as two eggs a day are unsuitable for hatching. It has been shown that of the fertilized eggs of this type only 13–26 % will give a viable chick. With regard to the reasons for the high embryonic mortality in 'first' eggs it can be said that:

1. The experimental results do not prove that either the delayed escape of carbon dioxide or the prolonged stay of the egg at body temperature have any adverse effect on hatchability.
2. The porosity of the shell of 'first' eggs is less than half that of normal egg shells of the same hens; this lower porosity can be explained by pore blockage caused by extra shell deposition.
3. The hatching results of the eggs of which half the shell surface had been coated with paraffin confirmed the assumption that the blocking of the pores by the extra shell deposition is the main reason for the high embryonic mortality in 'first' eggs.

Summary

It has been shown that only a few viable chicks are obtained from the shell eggs which can easily be recognized by their appearance as the first of a pair. The influence on the hatching results of the longer stay of these eggs in the oviduct was investigated by holding normal eggs for 10–11 hours at body temperature immediately after laying and by preventing the escape of CO_2 during this period. In order to study the effect of the extra shell deposition on the hatchability of first eggs of a pair, the shell pores were counted and the porosity was measured. The effect of the pore blockage was investigated by coating whole or half the surface of the shell of normal eggs. This coating was done immediately after laying and just before placing the eggs in the incubator.

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5 The relationship between ovulation interval of White Plymouth Rock pullets and the laying of abnormal eggs

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Introduction

When selecting for fast-growing broiler mothers one is continually faced with the problem of unsatisfactory production of hatching eggs and the occurrence of a great number of abnormal eggs. Since only the double-yolked eggs are laid in the nests these are the most familiar type of abnormal egg. Usually the contrast is known between selection for growth rate and selection for a high production of hatching eggs but this knowledge is not concerned to the cause of the phenomenon. It is quite possible that an insight into the cause of abnormal eggs may provide a key to the improvement of the production of hatching eggs by broiler mothers without loss of growth rate.

For these reasons further research was conducted into the cause of the formation of the most important types of abnormal eggs. On the one hand the link was stressed between the different abnormalities and on the other the relation to the production of normal eggs. The experiment described was based on the hypothesis that the most frequently occurring forms of abnormal eggs from healthy hens are mainly due to one and the same cause, i.e. too short an interval between two succeeding ovulations.

Literature

The oviduct is usually differentiated in the infundibulum, magnum, isthmus, shell gland and vagina (Warren & Scott, 1935a). Both the length of the whole oviduct and the different parts may vary considerably from one hen to another (Asmundsen & Burmester, 1936; Curtis, 1915), but according to Warren & Scott (1935a) the relative lengths of the parts remain fairly constant in birds showing varying lengths of the entire organ. Despite wide variation in the lengths of the oviduct, no data are known from which it may be inferred that this variation manifests itself in the time of the egg spent in the oviduct. According to Fraps (1955) the oviducal term should be related to the length of a sequence. Warren & Scott (1935a) observed the ovum passing the infundibulum on an average in 18 minutes (5–40 minutes), the magnum in 174 minutes (165–180 minutes), the isthmus in 74 minutes (65–95 minutes) and staying about 20 hours and 40 minutes in the shell gland and vagina (19 hours 33 minutes – 21 hours 44 minutes) of which no more than 10 minutes spent in the vagina alone. It was subsequently observed that the fully formed eggs passed the vagina in about 1 minute (Phillips & Warren, 1937). In earlier research most attention was paid to the time spent in the shell gland, and the great variations that may occur were often pointed out. Warren & Scott (1935b) reported in another study that the egg spends on an average 20 hours in the shell gland with a variation from 18–22 hours. The same average was found by Bradfield (1951), but with a spread of only plus or minus one hour. The average value of 20 hours and 40 minutes

given by Burmester et al. (1939) is the same as that found by Warren & Scott (1935a). It is noticeable that all these workers found that the egg stayed in the shell gland for an average time of from 20–21 hours, notwithstanding the variation observed between individual birds and the fact that they worked with different strains.

The literature contains several direct and indirect references to the influence of the ovary on the development and capacity of the oviduct to work. The yolk is picked up by the infundibulum soon after its ovulation and the entire oviduct starts to move; even the shell gland undergoes constant contractions (Warren & Scott, 1935a; Phillips & Warren, 1937). In view of these observations it may be imagined that an ovulation occurring before the oviposition of the preceding egg may be a stimulus to the laying of the egg in the oviduct. This assumption is supported by the experience that an injection with luteinizing extract is often followed both by an ovulation and a premature oviposition (Fraps, 1942; Bastian & Zarrow, 1955). The ovary influences the oviduct even after ovulation. It has been established that the last ruptured follicle plays an important part in determining the time of oviposition of the corresponding egg (Rothchild & Fraps, 1944a, 1944b; Conner & Fraps, 1954; Wood-Gush & Gilbert, 1964; Gilbert & Wood-Gush, 1965).

Although the oviducal function is greatly affected by the ovary in this way, it is probable that the effect is not mutual. As early as 1914 Pearl & Curtis demonstrated that the development of the ovary in no way depends on the oviduct. Even entire removal of the oviduct had no effect on the ovary. This result is confirmed by the work of Wood-Gush (1963), if it is assumed that nesting behaviour represents ovary activity (Wood-Gush & Gilbert, 1964). On the other hand Huston & Nalbandov (1953) concluded that the presence of a bead in the magnum or a thread through the wall of the magnum caused in most treated birds an almost complete recession of lay during the first 25 days after the operation. They attributed this phenomenon to an inhibitory effect of the bead or thread on LH-release. In view of these results Nalbandov (1959) assumed that the yolk in the magnum inhibits LH-release after being engulfed by the infundibulum. This inhibiting effect of the ovum during its passage through the magnum and isthmus lasts 5 hours at the most. Huston & Nalbandov's statements (1953), on which Nalbandov's theory rests, were not confirmed by the results of other experiments (Sykes, 1962; Lake & Gilbert, 1964). According to Farrington et al. (1966) there is some evidence that individual irritants do block ovulation, although their results are not convincing. However, Bullock & Nalbandov (1967) were also unable to demonstrate any ovulation-inhibiting effect of a thread in the magnum, and they denied that the oviduct played an essential part in the LH-release mechanism.

Assuming the oviduct to have no effect on the ovary it may be taken for granted that with regard to the oviduct, any length of time in principle is possible for the interval between two succeeding ovulations. The length of the interval is defined by the interplay between (1) the time that elapses between one follicle reaching maturity to the next (2) the lighting conditions of the bird. It is generally accepted that the release of LH is confined to the hours of darkness. This theory is especially based on the results obtained by changing the period of darkness; such experiments were initiated by Warren & Scott (1936). It is not quite clear when the first ovulation in a given period can occur. But it may be concluded from the work of Rothchild & Fraps (1949) that the first ovulation in a clutch can be expected from 7 hours after the onset of the period of darkness, with an average time elapse of 9 hours. Other work by Fraps et al. mentions 8 hours for this, with

a mean of 10 hours (Fraps & Dury, 1943; Fraps & Case, 1953). With the help of the oviposition times Fraps (1954) later estimated that the first ovulations occur 10 hours after nightfall. This time shows good agreement with the results of Bastian & Zarrow (1955). When delaying ovulation the latter found a mean time lapse of 9 hours between the onset of darkness and the ovulations. Since LH-release is restricted to night-time, when the bird is asleep, the last possible release is at the end of this period. According to Fraps et al. (1942) injection with a luteinizing preparation may be followed by an ovulation even after 9–10 hours. If this result can be considered as representative of natural conditions, the last possible ovulation can be expected at about 10 hours after the end of the night. This time is 1–2 hours later than the 8 hours which can be inferred from Fraps' (1955) estimations.

Material and methods

The research was performed with pure White Plymouth Rocks belonging to our Institute's experimental lines. All hens were intermingled during both the rearing and laying periods. The flock consisted of four batches respectively hatched on Jan. 6th, Jan. 20th, Feb. 3rd and Feb. 17th, 1971. Up to the age of 18 weeks all hens were kept on the floor and thereafter placed in individual laying cages, viz. half of the hens in 30 X 45 laying cages and the other half in cages measuring 25 X 40 cm. With the help of artificial light the birds received a minimum daylength of 14 hours, during both the rearing and laying periods. Feeding was invariable ad libitum, the ration consisting of 2700 kcal ME/kg, 15.2 % crude protein, 2.65 % Ca and 0.5 % P. The mean bodyweight was 1100 g at eight weeks and 3260 g at 34 weeks. The average age at the laying of the first egg was 140 days. During the first 80 days of a hen's lay the mean production was 55 yolks, of which 41 were found in normal eggs. The mortality was 4.7 % during 0–18 weeks of age and 7.4 % from 18–60 weeks.

From 7 p.m. on July 19th to 8 a.m. on July 24th the egg production of 1003 hens was checked hourly, the average age of the pullets being 25 weeks. Both the appearance of the laid egg and the time of finding were recorded for each bird, this representing the time of laying within an accuracy of one hour. The abnormal eggs were allotted to the different classes in accordance with the following criteria:

1. amount of shell formation (calcification);
2. shape (a normal shape, the appearance of a compressed side or of a bulge as described by van Middelkoop, 1971);
3. occurrence of yolk material on the outer surface of the egg;
4. number of yolks in the egg.

During the hourly check of egg production the sunrise varied from 4.30 a.m. to 5 a.m. and sunset from 8.30 p.m. to 9 p.m. This means that the pullets received a day of about 16 hours and a night of 8 hours.

So far as is known, it has not hitherto been possible to measure ovulations directly. In studies in which time of ovulation is an important factor, this time is often estimated by palpating the eggs hourly via the colon. The drawback of this method is that it is hardly practicable and according to our own experience it is somewhat unsatisfactory when the oviduct contains two eggs. For these reasons, and the fact that it is only a rough estimate, the hens were not palpated in this experiment.

Results and discussion

Throughout the experiment a total of 3346 yolks were found both in and on the eggs collected. Table 1A gives a survey of the chief classes into which the 'eggs' were divided. Only 72 % of yolks were found in apparently normal eggs. This figure is much lower than the 83 % found elsewhere with broiler mothers of about the same age (Jaap & Muir, 1968). It seems very likely that the difference is mainly due to the use of a more precise

Table 1. Survey (A) of all eggs collected and (B) of the abnormal eggs of which could be deduced that the ovulation in question occurred shorter before or after another ovulation than the time needed to form a normal egg.

Type of egg	A. All eggs collected		B. Abnormal eggs shown to be related to relative short ovulation interval	
	number of eggs	number of yolks	number of eggs	number of yolks
Double-yolked eggs				
Hard shelled and normal shaped	95	190	95	190
Not hard shelled and/or otherwise abnormal	5	10	5	10
Single-yolked eggs				
Hard shelled				
normal shape	2412	2412	irrelevant	irrelevant
normal shape, but with additional shell deposition	150	150	118	118
normal shape, but soiled with yolk material	5	10	1	2
compressed-sided	95	95	86	86
showing a bulge	8	8	7	7
Soft shelled				
normal shape	45	45	45	45
normal shape, but soiled with yolk material	11	22	11	22
compressed-sided	32	32	27	27
showing a bulge	14	14	13	13
Membranous				
normal shape[1]	274	274	200	200
normal shape but soiled with yolk material	25	50	25	50
compressed-sided	25	25	16	16
otherwise abnormal	2	2	0	0
Various abnormalities	7	7	0	0
Total	3205	3346	649	786

1. This class also includes the broken eggs, as it could not be established whether these eggs had a compressed side.

distinction between the different classes of eggs collected. For such a distinct criterion as the percentage of total yolk production found in double-yolked eggs, our figure of 6.0 % does not greatly differ from Jaap & Muir's 6.5 %.

As was pointed out in the discussion of the data in the literature, the length of the interval between two succeeding ovulations is mainly determined by the coordination of (1) the difference in maturity between the follicles in the ovary and (2) the day-and-night rhythm. On account of the results of the research cited it is assumed in this paper that ovulations may occur from 10 hours after nightfall to 10 hours after daybreak. As regards the conditions under which the present experiment was carried out, this means that the 'ovulation period' ranges from 7 a.m. to 3 p.m.

Ovulation period

It is unfortunate that we do not know to what extent this deduced ovulation period tallies with the facts. An indirect check of ovulation can only be made with the help of oviposition times of the normal eggs and time taken to form a normal egg. This check is hindered by the fact that the time needed for the formation of normal eggs is not constant but is rather variable. Warren & Scott (1935a) give a mean duration of about 25 hours for good layers, whereas Rothchild & Fraps (1949), citing unpublished data, give figures of 27–29 hours for the first egg of a sequence. There are also estimated values for oviducal terms ranging from 24½–28 hours (Fraps, 1955). From Kappauf's work (1971) it may be concluded that a range of from 25–27 hours is needed for egg formation. From this article can be inferred that the time from oviposition to the next ovulation within a sequence is not constant but may increase from 0.5 to 1.5 hours.

In other research (unpublished) we estimated with poor laying broiler mothers that about 27 hours were required for normal egg formation. All things considered it seems justified to use a figure of from 24–27 hours for the main oviducal term of normal eggs. These figures presuppose that normal eggs are laid from 7 a.m. to 6 p.m. proceeding from the established ovulation period. The expected time of lay of normal eggs showed good agreement with the period at which they were actually found (Fig. 1), so this is no reason for rejecting the deduced ovulation period.

Oviducal stay of membraneous and soft-shelled eggs

Since the amount of shell deposition is an index of the time spent in the shell gland, the oviducal term of prematurely laid eggs can be deduced from its shell thickness, and the ovulation time can be estimated provided the time of laying is known. When apart from its shape, the laying of a membranous or soft-shelled egg by healthy hens is mainly due to premature expulsion, the time of oviposition of such eggs can be related to the ovulation time and vice versa. This assumption can be checked by comparing the expected laying time, based on the established ovulation period together with the theoretical oviducal stay deduced from the various stages of normal egg formation, with the time when the membranous and soft-shelled eggs are actually found. But before doing this it is necessary to define clearly what is meant by 'membranous' or 'soft-shelled' from which the oviducal term can be deduced. In this paper 'membranous' is a practical concept and is not limited to eggs without any shell deposition. Consequently the line of

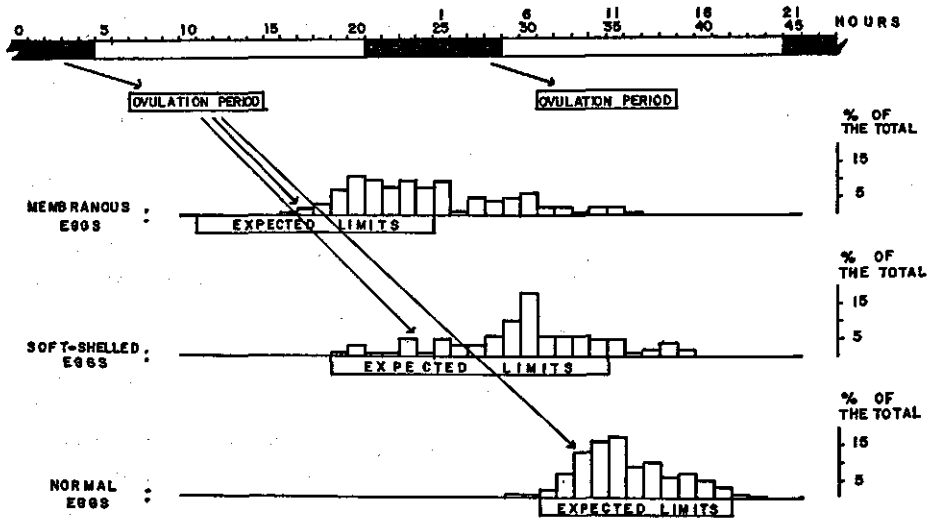

Fig. 1. Ovulation period and expected time of laying.

demarcation between a membranous and a soft-shelled egg is where it changes from a soft and flexible state into an egg with a rigid shell. According to Burmester et al. (1940) this state is reached between the 7th and 9th hour of uterus development, corresponding to an oviducal term of 11–13 hours. Evers (1967) established that about 15 hours before the normal expected oviposition the mammilary knobs fuse and the palisade layer starts to form, resulting in an inflexible egg shell. From this it can be inferred that after an oviducal stay of 10–12 hours the egg reaches the soft-shelled stage.

Having regard to these values as compared to other data (Conner & Fraps, 1954; Conrad & Scott, 1938; Rzasa & Ewy, 1970), it may be stated that the oviducal term of a membranous egg is from 4 (Warren & Scott, 1935a) to 10 hours, and that of a soft-shelled egg from 12–18 hours (Rzasa & Ewy, 1970); hard-shelled eggs having a minimum stay of 20 hours (Rzasa & Ewy, 1970). In setting these limits the indistinct transition between the different classes of shell thickness is omitted. As a result the laying of membranous eggs is expected to be limited to the period from 11 a.m. – 1 a.m. and that of soft-shelled eggs from 7 p.m. to 11 a.m.; the time between cessation of egg formation and oviposition is ignored as being an unknown factor. In the case of the soft-shelled eggs the expected period has shown to be in fair agreement with the actual oviposition times: whatever their shape, 85 % of the soft-shelled eggs had been laid between 8 p.m. and 11 a.m. (Fig. 1). But a similar agreement between the theoretical and actual period was not observed in the case of membranous eggs. They were found to have been laid between 5 p.m. and 11 a.m., and only 70 % of these eggs between 5 p.m. and 1 a.m. The question arises as to how far this discrepancy in the limiting time can be attributed to the assumed values of 4–10 hours for the oviducal term. In setting the lower limit, the time taken is that for an egg normally arriving in the shell gland (Warren & Scott, 1935a).

It is possible that an egg may not be expelled from oviduct and laid as a membranous one before plumping is finished. According to Sykes (1967) the introduction of an

uterine irritant results in the laying of very thin-shelled eggs due to premature expulsion, but after a stay in the shell gland corresponding to the time required for maximum accumulation of albumen. Although Sykes' observations are based on unnatural conditions, it might be imagined that also under normal conditions an egg will not be directly expelled immediately after entry in the shell gland but after a latent period. To correct the lower limit of the oviducal term of membranous eggs for the time needed for plumping we have to add 6–8 hours (Scott, 1938 cited by Burmester et al., 1939; Burmester, 1940; Burmester et al., 1940; Bradfield, 1951) thus arriving at 10–12 hours. Taking 10 hours for the lower limit and for the upper the value of 13 hours derived from Burmester et al. (1940), the laying of membranous eggs may be expected to occur in the period from 5 p.m. to 4 a.m. With exception of the upper limit this period is found to agree with the facts (Fig. 1). The difference of the upper limit from the observed value is 7 hours, which is too great to be ascribed to the indistinct transition from a still membranous to a soft-shelled egg.

The fact that membranous eggs can be laid much later than expected may indicate that the laying of these eggs may not only be due to premature expulsion, but also that a somewhat different oviducal secretion may be a cause. Some support to this theory is provided by the observations that in 10 or even 5 hours time a membranous egg may be followed by a more or less hard-shelled egg with a compressed side. This phenomenon can be explained as follows: one egg leaves the isthmus while another is still in the shell gland and not developed much beyond the plumped stage. The second egg grows by plumping while being compressed against the taut first one. As a result the shell membranes stretch disproportionally, giving rise to the compressed shape (van Middelkoop, 1971). Presumably owing to stimuli connected with the second ovulation, no appreciable amount of shell is deposited on either the first or second egg. After plumping of the second egg is completed, the first is expelled and laid as a membranous egg. It may be assumed that a similar membranous egg has been in the oviduct for as long as 16 hours or even longer. It is not yet known how long an egg which has been forced out of the shell gland by another egg can remain in the vagina before being laid. If there are more cases in which the oviducal stay of membranous eggs may be estimated at about 16 hours, this could explain why membranous eggs are laid so much later than expected.

Since the most frequent oviposition times usually show marked differences, depending on the amount of shell thickness, it may be assumed that the laying of membranous or incompletely calcified eggs is mainly connected with the premature expulsion of the egg. Although the length of the oviducal stay of a membranous egg is not well established, in this paper a time ranging from 4–10 hours will be taken for the oviducal stay when estimating the ovulation time in question from the time of oviposition, because these limits correspond to the membranous stage in normal egg formation without any correction for possibly abnormal oviducal secretion. In this way an attempt is made to use data as unbiased as possible for demonstrating the relationship between ovulation rate and egg formation.

Ovulation interval and egg formation

Ovulations can be roughly divided into two groups according to their rate of succession, viz.:

a. ovulations succeeding each other in the same ovulation period.
b. ovulations occurring in two different periods.

a. The best known example of two ovulations in the same period is the laying of double yolked eggs (Bonnett, 1883; Warren & Scott, 1935a; Buss, 1963). It is only by means of the double-yolked eggs collected during this experiment, that it can be shown that 100 ovulations occurred in rapid succession so that neither yolk led to the formation of normal eggs. The other cases, in which ovulations succeed each other in the same period, cannot always be seen directly from the eggs. Since the ovulation times are unknown these have to be deduced and this was done by subtracting the estimated oviducal term of the laid egg from the oviposition time. As the oviducal term is estimated either the upper or the lower limit can be used for it. To estimate the interval between two succeeding ovulations the safest way is to subtract the minimum oviducal stay from the oviposition time of the 'second' egg, and the maximum oviducal stay from the laying time of the 'first' egg. Apart from the double-yolked eggs, it can be shown in this way that 154 ovulations succeeded one another in the same period. It is noticeable that only 25 of the 308 ovulations in question resulted in normal egg laying. Instead of making the safest estimate it is also possible to subtract the minimum oviducal stay of the laying time of the 'first' egg and reduce the oviposition time of the 'second' egg by the maximum duration. This broad estimate must be made in such a way as to exclude the risk of it being so wide that it includes the possibility of two ovulations falling in separate periods. With this reservation it is found in this way that 198 ovulations are succeeded by one another in the same period. Only 27 normal eggs were formed from the 396 ovulations concerned. Since the established ovulation period was only 8 hours, the above results support the hypothesis that the laying of abnormal eggs is caused by a too rapid succession of ovulations.

b. With regard to the other group of ovulations, i.e. those occurring in separate periods, there is some difficulty, i.e. the estimate of the time of ovulation corresponding to hard-shelled eggs with additional shell deposition. These eggs remain in the shell gland far longer than the normal time (van Middelkoop, 1971, 1972), but the exact period has not yet been established. With a few exceptions it has only been ascertained that these eggs stay less than two days in the oviduct (unpublished). In view of the cause of the extra shell deposition on the hard-shelled egg together with the origin of the compressed side of the succeeding egg (van Middelkoop, 1971), it wil be evident that we are concerned here with an ovulation occurring before the preceding egg has been laid. The question, however, is why an egg laid with additional shell deposition was held in the shell gland longer than the normal term. It is suggested that an egg is held in the shell gland when the following ovulation occurs some hours before the preceding egg should have been laid as a normal one. This would mean that in these cases the ovulations are separated by intervals of about 20–22 hours and this can only be the case when these events occur in two separate ovulation periods. As a result of this type of ovulation inferred from the pairs of eggs laid within 20 hours of each other and of which the first was a hard-shelled one with additional shell deposition, a total of 217 yolks are lost as abnormal eggs. Of the 2412 normally laid eggs, in 49 cases only the interval between two ovulations was less than 24 hours, viz. 4.1 % of the total. In 45 of the 49 cases the intervals exceeded 20 hours. It is concluded from these results that a normal egg will not

be laid within 24 hours after another normal egg. Heywang (1938) and van Albada (1958) also found that in all cases the interval between the laying of two normal eggs exceeded 23 hours. Taking an oviducal term of 24 hours for the first egg laid and a stay of 27 hours for the second, this would imply that the ovulations corresponding to normal eggs are separated by at least 21 hours.

Remaining group

Having regard to the yolks found in and on the eggs collected, it can be said that at least 3346 ovulations occurred during the experimental period. Hitherto it could be inferred that 786 of the 934 yolks lost in abnormal eggs were related to ovulations occurring not more than 20 hours before or 20 hours after another ovulation (Table 1B). Besides the hard-shelled eggs with additional shell deposition and the related compressed-sided eggs, there remain 148 abnormal eggs which have not been shown to be associated with a relatively short ovulation interval. In the present experiment unfortunately it was impossible to demonstrate that these eggs had any such relationship. The main reason for this gap is the impossibility of recording ovulations directly and the fact that this drawback cannot always be overcome by deducing the oviducal stay from the oviposition time, as for instance in case of eggs soiled with yolk material and internal laying.

Eggs soiled with yolk material

The unacquaintedness with the actual ovulation time is a particular drawback in the case of eggs soiled with yolk material. Most of these soiled eggs were membranous. It is impossible that in these cases the succeeding ovulation occurred before the preceding egg was laid, but the length of the ovulation interval is unknown. With regard to the origin of the soiled eggs, it was first assumed that the contractions of the shell gland associated with the new ovulation (Warren & Scott, 1935a; Phillips & Warren, 1937) gave rise to the laying of the egg in the shell gland. It may be imagined that the yolk which has just been engulfed is pressed more rapidly through the oviduct as a result of the induced oviposition. The yolk overtakes the egg being laid and is broken against it in turn. Sometimes some small kind of membrane is found on the soiled egg in addition to the yolk material. Microscopic study of such a membrane revealed that it consisted of vitelline membrane on which was deposited a thin layer of shell membranes and some crystals. If it was a quite normal oviducal secretion, these traces would indicate an abnormally rapid passage of the yolk through the oviduct and give some support to the above assumption. Hence it may be expected that eggs soiled with yolk material are mainly laid within the limits of the ovulation period. It was found, however, that this type of egg is laid more or less around the clock and that the laying time seemed to be more closely related to the amount of shell formation of the soiled egg itself. If we stick to the established ovulation period, these observations indicate that the yolk may stay in one or other region of the oviduct for some time without appreciable secretion on it. Since we have insufficient data on the origin of soiled eggs, it can only be stated here that they are associated with a relatively short ovulation interval because no completely formed eggs of this type are laid.

Internal laying

As the ovulations could not be recorded directly it is not known in how many cases the yolk was not engulfed by the infundibulum after its ovulation. Consequently it is not known how many yolks are lost in this way to normal egg production. According to Wood-Gush & Gilbert (1970) it may amount 5 % of the total egg production or even much more. This statement, however, is biased by the assumption that all eggs had been laid on the trap-nest; the authors do not report whether they noted which yolks had been engulfed, but were laid in the litter as abnormal eggs. More important than the estimate in this paper of the loss due to internal laying is the fact that the effect of such ovulations on the formation of the preceding or succeeding egg is impossible to establish. The impossibility, in many cases, of inferring that abnormal eggs are associated with a high ovulation rate is due to the lack of data on ovulations resulting in internal laying.

Remark

Some support of the hypothesis that the laying of abnormal eggs is associated with a short ovulation interval is provided by the work of Sheldon et al. (1969). When selecting White Leghorns for a short oviposition interval which in fact means a selection for high ovulation rate, they noticed a remarkable increase in the occurrence of abnormal eggs. It is also noticeable that an oviposition interval of 20 hours or less was regarded as undesirable. This figure tallies almost exactly with the time interval between the laying of an egg with additional shell deposition and a compressed-sided shell egg (van Middelkoop, 1971).

Summary

Egg laying and the appearance of the eggs were recorded hourly for each hen over a single period of 4½ days. The time which was needed to form a normal egg and the times of laying of normal eggs were taken to check whether the theoretically expected ovulation period tallied with the facts. The time of laying of membranous and soft-shelled eggs was then inferred from the established ovulation period and the process of normal egg formation. Comparison of this period with the time the eggs were actually laid showed that the laying of this type of eggs did, in fact, result from premature expelling, but that this was not the only cause.

After the oviducal term of the different types of abnormal eggs was established, the ovulations in question were inferred from the oviposition times. Together with ovulations connected with normal egg laying it could thus be deduced which eggs were associated with different ovulations occurring in the same period and which eggs resulted from ovulations occurring at different periods.

There are the following conclusions:

a. Healthy broiler mothers may lay many membranous and soft-shelled eggs as a result of premature expulsion, and it seems that somewhat modified oviducal secretion is a further factor to be considered.

b. A large proportion of the yolks lost in abnormal eggs originates from different ovulations occurring in the same ovulation period.

c. From another part of these yolks, it can be said that the ovulations in question

occurred in two separate periods indeed, but they are still some hours less apart from each other than the time needed for the formation of a normal egg.
d. Normal egg laying results from an ovulation occurring at least 21 hours before or after another ovulation.

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6 Selection trial on the laying of normal and of abnormal eggs of White Plymouth Rock pullets and estimation of genetic parameters

Introduction

Until now, conventional selection procedures used to improve performance in broiler stock have not fully succeeded in raising both hatching egg production and growth rate in White Plymouth Rock pullets. At the Ploufragan Testing Station (Anonymous, 1965–1969), for instance, the total egg production of comparable entries appeared to have increased 2–5 eggs per year from 1965 to 1969. The improvement in egg production is very low; it is also quite possible that the change was mainly environmental. In the same period, the mean bodyweight of those hens at 52 days of age increased 50–60 grams each year, being a more satisfactory result.

Selection for both a high growth rate and a high egg production will only be successful if negative genetic correlations due to pleiotropic effects or close linkage play a minor role. Data from the literature give no conclusive answers for the estimates of genetic correlation coefficient, because they range from slightly positive to minus one (Jaap et al., 1962; Merritt, 1968; Kinney et al., 1968; Kinney & Shoffner, 1965; Friars et al., 1962; Hale, 1961; Merritt et al., 1966). Realized correlated responses in selection trials were shown to be negative (Jaap et al., 1962; Merritt et al., 1966; Siegel, 1963, 1970; Ideta & Siegel, 1966). This result may also be caused by recombination of chromosomes through the release of the preceeding selection pressure (Dickerson, 1963).

Important to note is the fact that one normally speaks about egg production without further specification. Since the work of Jaap and co-workers (Jaap & Clancy, 1968; Jaap & Muir, 1968) it has become evident that with meat-type pullets, egg production should be distinguished into normal and abnormal egg laying. Not only has this distinction seldom been made, but most abnormal eggs are not noticed when the hens are not housed in batteries. If so, a considerable proportion of them are not recorded, not being laid in the nest (Wood-Gush, 1963; van Middelkoop, 1971). Therefore it could be imagined that research on ways of improving hatching egg production has to be directed to a better understanding of the physiology of egg production in meat-type pullets. In this way, other criteria of selection may be detected. Insufficient is yet known about how far the laying of a moderate number of normal and a relatively large number of abnormal eggs is predisposed by the hen's hereditary pattern. Nor is it clear whether a distinction between the different types of abnormal eggs has to be made. The few literature that have been published are not only very concise, but were also dealing only with double-yolked eggs (Lowry, 1967; Lowry & Abplanalp, 1967, 1968; Tardatjjan, 1968; Corcelle, 1969). A selection trial was therefore started in order to study the genetical aspects of the laying of two important types of abnormal eggs.

The first problem met with in selection for the laying of abnormal eggs is the choice of the correct criterion. When the trial was started in 1969 not enough was known about the

subject. This was still so when the parents of the next generation had to be selected. In the first instance, it was decided to try and select for the laying of membraneous eggs, besides the development of a double-yolk line. However as the laying period progressed, the lay of membraneous eggs appeared to be associated with quite different types of laying patterns. It was found for instance that these eggs could be laid together with another membraneous or soft-shelled egg on the same day, but also as a 'second' egg thus constituting part of the phenomenon of the production of two eggs on one day (van Middelkoop, 1971, 1972b).

The laying of two eggs a day appeared to be clearly distinguishable from the other types of abnormal egg laying: the frequency of most other types decreases with the progress of the laying period, while the frequency of first eggs remains fairly constant throughout (Fig. 1). For this reason, selection for laying of membraneous eggs was discontinued in 1970, and was replaced by selection for the laying of two eggs a day. Simultaneously with the development of 'abnormal egg' lines a counterpart was developed by selecting for the production of normal eggs only.

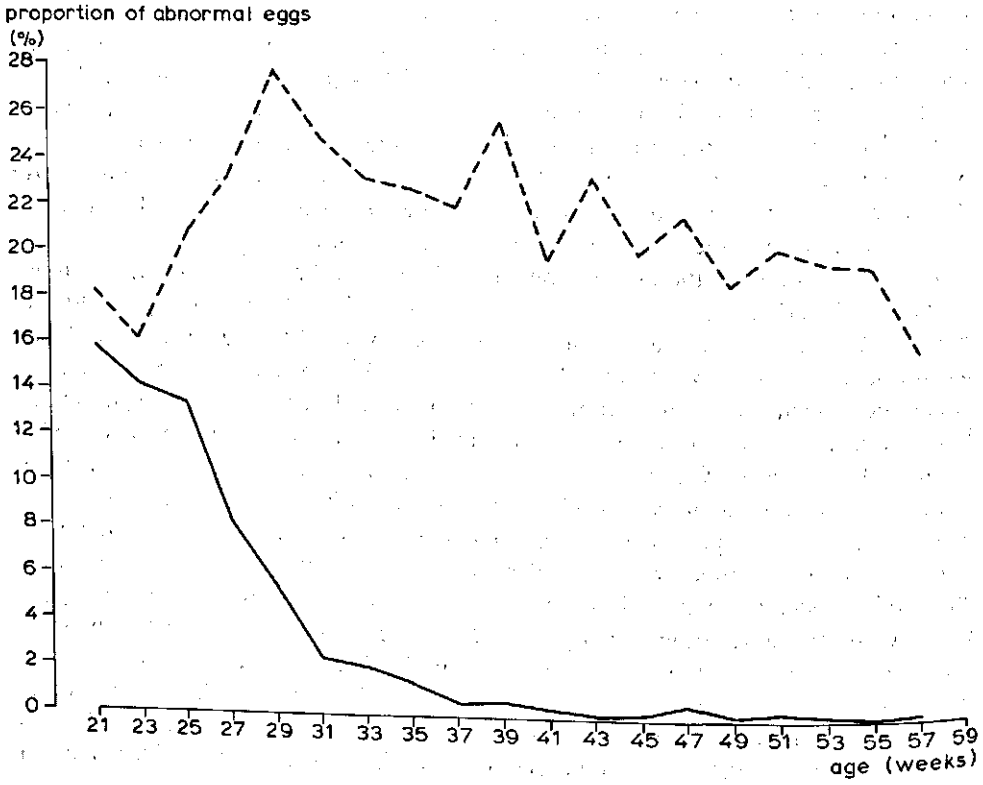

Fig. 1. The proportion of abnormal eggs/total egg production (%) during the laying period. Double-yolked eggs laid in the DY line of 1972 hatch (——); 'first' eggs laid in the TE/D line of 1972 hatch (– – –).

The trial

Housing and feeding

A new generation was hatched in January/February of each year during the course of the trial. The number of pullets desired was obtained in three to four hatches, which were hatched at two-week intervals. The pullets and cockerels of each hatch were reared together until eight weeks of age and were then weighed individually and the sexes housed separately. Until the age of 18 weeks, the hens were kept on the floor in natural daylight. At that time, the pullets were removed to another daylight poultry house and placed in individual laying cages to avoid floor egg problems. With artificial light, the pullets received a minimum daylength of 14 hours, during both the rearing and the laying period. Feeding was invariably ad libitum. Only one important change was made in the composition during the course of the trial: the ration having more metabolizable energy and crude protein in 1970 and subsequent years, than in 1969. Table 1 gives a survey of the average energy and protein content.

Unfortunately the pullets of the 1970 hatch received not enough calcium in their ration during the first part of the laying period. This period ranged from 18–31, 18–29, 18–27 and 18–25 weeks for the four batches, respectively. The calcium content amounted to only 0.9 % of the ration during this period. As soon as the deficiency was realized, it was corrected and the level raised to 2.7 %. As far as could be checked by comparison of the egg production data before and after correction, no interaction could be assumed to exist between strain within batch and the calcium content of the ration, at least in egg production. The total production, however, did not reach the level of other years during the trial, because several pullets stopped laying for some time. As to a possible interaction between unidentified batch influences and the different lines, it is important that the pullets of all lines were spread rather well over the batches.

Recording egg production

The production as well as the type of egg laid was recorded daily for each hen, whereby the eggs, according to type, were classified as follows:

a. normal eggs;
b. hard-shelled eggs, but with additional shell deposition typical for the first egg of pairs ('first' eggs) (van Middelkoop, 1971, 1972a);
c. soft-shelled eggs;

Table 1. Average energy content and percentage crude protein of the rations fed.

	Rearing period 0–8 w		Rearing period 8–18 w		Laying period	
	hatch 1969	hatch 1970–2	hatch 1969	hatch 1970–2	hatch 1969	hatch 1970–2
ME (kcal)/kg	2750	2750	2700	2850	2550	2700
Crude protein (%)	18.1	18.1	14.7	16.0	14.1	15.2

d. membraneous eggs;
e. double-yolked eggs;
f. eggs with an abnormal shape, i.e. being compressed sided or showing a bulge on one side (van Middelkoop, 1971, 1972b);
g. eggs soiled with yolk material (van Middelkoop, 1971, 1972b).

Thus the number as well as the different types of eggs and the sequence of laying was known for each hen. The latter will subsequently be denoted in this report as the laying pattern.

Double-yolked eggs were established on the basis of their size but 'normal' eggs were broken open if there was the slightest doubt. At the beginning of the laying period, all eggs were regularly broken open in order to check the accuracy of the detection of the double-yolked eggs. After being recorded, the eggs that had dropped through the wire cage floor were covered with peat dust to avoid noting them twice.

Control strain

Introduction

In selection trials in general and also in this study, the need for a control strain is felt in order to be able to correct for the influence of the environment, which varies from year to year. As long as so little is known about the cause of abnormal egg laying, it is hard to conclude whether the change in the laying pattern from generation to generation should mainly be ascribed to the effects of selection or to a change in the environment. Instead of the maintenance of a control strain a divergent selection could also be considered. An objection, however, is that it can only be used if it is known that no environmental interaction exists (Falconer, 1960). Moreover the number of selected lines needed would have to be increased. Another equally important condition is that selection in the opposite direction has to be possible. Selection for abnormal egg laying does not give problems, but selection against this character may do, because the limit of the laying of normal eggs only will probably soon be approached. Although selection for the laying of normal eggs only could be considered as divergent selection for abnormal egg laying, yet a control strain has been maintained for safety.

Design and stability

The control strain was built up from the 1969 hatch of the Institute's White Plymouth Rock strain, just before the start of the selection with the experimental lines. The hens which were intended to form the control strain, were chosen as follows: from each of the 20 half-sib groups, one full-sib family, and from this family one male was taken at random. The females were obtained in a similar way: from each half-sib group, three full-sib families were chosen at random, each one providing one breeder hen. Although one cockerel had been taken from each sire, the number of female breeders was thus reduced from 141 to 60, because a greater number will hardly be able to increase the effective population size.

Pairing of the males and females was at random, but no full or half sib mating was allowed. Halfway through the hatching season, the cocks were shifted to other females so

as to get a broad basis for the foundation of the control strain. In the subsequent generations, the parents used for the breeding of the control strain were obtained by taking at random one cockerel from each sire and one pullet from each dam as had been done for the foundation of this strain.

When a breeder cock or hen had no offspring at the time of selection, those families were not replaced by birds from other descent. If this were to be done, the influence of one parent would be doubled in the next generation while that of the others remained unchanged. If the birds were not replaced, the influence of all parents increased proportionately.

An estimate of the genetic stability of the control strain was made by comparing the actual mean performance in the successive years with the mean corrected for natural selection. In order to do so, all ancestors of the hens in the 1972 hatch were traced back to 1969. All families that did not have offspring in 1972 were eliminated over the previous generations and the mean performance calculated again for each trait. It should be noted that theoretically this procedure was not correct, because in this way the correction for natural selection was based upon the surviving families instead of the original ones and thus the original strain was converted into a somewhat fitter strain. Owing to this correction it is quite well possible that the corrected strain is influenced by the environment to another extent than the original strain. However a correction performed for the missed influence of the families lost is impossible, because the performance of hens which do not exist cannot be measured.

In Table 2A, the mean performance of six traits of the complete flocks of 1969, 1970 and 1971 is given together with the means based on the families still present in 1972. Accepting this check on genetic stability, the observed changes in annual means proved to be of minor importance. A better idea of the success of maintaining a control strain is given, when the changes are expressed in terms of variance due to genetic drift. The variance due to genetic drift (S_d^2) does provide a yardstick for the genetic changes, which can be expected on account of the effective size of the control strain and the heritability of the trait concerned. Since the error from drift can increase with successive generations a maximal observed drift of 3 to 6 S_d has not to be unusual. Estimating S_d^2 from $\sigma_d^2 = h^2\sigma^2/2N$ (Gowe et al., 1959; Hill, 1972a) the desired σ^2 was obtained from analysis of variance and N by calculating the weighted effective population size (N_e) of all generations, t, by means of

$$\frac{1}{\bar{N}_e} = \frac{1}{t}\left[\frac{1}{\bar{N}_{e_1}} + \ldots + \frac{1}{\bar{N}_{e_t}}\right] \quad \text{(Falconer, 1960)}$$

where

$$\frac{1}{\bar{N}_{e_t}} = \frac{3}{16N_m} + \frac{1}{16N_f} \quad \text{(Gowe et al., 1959).}$$

For h^2, an arbitrary value has been taken of only 0.2 even for the trait bodyweight at 8 weeks of age so as to have a low estimate of S_d^2. According to this procedure the maximum difference between the actual and the corrected mean of the control strain was found to be less than 4.7 S_d (Table 2B). So this comparison does not indicate that the loss of some families during the maintaining of the control strain did reduce the value of

Table 2. Stability of control strain.

A. Absolute changes

Character		Year of hatch 1969	1970	1971	1972
Age at first egg (days)	actual	151.0	145.4	146.3	145.2
	corrected	149.6	144.4	146.0	145.2
	difference	−1.4	−1.0	−0.3	0.0
Normal eggs laid in 80 days	actual	39.6	30.2	39.0	42.7
	corrected	40.6	30.2	39.0	42.7
	difference	+1.0	+0.2	0.0	0.0
'First' eggs laid in 80 days	actual	1.3	1.7	2.4	2.5
	corrected	1.3	1.6	2.3	2.5
	difference	0.0	−0.1	−0.1	0.0
Double-yolked eggs laid in 80 days	actual	1.3	1.8	2.0	2.4
	corrected	1.1	1.7	2.0	2.4
	difference	−0.2	−0.1	0.0	0.0
Total number of yolks produced in 80 days	actual	49.2	45.4	54.2	58.4
	corrected	49.8	45.2	54.0	58.4
	difference	+0.6	−0.2	−0.2	0.0
Bodyweight at 8 w (g)	actual	1017	1075	1105	1098
	corrected	1001	1079	1108	1098
	difference	−16	+4	+3	0

B. Changes expressed in terms of genetic drift (S_d).

	N_e	S^2 (min)	S_d^2.	Largest observed difference
Age at first egg (days)	80	145	0.18	$3.3\,S_d$
Normal eggs laid in 80 days	80	127	0.16	$2.5\,S_d$
'First' eggs laid in 80 days[1]				
Double-yolked eggs laid in 80 days[1]				
Total number of yolks produced in 80 days	80	170	0.21	$1.3\,S_d$
Bodyweight at 8 w (g)	80	9127	11.41	$4.7\,S_d$

1. Double-yolked egg production and laying of 'first' eggs are omitted, being distributed very skewly.

Symbols: N_e = effective population size; S^2 (min) = lowest observed variance.

the control strain for estimating the influence of the annual differences in environmental circumstances on the mean performance and that the control strain remained almost unchanged during the trial. This result confirms the work of Bowman & Powell (1971) about the efficiency of maintaining control strains and consequently this strain can provide useful data for the estimation of the influences of environmental changes.

Selection procedures

It was decided to select hens on the basis of the performance during an equal number of days for each hen after it laid its first egg to try and eliminate the influence of differences in precocity on the length of the laying period taken. The length of the period for which the data about a pullet's laying performance and pattern could be collected was closely connected to the housing facilities during both the rearing and the laying period. Because it was not possible to dispose of light-controlled rearing and laying houses, the breeding of the new generation had to be at the same date from year to year so as to minimize seasonal influences. Consequently the collection of data for selection had to be discontinued when the pullets of the youngest batch had reached the age of 38 weeks. Calculated from the time at which a hen laid its first egg, almost all the pullets were found to have completed a laying period of 80 days at that age. This length of 80 days has been maintained throughout the whole trial.

The selection of the cockerels had to take place after a recording period of 56 days. This difference in time at which the males and females were selected was due to the fact that artificial insemination was practised. The hens can be inseminated immediately after they have been selected. The cockerels, however, have to be placed in cages some time before and need to be trained before they can be used.

For the choice of the breeder hens, mass selection was preferred above other methods. This procedure was mainly chosen for two reasons, the first being the expectation of a relatively high heritability for the trait 'production of double-yolked eggs' (Lowry, 1967; Lowry & Abplanalp, 1967; Tardatjjan, 1968; Corcelle, 1969) and 'production of normal eggs.' For the latter trait, slight selection had been practised so far. The other reason was the fact that family selection caused more inbreeding than individual selection, if the intensity of selection were the same.

Males were selected on the basis of the performance of their full sisters in order to avoid a high degree of inbreeding. For the same reason, the cockerels taken were descended from 10 sires at least. Only for one line in one year was this number less, namely seven.

Selection lines

In total, three lines were selected from the original Plymouth Rock strain of the Institute. For convenience, these lines will be further denoted as DY (Double-Yolked egg line), as TE/D (Two Eggs a Day line) and N (Normal egg line). Because the procedures used in the selection of the lines differed in some respects, they will be dealt with separately.

In the DY line the hens were selected on the basis of the laying of a large number of double-yolked eggs, regardless of the performance for other traits. Because the proportion of birds of the 1969 flock laying at least one double-yolked egg exceeded the number of breeder hens, all hens could be selected on account of their own performance. Although the proportion selected each year was about the same, the number of effective breeders (males and females) and the number of offspring were rather variable (Table 3). This variability was mainly due to some cocks of low fertility.

Selection in the N line was based on the production of a large number of normal as

Table 3. Number of all surviving pullets of each line and the number of effective breeders.

		1969	1970	1971	1972
Control strain	all pullets	450	257	210	220
	female breeders	47	41	40	–
	male breeders	20	16	15	–
DY line	all pullets	–	150	181	259
	female breeders	28	46	39	–
	male breeders	9	12	10	–
TE/D line	all pullets	–	–	196	164
	female breeders	–	51	35	–
	male breeders	–	11	9	–
N line	all pullets	–	273	319	429
	female breeders	49	59	56	–
	male breeders	10	14	13	–

well as the laying of practically no abnormal eggs. The number of breeder birds in this line varied less from year to year than in the DY line, the reason being that less difficulties were encountered with fertility. Since enough laying cages were available, the total number of hens was increased each year (Table 3).

The procedure used in selecting the TE/D line needs more comment. The first distinction from the two other lines (DY and N) was the difference in starting year. In 1970, after the selection of the males and females for each line as described above, the rest of the birds were pooled so as to provide the breeder birds for the development of the TE/D line. A comparison of several characters of the pooled population with the first generation of the control strain showed that the differences were very small. Hence the control strain could also be considered to be right as a control for the TE/D line. Another important distinction of this line from the others was the fact that it was not directly selected for the laying of two eggs a day. This had been performed for practical reasons by selection for the laying of 'first' eggs. As with the DY line, all breeder hens could be selected on account of their own performance.

Results and discussion

Collecting period

As in selection of the breeders, a fixed period was taken for each hen after it laid its first egg in order to be able to measure the selection response, again with the aim of ruling out the influence of differences in precocity on the length of the completed laying period. The data were collected until two different end-dates, one being 80 and the other 200 days after a pullet laid its first egg. The long period of 200 days was important, because it gave a measure of the persistency of a character. With this I could check whether one particular type of abnormal egg laying passes into another in the course of time. For the laying of two eggs a day for instance, it is not known whether this type of laying is preceded by another one in the beginning of the laying period. Therefore it

could very well be possible that a period of 80 days is in itself too short to measure a hens inherent ability to lay two eggs a day.

In the first year of the trial no data were collected for a 200 days laying period. Although in itself this is regrettable, it was of no consequence for the laying of two eggs a day, because selection for this trait started in 1970.

Frequency distributions

The first investigation to be carried out when studying the heritability of a character is to look at its frequency distribution. When this is done with the traits 'double-yolked eggs' and the 'laying of two eggs a day', a continuous distribution is found, which is, however, very skew. Furthermore the zero class appears to be a very important one, even after a few generations of selection (Figs 2 and 3). The question arises as to the cause of this kind of distribution. Is it due to scale effects? Are we dealing with threshold characters? Or do genotype x environment interactions play a role?

When the laying of double-yolked eggs is seen as some type of egg production, just as in normal egg laying, it can be presumed that there is no reason until now to suppose that the skewness of the distribution is caused by scale effects. As far as the laying of two eggs a day is concerned, the same statement can be made. Therefore when we consider the

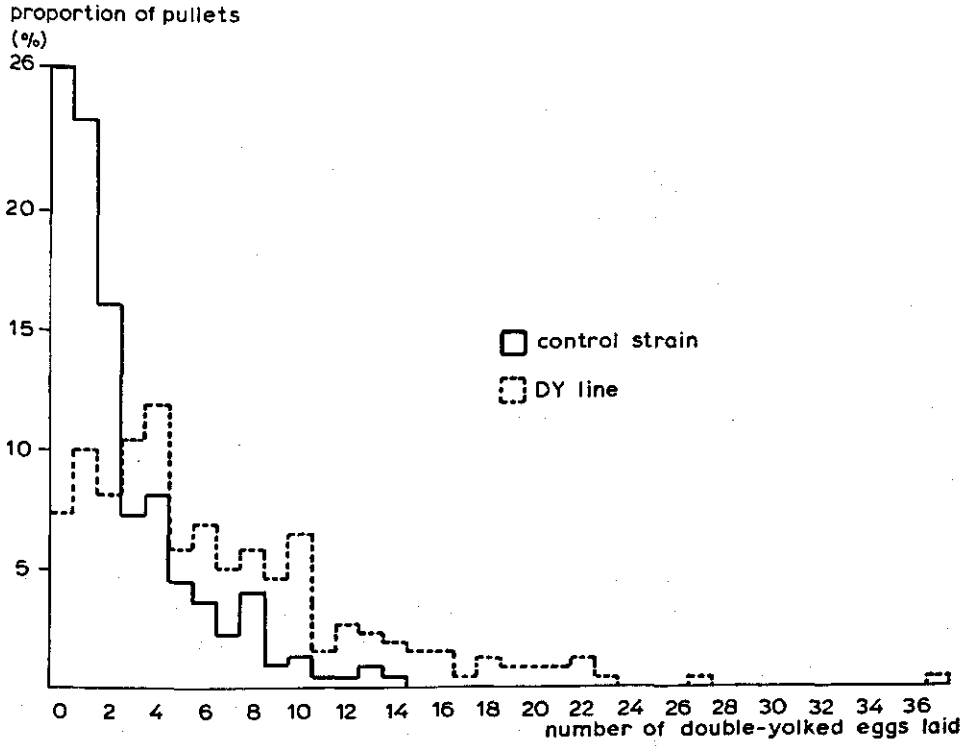

Fig. 2. Frequency distribution of hens laying different numbers of double-yolked eggs in 200 days of production from the 1972 hatch.

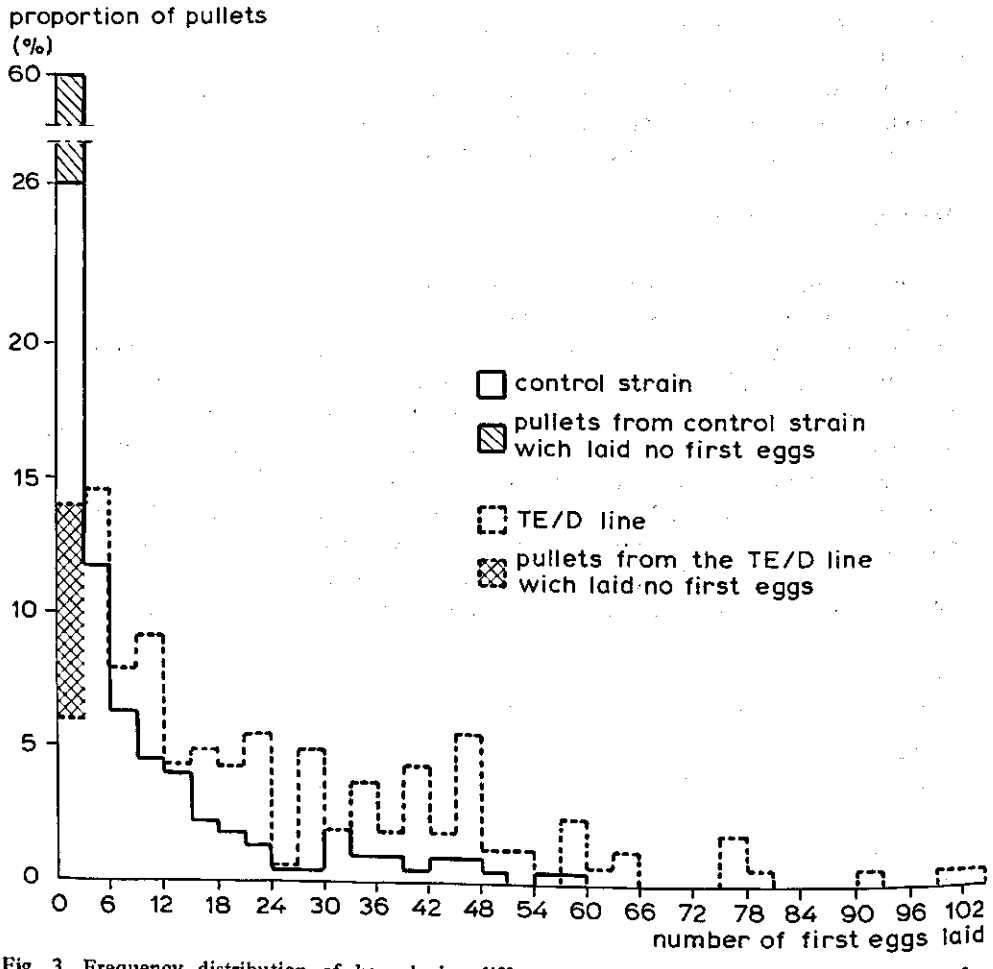

Fig. 3. Frequency distribution of hens laying different numbers of 'first' eggs in 200 days of production from the 1972 hatch.

laying of normal eggs, double-yolked eggs and the laying of two eggs a day as analogous types of egg production no reason exists to apply a scale transformation in the case of the latter two. A skew distribution in itself provides no sufficient reason for this correction because the risk exists that it obscures rather than illuminates the description of the genetic properties of a population (Falconer, 1960).

Because the zero class was found to be an important one, it should be checked whether the genetic background of this class could be considered in the same way as the other classes, or if it should be treated as representing all classes ranging from minus infinity to zero. This would mean that variation in the character concerned was continuous but that it was broken by a threshold. With the traits double-yolked eggs as well as laying of two eggs a day, one could imagine that this was so, because with both of these characters the interval between the two ovulations seemed to remain below a certain limit. But the clue to it lies in the question why two ovulations follow each other

so rapidly. This rapid succession of ovulations can in principle be due to an excessive yolk formation in the ovary together with a large number of follicles in the rapidly growing phase resulting in the maturation of more than one follicle at the same ovulation period as well as to the fact that the follicles in the ovary enter the rapidly growing phase at fairly irregular intervals. To gain more insight into the cause of the occurrence of two rapidly succeeding ovulations, more research is necessary in this field first. The possibility of an invisible (negative) scale as a basis for the visible part of the distribution for double-yolked eggs or for two eggs a day should not be excluded a priori.

The third possibility mentioned was a decreasing or progressing environmental influence with increasing class number, which could be a cause of the skewness of the distribution. The laying of double-yolked eggs is largely restricted to the beginning of the laying period and the more that maturation is postponed the less double-yolked eggs are laid. Therefore it may be supposed that only the 'proper' double-yolked egg layers are still able to show this ability after a delayed maturity and that the 'improper' double-yolked egg layers fail to do so because of negative environmental influences. The following explanation could be given: laying of one, two or at most three double-yolked eggs may be due to chance, the chance increasing with higher yolk production, while the laying of a fair number of such eggs is controlled by specific 'double-ovulation' alleles. If this explanation be valid, the frequency distribution of the 'proper' double-yolked egg layers would be influenced by the environment through the total yolk production in such a way that the originally normal distribution is made skew and a scale transformation would give more weight to the 'chance' double-yolked egg layers. Hence the scale was not transformed.

With the trait 'laying of two eggs a day' the case seems to be somewhat different. This abnormality is found throughout the whole laying period and needs only a two-egg sequence for its expression. Because a two-egg sequence is soon reached, this abnormality is supposed to be influenced only to a small extent by the environment. Therefore the same can be said of the frequency distribution of the hens.

Selection results

In order to get a first rough picture of the influence of the selection on laying pattern, a survey is given in Table 4 of the average result of each character per generation for each line. The lines soon diverged for the traits selected, so that high heritabilities may be expected.

Realized heritabilities

In view of the shape of the frequency distributions, it was decided to estimate the heritability from the selection response. This method has the advantage of being less influenced by an abnormal shape of the frequency distribution, while an estimate of the realized heritability represents the best progress breeders can achieve in practice. But this method means that only one estimate can be derived from each succeeding generation without further information. An improvement of the accuracy of the estimate can be achieved by fitting a regression line of the cumulative response over several generations to the cumulative selection differential (Falconer, 1954; Hill, 1972b). Besides the improve-

Table 4. Number of eggs for each trait, line and generation (mean ± standard error).

Line	80 days				200 days			
	1969	1970	1971	1972	1969	1970	1971	1972
Double-yolked eggs								
Control	1.3 ± 1.8	1.8 ± 2.4[a]	2.0 ± 2.2[a]	2.4 ± 2.8	–	2.0 ± 2.2[a]	2.2 ± 2.4	2.5 ± 2.9
DY	–	2.8 ± 2.9	3.5 ± 3.6	6.0 ± 5.0	–	3.1 ± 3.3	4.0 ± 4.1	6.5 ± 5.7
TE/D	–	–	1.7 ± 2.1[ab]	1.7 ± 2.1[a]	–	–	1.7 ± 2.2[a]	1.8 ± 2.3[a]
N	–	1.6 ± 1.9[a]	1.4 ± 1.9[b]	1.4 ± 1.9[a]	–	1.7 ± 2.1[a]	1.5 ± 2.0[a]	1.4 ± 2.0[a]
'First' eggs								
Control	–	1.7 ± 2.7[a]	2.4 ± 3.7[a]	2.5 ± 4.3[a]	–	4.6 ± 6.7[a]	6.7 ± 9.8[a]	6.4 ± 11.1
DY	–	1.4 ± 2.3[a]	2.4 ± 3.5[a]	3.2 ± 5.1[a]	–	3.9 ± 5.8[a]	7.1 ± 10.0[ab]	9.5 ± 14.5
TE/D	–	–	3.6 ± 5.0	9.0 ± 9.2	–	–	9.3 ± 13.3[b]	21.9 ± 21.8
N	–	1.5 ± 2.3[a]	1.4 ± 2.6	1.5 ± 2.8	–	4.0 ± 6.5[a]	3.9 ± 7.1	3.4 ± 6.8
Normal eggs								
Control	39.6 ± 15.2	30.0 ± 11.3[a]	39.0 ± 14.4[a]	42.7 ± 12.9	–	77.3 ± 31.0[a]	94.1 ± 34.3[a]	99.5 ± 35.0
DY	–	29.4 ± 10.9[a]	36.7 ± 13.0[a]	37.7 ± 13.0[a]	–	77.6 ± 27.6[a]	88.3 ± 33.0[a]	91.4 ± 33.8
TE/D	–	–	38.2 ± 14.2	35.0 ± 15.7	–	–	88.5 ± 35.2	78.0 ± 37.4
N	–	33.6 ± 11.8	45.3 ± 13.6	51.3 ± 10.6	–	87.4 ± 31.4	105.6 ± 32.3	118.8 ± 26.5

Numbers with the same superscript for a trait in each column do not differ significantly ($P > 0.05$).

ment of the estimate, we can now also give confidence limits. However, it is only justified to give a regression line if the changes in genetic variances and covariances during the trial can be neglected. The frequency distributions and the phenotypic variances per generation of the traits 'laying of double-yolked eggs' and 'laying of two eggs a day' (Table 4) show that important alterations did occur. As these alterations in the selection lines far exceed those in the control strains, they could hardly be due to environmental influences only. The question arises whether the variance due to the environment (S_e^2) did increase proportionally to the genetic variance (S_g^2). One can well imagine that as far as the traits 'lay of double-yolked eggs' and 'lay of two eggs a day' are concerned, the chance exists that the expression of environmental influences increases with a decreasing number in the zero class. If so, heritability should not necessarily have changed much during the trial and it may be reasonable to calculate a weighted average.

Considering the above, realized heritabilities are estimated per selected generation, as well as over the whole period by means of the regression of the cumulative response to the cumulative selection differential. The estimates of the realized heritability are based on the ratio between the realized selection differential and the observed mean performance of the progeny. Because we are dealing with sex-limited characters, the selection differentials for the males were approached by the data for their full sisters. To minimize the influence of different environmental conditions from year to year, the observed response as well as the effective selection differential have been corrected, using the data from the control strain. The regression coefficient of the cumulative response on the cumulative selection differential and the estimated standard error are calculated by the method of Hill (1972b).

$$h^2 = b = \sum_{i=1}^{t} X_i S_i \; / \sum_{i=1}^{t} S_i^2$$

$$s^2 = V(b) = \left[\sum_{i=1}^{t} \sum_{j=1}^{t} S_i S_j \min(i, j)\, \sigma_d^2 \; / \sum_{i=1}^{t} S_i^2 + \sigma_e^2 \right] \sum_{i=1}^{t} S_i^2$$

t = Number of selected generations
X_i = Performance
S_i = Cumulative selection differential
S_j = Cumulative selection differential
$\min(i, j)$ = The lower i or j
σ_d^2 = Variance due to genetic drift
σ_e^2 = Variance due to error

A summary of the estimates is given in Table 5A.

Table 5. Estimates of the realized heritability. 1969/70, for instance, refers to parents selected in 1969 and offspring hatched and observed in 1970.

Line		80 days				200 days			
		1969/70	1970/71	1971/72	cumulative	1969/70	1970/71	1971/72	cumulative
A. Selection results expressed as mean performance.									
Double-yolked eggs									
DY	eff. S	2.5	1.5	3.6		–	1.7	4.1	
	obs. R	1.0	0.6	2.1		–	0.6	2.2	
	h^2	0.39	0.36	0.59	0.45 ± .12	–	0.38	0.53	0.48 ± .16
'First' eggs									
TE/D	eff. S	–	3.9	4.3		–	6.8	12.4	
	obs. R	–	1.2	5.4		–	2.6	12.9	
	h^2	–	0.31	1.24	(0.70 ± .17)	–	0.38	1.05	(0.76 ± .18)
Normal eggs									
N	eff. S	10.9	7.7	7.9		–	9.1	10.7	
	obs. R	3.6	2.6	2.2		–	1.4	7.8	
	h^2	0.33	0.34	0.28	0.33 ± .08	–	0.15	0.73	0.41 ± .26
B. Selection results expressed as the median value.									
Double-yolked eggs									
DY	eff. S	3.0	2.3	4.5		–	2.6	5.3	
	obs. R	0.8	0.4	2.1		–	0.4	2.1	
	h^2	0.26	0.18	0.48	0.31 ± .09	–	0.16	0.39	0.30 ± .11
'First' eggs									
TE/D	eff. S	–	4.7	6.3		–	8.9	17.9	
	obs. R	–	0.6	4.8		–	1.5	11.9	
	h^2	–	0.13	0.77	(0.44 ± .12)	–	0.17	0.67	(0.47 ± .13)

The effective selection differentials of the trait 'laying of double-yolked eggs' based on data for a 200-day collection period are somewhat higher than those based on a collection period of 80 days. This difference can be attributed mainly to the fact that not all the hens lay their double-yolked eggs within the first 80 days of the production period and this applies especially to the hens laying the greatest number of this kind of eggs. As to the heritability estimates from generation to generation, it can be seen that there is hardly any consistent change as was observed in other research (Lowry, 1967; Lowry & Abplanalp, 1967). It is therefore assumed that we are justified in calculating the regression of cumulative response against the cumulative selection differential in order to obtain the best possible estimate.

In contrast to the laying of double-yolked eggs, the laying of two eggs a day continued the whole laying period. Therefore it could be expected that data obtained from a laying period of 200 days should be more accurate than those obtained during the first 80 days. Sampling error could cause an extreme estimate of heritability when the data about the laying of two eggs a day are based on the short period and an estimate of 1.24 could be explained in this way. Because the data of only two generations are available and these figures are very different from each other, there is no justification for pooling them and they must be considered separately. An estimate of 1.05 based on a laying period of 200 days as was found in the column 1971/1972 of Table 5A is extremely high indeed and can be ascribed to chance.

However other explanations can also be given from the manner of collecting data as well as from gene action: the laying of two eggs a day has been measured by means of the oviposition of hard-shelled eggs characterized by additional shell deposition ('first eggs'). Although acceptable for most purposes, this method is not entirely reliable when the laying pattern is of common occurrence. Sometimes it has been observed that the second egg of a pair that can be identified by its compressed-sided shape (van Middelkoop, 1971) also showed the additional shell deposition characteristic for the first egg of a pair. Because these eggs are abnormal in two ways and are followed by an egg with a compressed side, this phenomenon of overlapping egg-pairs can be explained in the same way as single egg-pairs (van Middelkoop, 1971). The clue is that these overlapping pairs contain two 'first' eggs within a sequence of three eggs against one in the other case. The result is not only that the number of times 'two eggs a day' were laid is miscalculated by a factor of 1.5, but that it is also possible to get a large number of 'first' eggs without a notable increase in the number of sequences containing a 'first' egg. For the compressed-sided 'first' eggs have been counted in the same class as the normal shaped ones and overlapping egg-pairs did occur more often in 1972 than in 1971, it could be a cause of the overestimation of the obtained response and for that reason of the realized heritability.

It is also possible to give an explanation based on genetics. Such a rapid increase in the heritability in two generations of selection is possible when the frequency of the alleles involved is very low in the initial generation and if most of the alleles are recessive. An increase up to a value of near 1.0 can be reached when the expression of the ability to lay two eggs a day is influenced only a little by the environment.

Finally I tried to check whether or not the traits 'laying of double-yolked eggs' or the laying of 'two eggs a day' have a threshold in the visible scale. At the same time, the influence of a skew distribution on the accuracy of the estimate of the realized heritability

will be discussed. For that purpose the response was based on the median instead of on the average value. The median better approaches the true average when negative values have to be ascribed to a certain proportion of the birds in the zero class. Should a threshold indeed exist, then a low response in the first year could be due to the influence of birds with a negative ability for the trait concerned when the results were based on the mean value. The influence of these can be eliminated to a certain extent with the median. The estimates from year to year would then be less variable, especially through a higher estimate based on the first selected generation.

For double-yolked eggs the results obtained by this procedure are similar to those based on the observed mean value and provide no reason for assuming the existence of a threshold. The only important difference is that this estimate provides a lower value for the realized heritability.

For the laying of two eggs a day as established from the number of 'first' eggs, estimates of heritability were lower than those based on the progenies mean performance as was so for double-yolked eggs. The differences between the two procedures were especially striking in last generation selected. Although for the above trait there is no more indication of a threshold than for double-yolked eggs, there is another fact needing special comment. Looking at Figure 3, one can see that a number of pullets of the TE/D line laid extremely many 'first' eggs. Such pullets have a great influence on the average value, because there are no extremes at the other end of the frequency distribution, which ends abruptly at zero.

The median value being less sensitive to abruptly skew distributions is a more suited yardstick to express the selection results obtained. Therefore the estimate of the realized heritability based on the median value best represents the real value. Hence the realized heritability for the laying of two eggs a day or, better, for the laying of 'first' eggs is expected to be between 0.5 and 1.0. This range is derived from the results with the last generation selected, because with that hatch the fewest pullets are found in the zero class. An analogous explanation can be given with the estimate of the heritability of double-yolked eggs and the estimate based on the median performance is seen as the best approximation.

In this study, additional data on the genetic basis of the laying pattern were also obtained from the selection in the normal-egg (N) line. Because all lines were reared and housed together for the whole trial, the results obtained in the N line provide clear proof of the existence of a genetic control over laying pattern. The estimate of the realized heritability of normal egg laying found in the above line of 0.3–0.4 agrees fairly well with estimates of this trait in egg-type pullets given by other authors (Kinney, 1969). This agreement confirms the supposition that the genetic basis of control over egg production in meat-type pullets differs little from that in specific laying hens. The difference in laying pattern usually found can be ascribed rather to a temporary surplus in yolk formation or to an irregular supply of mature follicles in the ovary. However the laying of two eggs a day should be viewed from a different angle.

Total yolk production and normal egg laying

It could be imagined that the genetic control of egg production could be traced back to the number of maturing and ovulating follicles in the ovary and that the rate of yolk

Table 6. Estimates of heritability and standard error from daughter/dam regression.

Line	80 days				200 days			
	1969/70	1970/71	1971/72	wtd mean	1969/70	1970/71	1971/72	wtd mean
Total number of yolks								
Control	0.26 ± 0.21	0.22 ± 0.23	0.77 ± 0.19	0.42 ± 0.12	–	0.70 ± 0.26	0.93 ± 0.19	0.82 ± 0.16
DY	0.57 ± 0.36	0.55 ± 0.23	0.50 ± 0.17	0.53 ± 0.14	–	0.48 ± 0.28	0.85 ± 0.22	0.70 ± 0.17
TE/D	–	0.88 ± 0.30	0.42 ± 0.27	0.67 ± 0.20	–	0.50 ± 0.24	0.31 ± 0.22	0.41 ± 0.16
N	0.01 ± 0.41	0.39 ± 0.39	0.96 ± 0.30	0.27 ± 0.21	–	0.02 ± 0.29	0.83 ± 0.29	0.47 ± 0.20
				0.42 ± 0.09				0.58 ± 0.09
Normal eggs								
Control	0.58 ± 0.28	0.00 ± 0.25	0.65 ± 0.19	0.43 ± 0.14	–	0.35 ± 0.31	0.41 ± 0.22	0.38 ± 0.19
DY	0.60 ± 0.27	0.20 ± 0.37	0.54 ± 0.20	0.33 ± 0.16	–	0.03 ± 0.47	0.67 ± 0.25	0.39 ± 0.25
TE/D	–	0.62 ± 0.29	0.09 ± 0.32	0.29 ± 0.21	–	0.68 ± 0.23	0.02 ± 0.30	0.36 ± 0.19
N	0.47 ± 0.63	0.10 ± 0.44	0.83 ± 0.34	0.49 ± 0.26	–	0.62 ± 0.30	0.82 ± 0.30	0.73 ± 0.21
				0.41 ± 0.11				0.51 ± 0.11

Table 7. Estimates of heritability from analysis of variance.

	1969			1970			1971
Line	h_s^2	h_d^2	h_{d+s}^2	h_s^2	h_d^2	h_{d+s}^2	h_s^2
Normal eggs, 80 days							
Control	0.52 ± 0.26	0.41 ± 0.19	0.46 ± 0.15	0.55 ± 0.35	0.25 ± 0.38	0.40 ± 0.19	0.43 ± 0.33
DY	–	–	–	0.12 ± 0.34	0.74 ± 0.53	0.43 ± 0.23	0.51 ± 0.37
TE/D	–	–	–	–	–	–	0.58 ± 0.32
N	–	–	–	0.08 ± 0.23	0.85 ± 0.31	0.46 ± 0.16	0.36 ± 0.21
Normal eggs, 200 days							
Control	–	–	–	0.91 ± 0.54	0.57 ± 0.43	0.74 ± 0.27	0.45 ± 0.35
DY	–	–	–	0.35 ± 0.26	0.68 ± 0.47	0.17 ± 0.19	0.76 ± 0.48
TE/D	–	–	–	–	–	–	0.88 ± 0.44
N	–	–	–	0.46 ± 0.29	0.58 ± 0.26	0.52 ± 0.17	0.60 ± 0.30
Total number of yolks, 80 days							
Control	0.74 ± 0.30	0.40 ± 0.18	0.57 ± 0.17	0.64 ± 0.41	0.06 ± 0.32	0.29 ± 0.22	0.18 ± 0.28
DY	–	–	–	0.67 ± 0.45	0.45 ± 0.38	0.56 ± 0.25	0.15 ± 0.33
TE/D	–	–	–	–	–	–	0.71 ± 0.40
N	–	–	–	0.06 ± 0.22	0.72 ± 0.30	0.39 ± 0.15	0.50 ± 0.46
Total number of yolks, 200 days							
Control	–	–	–	1.41 ± 0.64	0.35 ± 0.33	0.88 ± 0.32	0.33 ± 0.38
DY	–	–	–	0.49 ± 0.42	0.31 ± 0.36	0.40 ± 0.24	0.34 ± 0.40
TE/D	–	–	–	–	–	–	1.22 ± 0.58
N	–	–	–	0.31 ± 0.24	0.74 ± 0.28	0.52 ± 0.16	0.78 ± 0.34

production determines whether normal or abnormal eggs will be laid. If so in the abnormal-egg lines, the heritability of yolk production would be expected to be higher than the heritability of normal-egg production. To compare the genetic basis of normal egg production with that of total yolk production, the heritability of the two traits has been estimated in all lines from the regression of daughter on dam as well as from variance analysis. The estimates derived from the offspring-parent relationship are calculated from the intra-sire regression of daughter on dam (Becker, 1964). With the other estimates, the variance components have been analysed after the method of King & Henderson (1954) to obtain the sire's component of variance and that of the dam.

As can be seen in Tables 6 and 7, the estimates vary widely, making conclusions difficult to draw in detail. In general, however, no systematic difference seems to exist between the estimate of heritability for normal egg production from that for total yolk production. Even when only the results of the first 80 laying days are considered, when the difference between normal egg production and the total number of yolks is largest, no such systematic trend could be detected. Because the different lines are developed from the same strain and have been selected for three generations, it may be justified to pool

		1972			Weighted mean		
h_d^2	h_{d+s}^2	h_s^2	h_d^2	h_{d+s}^2	h_s^2	h_d^2	h_{d+s}^2
0.39 ± 0.33	0.41 ± 0.18	0.03 ± 0.29	0.65 ± 0.35	0.34 ± 0.17	0.40 ± 0.14	0.42 ± 0.16	0.42 ± 0.07
0.02 ± 0.29	0.25 ± 0.21	0.58 ± 0.39	0.23 ± 0.20	0.41 ± 0.21	0.41 ± 0.22	0.25 ± 0.20	0.35 ± 0.12
0.39 ± 0.36	0.48 ± 0.20	1.12 ± 0.64	0.06 ± 0.23	0.59 ± 0.33	0.80 ± 0.37	0.26 ± 0.20	0.53 ± 0.18
0.36 ± 0.23	0.36 ± 0.13	0.54 ± 0.28	0.22 ± 0.15	0.38 ± 0.15	0.31 ± 0.15	0.47 ± 0.13	0.40 ± 0.09
					0.43 ± 0.10	0.38 ± 0.08	0.42 ± 0.07
0.68 ± 0.36	0.56 ± 0.20	0.69 ± 0.43	0.19 ± 0.23	0.44 ± 0.22	0.70 ± 0.24	0.48 ± 0.18	0.58 ± 0.13
0.56 ± 0.33	0.66 ± 0.27	0.32 ± 0.27	0.30 ± 0.23	0.31 ± 0.16	0.27 ± 0.18	0.50 ± 0.20	0.41 ± 0.13
0.18 ± 0.29	0.53 ± 0.24	1.00 ± 0.55	0.00 ± 0.26	0.50 ± 0.29	0.93 ± 0.36	0.11 ± 0.19	0.52 ± 0.18
0.39 ± 0.22	0.49 ± 0.17	0.34 ± 0.23	0.42 ± 0.20	0.38 ± 0.14	0.47 ± 0.15	0.47 ± 0.13	0.47 ± 0.09
					0.57 ± 0.10	0.41 ± 0.09	0.49 ± 0.07
0.36 ± 0.36	0.27 ± 0.17	0.40 ± 0.42	0.99 ± 0.37	0.69 ± 0.22	0.51 ± 0.17	0.42 ± 0.17	0.49 ± 0.07
0.73 ± 0.41	0.44 ± 0.21	0.08 ± 0.18	0.87 ± 0.37	0.39 ± 0.15	0.25 ± 0.16	0.71 ± 0.22	0.45 ± 0.12
0.52 ± 0.36	0.61 ± 0.24	0.06 ± 0.15	0.13 ± 0.31	0.04 ± 0.14	0.41 ± 0.20	0.36 ± 0.24	0.38 ± 0.15
0.02 ± 0.18	0.24 ± 0.23	0.55 ± 0.32	0.52 ± 0.19	0.54 ± 0.17	0.36 ± 0.20	0.41 ± 0.13	0.39 ± 0.11
					0.41 ± 0.09	0.47 ± 0.09	0.44 ± 0.07
0.79 ± 0.36	0.56 ± 0.21	0.54 ± 0.43	0.62 ± 0.31	0.58 ± 0.22	0.82 ± 0.26	0.60 ± 0.20	0.67 ± 0.15
0.90 ± 0.39	0.62 ± 0.24	0.04 ± 0.18	0.70 ± 0.34	0.33 ± 0.14	0.28 ± 0.16	0.69 ± 0.21	0.46 ± 0.13
0.10 ± 0.24	0.66 ± 0.30	0.12 ± 0.22	0.18 ± 0.34	0.15 ± 0.16	0.78 ± 0.29	0.13 ± 0.21	0.46 ± 0.19
0.13 ± 0.17	0.45 ± 0.18	0.33 ± 0.23	0.52 ± 0.21	0.43 ± 0.14	0.48 ± 0.15	0.47 ± 0.13	0.47 ± 0.09
					0.59 ± 0.10	0.49 ± 0.09	0.51 ± 0.07

all single estimates. In doing so, it appears that the average estimate of normal egg production and that of total number of yolks are about equal and that it makes no difference whether they relate to a production period of 80 or 200 days. Nor does it matter whether they are based on a daughter-dam regression or on variance analysis. This result could indicate that the genetic basis controlling yolk production as such is not different from that controlling the formation of normal eggs from the engulfed yolks. Therefore it should be borne in mind that it is quite possible that other genetic factors are involved in abnormal eggs, for instance factors controlling the regular supply of mature follicles in the ovary. This is supported by the same total yolk production in 200 days after selection for normal eggs and after selection for double-yolked eggs, whereas the production up to 80 days differed significantly between those lines in 1971 and 1972 (Table 12).

Correlations

Correlations have been calculated between total number of yolks, double-yolked eggs, two eggs per day and normal eggs, and in addition between these traits and 8-week bodyweight. The phenotypic correlations (r_p) of double-yolked eggs and two eggs a day with the other traits were calculated according to the method of Spearman because of the abnormal shape of the frequency distributions of these traits. A summary of the phenotypic correlations is given in Table 8. All correlations are given per generation, as a weighted mean per line, and as a weighted mean over all lines. Summarizing the single estimates into a weighted value, however, was only justified in the absence of differences between years and lines with regard to the real value of r_p. Because the real value was not known, the pooled values were not tested for significance. The best is to look at the estimates per line and per generation. From Table 8, it can be seen that r_p in all lines between total number of yolks and number of double-yolked eggs laid estimated over a period of 200 days is not as large as for the r_p between total number of yolks and number of normal eggs laid. For the first 80 days of lay, the same is seen only in the control strain and in the N line. Therefore these correlations do not give an indication of the laying of double-yolked eggs being caused simply by an overformation of yolks in the ovary, through which the chance of the occurrence of double ovulations increases. A shift rather seems manifest in the direction of an increased yolk production at the beginning of the laying period (Table 12). In this way can be explained too why Abplanalp (1970) found that double-yolked egg laying in White Leghorns can be markedly increased without the yolk production being increased. A positive correlation was found too between the laying of two eggs a day and the total yolk production, but was not strikingly large.

The genetic relationship (Table 9) has been estimated by means of the rank correlation between the mean half-sib values, which are a measure of the breeding value of the sires. Hence it was assumed that the rank correlation of two traits based on those means is a fair approximation of the genetic correlation (r_g). If this procedure is correct the values of r_g for total number of yolks with double-yolked eggs as well as for normal eggs show the same pattern as the phenotypic correlations. The weighted mean value of r_g was calculated, but its significance was not tested, for it is doubtful whether r_g has the same value in the successive generations, because it is unknown if the r_g is based upon linkage between alleles or upon pleiotropic effects. The calculated correlations between double-

Table 8. Survey of phenotypic correlation coefficients (* $P < 0.05$, ** $P < 0.01$).

Line	80 days					200 days				
	1969	1970	1971	1972	wtd mean	1969	1970	1971	1972	wtd mean
Total yolk number X double-yolked eggs										
Control	+0.36**	+0.44**	+0.54**	+0.47**	+0.44	–	+0.30**	+0.35**	+0.27**	+0.30
DY	–	+0.53**	+0.57**	+0.77**	+0.66	–	+0.32**	+0.43**	+0.55**	+0.46
TE/D	–	–	+0.51**	+0.49**	+0.51	–	–	+0.34**	+0.32**	+0.33
N	–	+0.36**	+0.49**	+0.47**	+0.45	–	+0.30**	+0.43**	+0.35**	+0.36
					+0.50					+0.37
Total yolk number X two eggs a day										
Control	+0.26**	+0.23**	+0.28**	+0.10	+0.23	–	+0.28**	+0.24**	+0.05	+0.20
DY	–	+0.23**	+0.15*	+0.16**	+0.18	–	+0.14	+0.08	+0.21**	+0.15
TE/D	–	–	+0.09	+0.12	+0.10	–	–	+0.09	+0.14	+0.11
N	–	+0.17**	+0.13*	+0.10*	+0.13	–	+0.13*	+0.12*	+0.01	+0.08
					+0.17					+0.13
Total yolk number X normal eggs										
Control	+0.84**	+0.62**	+0.66**	+0.55**	+0.72	–	+0.84**	+0.78**	+0.78**	+0.80
DY	–	+0.54**	+0.46**	+0.13*	+0.35	–	+0.77**	+0.71**	+0.60**	+0.68
TE/D	–	–	+0.59**	+0.19*	+0.43	–	–	+0.73**	+0.43**	+0.61
N	–	+0.58**	+0.78**	+0.58**	+0.66	–	+0.85**	+0.86**	+0.81**	+0.84
					+0.61					+0.78
Double-yolked eggs X two eggs a day										
Control	+0.23**	+0.10	+0.13	−0.00	+0.14	–	+0.07	+0.12	+0.00	+0.07
DY	–	+0.12	+0.10	+0.10	+0.10	–	+0.11	+0.04	+0.12	+0.09
TE/D	–	–	+0.03	+0.12	+0.07	–	–	+0.01	+0.16*	+0.08
N	–	+0.06	+0.17**	+0.16**	+0.13	–	+0.09	+0.15**	+0.18**	+0.15
					+0.12					+0.11
Double-yolked eggs X normal eggs										
Control	−0.00	−0.08	+0.01	−0.10	−0.04	–	+0.08	+0.09	+0.02	+0.06
DY	–	−0.06	−0.23**	−0.28**	−0.21	–	−0.00	+0.01	+0.01	+0.01
TE/D	–	–	+0.06	−0.11	−0.02	–	–	+0.11	−0.04	+0.04
N	–	−0.17**	+0.05	−0.04	−0.05	–	+0.04	+0.21**	+0.09	+0.11
					−0.07					+0.07
Two eggs a day X normal eggs										
Control	−0.06	−0.11	−0.09	−0.27**	−0.12	–	−0.09	−0.20**	−0.37**	−0.22
DY	–	−0.06	−0.27**	−0.32**	−0.24	–	−0.23**	−0.38**	−0.38**	−0.34
TE/D	–	–	−0.39**	−0.79**	−0.61	–	–	−0.46**	−0.75**	−0.61
N	–	+0.20**	−0.18**	−0.30**	−0.24	–	−0.22**	−0.22**	−0.37**	−0.28
					−0.25					−0.33

Table 9. Survey of estimated genetic correlation coefficients.

Line	80 days					200 days				
	1969	1970	1971	1972	wtd mean	1969	1970	1971	1972	wtd mean
Total yolk number × double-yolked eggs										
Control	+0.70**	+0.64**	+0.53	−0.19	+0.55	–	+0.33	+0.29	−0.27	+0.18
DY	–	+0.68	+0.30	+0.60	+0.51	–	+0.76*	+0.21	+0.35	+0.44
TE/D	–	–	+0.40	+0.43	+0.41	–	–	+0.55	+0.13	+0.41
N	–	+0.50	+0.35	+0.66*	+0.51	–	−0.17	+0.14	+0.60*	+0.21
					+0.52					+0.28
Total yolk number × two eggs a day										
Control	−0.22	+0.09	+0.26	−0.25	−0.04	–	+0.51*	+0.36	−0.09	+0.33
DY	–	+0.10	0.00	−0.06	+0.01	–	+0.38	−0.20	+0.02	+0.03
TE/D	–	–	+0.65*	−0.05	+0.27	–	–	+0.40	−0.14	+0.20
N	–	+0.71**	+0.35	−0.41	+0.43	–	+0.47	+0.50	−0.28	+0.26
					+0.12					+0.22
Total yolk number × normal eggs										
Control	+0.91**	+0.57*	+0.62*	+0.63*	+0.76	–	+0.80**	+0.60*	+0.67*	+0.72
DY	–	+0.46	+0.55	−0.20	+0.32	–	+0.69	+0.80**	+0.30	+0.65
TE/D	–	–	+0.22	−0.02	+0.13	–	–	+0.53	+0.48	+0.51
N	–	+0.04	+0.60*	+0.84**	+0.57	–	+0.73**	+0.69**	+0.92**	+0.81
					+0.59					+0.72
Double-yolked eggs × two eggs a day										
Control	−0.20	−0.11	−0.06	−0.18	−0.14	–	−0.10	−0.28	−0.19	−0.18
DY	–	+0.05	−0.55	+0.07	−0.22	–	−0.26	−0.28	+0.16	−0.14
TE/D	–	–	+0.14	+0.29	+0.20	–	–	+0.16	+0.31	+0.22
N	–	+0.37	+0.50	−0.20	+0.25	–	+0.29	+0.18	−0.29	+0.06
					−0.00					−0.04
Double-yolked eggs × normal eggs										
Control	+0.46*	+0.10	+0.16	−0.32	+0.19	–	+0.01	+0.12	−0.14	+0.01
DY	–	+0.02	−0.16	−0.65	−0.30	–	+0.57	−0.18	−0.42	−0.05
TE/D	–	–	+0.21	−0.36	−0.01	–	–	+0.01	−0.14	−0.05
N	–	−0.50	−0.27	+0.37	−0.15	–	−0.67*	−0.17	+0.50	−0.14
					−0.02					−0.06
Two eggs a day × normal eggs										
Control	−0.31	−0.16	−0.04	−0.64*	−0.28	–	+0.17	−0.21	−0.67*	−0.18
DY	–	−0.49	−0.17	−0.18	−0.26	–	+0.16	−0.34	−0.76*	−0.40
TE/D	–	–	−0.17	−0.98**	−0.76	–	–	−0.33	−0.90**	−0.65
N	–	−0.41	−0.33	−0.61*	−0.45	–	−0.08	−0.01	−0.48	−0.20
					−0.40					−0.32

yolked eggs, two eggs a day and normal eggs are given indeed, but it is dangerous to use these data without comment. A negative or a low positive correlation between these traits can easily be found through the fact that these traits are not entirely independent. If the incidence of one of these traits increases, the other ones automatically decrease: the total number of yolks, being a constant for each hen, constitutes the sum of the number of yolks in normal eggs, double-yolked eggs, two eggs a day, as well as in other forms of abnormal eggs. If one only considers the estimates based on a 200-day laying period in the control strain – this period is fairly long and the incidence of abnormal eggs in this strain is not to high – no clear correlation seems to exist between the laying of two eggs a day and of double-yolked eggs. If a correlation is still assumed to exist, the genetic one is expected to be negative and the phenotypic one positive.

The last, but not the least important, question to discuss is the relation – phenotypic as well as genetic – between 8-week bodyweight and the laying pattern. Of these two correlations, the genotypic one is most important, because it may tell us how far high growth rate can be combined with high egg production in one hen. As to the phenotypic values, the r_p of 8-week bodyweight with double-yolked eggs and with two eggs a day is found to be positive, while with normal egg laying it was negative (Table 10). This finding supports the existing idea that the heavier the pullet, the fewer normal eggs laid.

Significant values for r_g are found only of 8-week bodyweight with total yolk production and with number of normal eggs (Table 11). Except in one estimate, these significant correlations were negative and therefore in agreement with other data about correlated responses (Jaap et al., 1962; Merritt et al., 1966; Ideta & Siegel, 1966; Siegel, 1963, 1970). A clear explanation of this exception cannot be given. That no significant genetic correlations were found between 8-week bodyweight and the laying of double-yolked eggs and between 8-week bodyweight and the production of two eggs a day, does not, however, imply, that such correlations do not exist. It is even possible that abnormal egg laying and the 8-week bodyweight are positively correlated. Further research is necessary in order to find out whether genetic correlation – even a small one – exists between the laying of two eggs a day and 8-week bodyweight. The same remark has to be made about the correlation between the laying of double-yolked eggs and the 8-week bodyweight.

Correlated responses

The best way – and also the most practical one – of checking the influence selection for one trait has upon other traits is the comparison of the population means for the unselected traits from year to year. By this means, one can correct for the annual changes in environmental factors with the help of the performance of the pullets from the control strain. The summary given in Table 12 shows that the 8-week bodyweight of the pullets in the N line remained unchanged, while the normal egg production was significantly higher than for the control strain. This result has also been found by Jaap & Khan (1972). Pullets from the DY and the TE/D line did reach a significant higher 8-week bodyweight in 1972 (Table 12). These correlated results for the abnormal-egg lines are in agreement with those of Udale et al. (1972), who selected for a higher juvenile bodyweight and observed a concomittant increase in abnormal egg laying during the first 29 days of lay.

Table 10. Phenotypic correlation between 8-week bodyweight and laying traits.

Line	80 days					200 days				
	1969	1970	1971	1972	wtd mean	1969	1970	1971	1972	wtd mean
Total yolk production										
Control	−0.09	−0.09	−0.08	−0.02	−0.08	–	−0.13*	−0.12	−0.10	−0.12
DY	–	−0.16	−0.01	+0.13*	+0.01	–	−0.13	−0.02	−0.01	−0.04
TE/D	–	–	−0.03	+0.11	+0.03	–	–	−0.12	+0.12	−0.01
N	–	+0.17**	−0.07	+0.09	+0.06	–	+0.07	−0.04	+0.03	+0.02
					−0.00					−0.04
Double-yolked eggs										
Control	+0.12**	+0.11	+0.02	+0.21**	+0.12	–	+0.10	+0.01	+0.23**	+0.12
DY	–	+0.05	+0.17*	+0.15*	+0.13	–	+0.07	+0.19**	+0.16**	+0.15
TE/D	–	–	+0.04	+0.14	+0.09	–	–	+0.04	+0.15	+0.09
N	–	+0.35**	+0.11	+0.26**	+0.24	–	+0.34**	+0.13*	+0.26**	+0.24
					+0.16					+0.17
Two eggs a day										
Control	+0.05	+0.04	−0.01	+0.18**	+0.06	–	+0.04	−0.03	+0.11	+0.04
DY	–	+0.00	+0.11	+0.08	+0.07	–	−0.05	+0.10	+0.01	+0.02
TE/D	–	–	+0.16*	+0.10	+0.13	–	–	+0.13	+0.16*	+0.14
N	–	+0.17**	+0.13*	+0.19**	+0.17	–	+0.18**	+0.08	+0.19**	+0.15
					+0.11					+0.09
Normal eggs										
Control	−0.07	−0.14*	−0.02	−0.19**	−0.10	–	−0.18**	−0.09	−0.19**	−0.16
DY	–	−0.21**	−0.22**	−0.22**	−0.22	–	−0.12	−0.15*	−0.17**	−0.16
TE/D	–	–	−0.16*	−0.09	−0.13	–	–	−0.20**	−0.07	−0.14
N	–	−0.12*	−0.15**	−0.10*	−0.12	–	−0.08	−0.06	−0.07	−0.07
					−0.13					−0.12

Table 11. Estimates of genetic correlation between 8-week bodyweight and laying traits.

Line	80 days					200 days				
	1969	1970	1971	1972	wtd mean	1969	1970	1971	1972	wtd mean
Total yolk production										
Control	−0.37	−0.20	−0.62*	−0.48	−0.40	–	−0.24	−0.63*	−0.42	−0.42
DY	–	−0.70*	−0.25	+0.23	−0.26	–	−0.90**	−0.23	+0.20	−0.40
TE/D	–	–	+0.03	+0.69	+0.33	–	–	−0.26	+0.79*	+0.24
N	–	+0.51	+0.19	+0.08	+0.27	–	+0.21	+0.20	+0.10	+0.17
					−0.11					−0.14
Double-yolked eggs										
Control	−0.40	+0.08	−0.17	−0.05	−0.17	–	+0.12	−0.27	−0.03	−0.04
DY	–	−0.60	−0.28	+0.27	−0.21	–	−0.60	−0.20	+0.38	−0.14
TE/D	–	–	−0.32	+0.40	−0.04	–	–	−0.36	+0.38	−0.08
N	–	+0.33	+0.27	+0.20	+0.27	–	+0.50	+0.29	+0.17	+0.33
					−0.04					+0.06
Two eggs a day										
Control	−0.38	−0.30	−0.13	+0.50	−0.18	–	−0.18	−0.11	+0.42	−0.01
DY	–	−0.43	+0.18	−0.31	−0.14	–	−0.52	+0.21	−0.32	−0.17
TE/D	–	–	+0.11	+0.05	+0.09	–	–	+0.26	+0.21	+0.24
N	–	+0.26	−0.07	−0.01	+0.06	–	+0.03	−0.25	−0.12	−0.12
					−0.07					−0.05
Normal eggs										
Control	−0.23	−0.34	−0.36	−0.51	−0.33	–	−0.39	−0.59*	−0.58	−0.50
DY	–	−0.38	−0.29	−0.40	−0.35	–	−0.81*	−0.18	−0.08	−0.38
TE/D	–	–	−0.19	−0.14	−0.17	–	–	−0.30	+0.12	−0.14
N	–	−0.14	+0.06	−0.12	−0.06	–	−0.05	+0.18	+0.11	+0.08
					−0.24					−0.25

Table 12. Performance of unselected traits given per line and per generation (mean ± standard error).

Line	1969	1970	1971	1972
8-week bodyweight				
Control	1017 ± 99.7	1075 ± 98.8[a]	1105 ± 95.5[a]	1098 ± 123.0[a]
DY	–	1115 ± 96.0	1116 ± 97.5[a]	1125 ± 91.9[b]
TE/D	–	–	1114 ± 89.8[a]	1138 ± 102.2[b]
N	–	1080 ± 98.6[a]	1082 ± 92.1	1081 ± 103.9[a]
Age at first egg				
Control	151 ± 13.0	145 ± 14.6[a]	146 ± 13.8[ab]	145 ± 12.0[a]
DY	–	144 ± 18.0[a]	145 ± 10.2[a]	147 ± 14.2[ab]
TE/D	–	–	145 ± 12.2[a]	148 ± 8.2[b]
N	–	145 ± 12.2[a]	148 ± 21.4[b]	147 ± 11.6[ab]
Total yolk number, 80 days				
Control	49.2 ± 14.9	45.4 ± 13.7[a]	54.2 ± 16.1[a]	58.4 ± 13.0[a]
DY	–	48.4 ± 14.9[a]	58.8 ± 15.5	68.1 ± 15.4
TE/D	–	–	55.0 ± 13.9[a]	59.6 ± 10.8[a]
N	–	47.0 ± 12.0[a]	54.4 ± 14.5[a]	60.4 ± 10.1[a]
Total yolk number, 200 days				
Control	–	100.4 ± 34.2[a]	119.4 ± 34.7[a]	123.2 ± 32.4[a]
DY	–	104.1 ± 37.3[ab]	122.2 ± 34.0[a]	135.6 ± 24.7[b]
TE/D	–	–	116.6 ± 32.2[a]	125.2 ± 35.5[a]
N	–	106.7 ± 32.3[b]	120.9 ± 32.6[a]	132.2 ± 27.1[b]

Numbers with the same superscript letter for a trait in each column do not differ significantly ($P > 0.05$).

The results in our DY line can be interpreted as confirmation of the findings of Lowry & Abplanalp (1967) who found an increase in adult bodyweight of 3 % after selection for double-yolked eggs in White Leghorns.

The selection trial thus shows that a marked improvement in normal egg production can be achieved by selection without a concomittant significant loss of 8-week bodyweight. Not yet certain, however, is whether there is no genetic correlation between juvenile bodyweight and normal egg laying and with the one or other type of abnormal egg production. Although no significant genetic correlation between abnormal egg laying and growth rate was found in this study, a positive relationship was expected. This expectation was based on the results of our own selection trial and also on those of Udale et al. (1972). Research on this subject with turkeys causes one also, to expect a positive genetic correlation between growth rate and abnormal egg laying (Nestor & Bacon, 1972). So further research is also needed on the cause of the laying of double-yolked eggs and of the laying of two eggs a day.

Conclusions from the trial

1. Laying of both normal and of abnormal eggs is controlled to an important extent by genetic factors. The heritability of the laying of normal eggs, double-yolked eggs and of

two eggs a day in the investigated strain were estimated to have values of about 0.4–0.5, 0.3–0.5 and 0.5–0.7, respectively.
2. Abnormal egg laying has at least two different genetic causes, which are manifest as the laying of double-yolked eggs and of two eggs a day. It is possible to select for the one trait without an important change in the incidence of the other.
3. Although specific genes are suspected to play an important role, a positive genetic correlation between total yolk production and abnormal egg laying seems to exist.
4. Selection results indicate that abnormal egg laying tends to be positively correlated with 8-week bodyweight, while normal egg production tends to be negatively correlated.

Summary

In 1969, a selection trial was started by the development of one line selected for double-yolked egg laying and another for the production of normal eggs from the Institute's White Plymouth Rock strain. Simultaneously a control strain was developed and kept together with the selection lines. In 1970, a third line had been initiated by the selection for the laying of two eggs a day.

The laying performance has been measured over fixed periods of 80 and 200 days after each hen laid its first egg. From the results obtained with those selection lines, realized heritability of the selected traits were calculated after correction for annual influences with the help of the control strain. Heritability of total yolk production and of normal egg laying were estimated, both from the regression of daughter on dam and from an analysis of variance. The estimates of these traits were similar, and the laying of double-yolked eggs and of two eggs a day were highly heritable. Therefore specific genes are expected to play an important role.

Phenotypic (r_p) and genetic (r_g) correlations were calculated between the traits normal egg production, double-yolked eggs, laying of two eggs a day, total yolk production and 8-week bodyweight. Both the r_g and r_p between total yolk production and the other laying traits proved to be positive, although the correlation with the laying of two eggs a day was markedly smaller.

The relations between 8-week bodyweight and the distinguished laying traits do not show a distinct pattern: both negative and positive values were found. The observed selection results show that normal egg production can be increased significantly without a change in growth rate from the control strain. Selection for double-yolked eggs and the laying of two eggs a day, however, resulted in a simultaneously higher 8-week bodyweight.

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7 Influence of the dwarfing gene on yolk production and its consequences for normal egg laying of White Plymouth Rock pullets

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Introduction

It has been regularly demonstrated that laying hens carrying the sex-linked dwarfing gene, *dw*, have not hitherto been able to compete in egg production with their normal-sized sisters or comparable groups (Hutt, 1953, 1959; Bernier & Arscott, 1960, 1972; French & Nordskog, 1969; Mérat, 1969, 1972; Rapp, 1970; Selvarajah et al., 1970; Mohammadian, 1971; Rajaratnam et al., 1971; Quisenberry, 1972). At best, the dwarfs laid at the same rate as the normal layers (French & Nordskog, 1969; Selvarajah et al., 1970). Studies on the influence of the dwarfing gene in meat-type pullets showed that fewer abnormal eggs are laid (Jaap & Mohammadian, 1969; Mohammadian, 1971), but no reduction in normal egg production was observed (Prod'homme & Mérat, 1969; Jaap & Mohammadian, 1969; Mohammadian, 1971). In one case it was even possible to demonstrate a significant increase in the laying rate (Ricard & Cochez, 1972).

The dwarfing gene is known to reduce the number of follicles in the ovary in their stage of rapid growth and to shorten the duration of this stage (Jaap & Mohammadian, 1969; Mohammadian, 1971). It is therefore probable that the different effect of *dw* on the normal egg production of meat- and egg-type pullets is affected via reduced yolk formation (Jaap, 1969; Mohammadian, 1971; Kuit & van Middelkoop, 1972). It has recently been shown that most abnormalities in egg formation and egg laying result from ovulations occurring at intervals of lesser than 20–21 h (van Middelkoop, 1972b), both yolks usually being lost for normal egg formation. Normal eggs are formed when the interval between two ovulations exceeds 21 h. A too rapid succession of ovulations is possible when an excessive number of follicles in the ovary is in their stage of rapid growth. From this it can be inferred that, when excessive yolk formation in the ovary is reduced by means of the effect of the dwarfing gene, *dw*, this results in fewer abnormal eggs and a simultaneous increase in the production of normal ones. The object of the present study was to test this hypothesis and to determine whether the effect of *dw* on normal egg production can be predicted from the laying pattern.

Materials and methods

Heavy *Dwdw* cocks from a commercial breeder were each mated to hens chosen from three different experimental White Plymouth Rock lines, which were selected from one starting-population for two generations on the basis of the following criteria:

a. line DY for the laying of Double-Yolked eggs;
b. line TE/D for the laying of Two Eggs a Day;
c. line N for the laying of only Normal hard-shelled eggs.

In total 9 *Dwdw* cocks and 108 *Dw-* hens were used, but 21 of the hens gave no

daughters at all, or after the rearing period the daughters appeared to be either *Dw-* or *dw-*.

The chicks were hatched on 26th October and 9th November, 1971. They were reared on the floor and fed ad libitum. A minimum daylength of 14 h was maintained. At the age of 16 w the birds were placed in individual laying cages, with the exception of pullets without a full sister of the other genotype, those with an extreme 8-w bodyweight within their group, or those culled at random when the number of full-sibs of one genotype exceeded five. After this procedure the experiment was started with 382 pullets.

During the laying period the hens were kept in natural daylight and provided with a minimum daylength of 14 h using additional artificial light. The birds were fed a ration containing 2700 kcal ME per kg and 15.4 % crude protein ad libitum.

The egg-laying pattern was registered by recording the type of eggs laid daily by each bird. The eggs were classified according to the amount of shell deposition, shape, presence of yolk material on the shell, and number of yolks they contained. Of the various types of eggs laid, we chose double-yolked eggs, hard-shelled eggs with additional shell deposition (van Middelkoop, 1971) and entirely normal eggs, as being most representative of the laying pattern:

– double-yolked eggs represent the occurrence of two ovulations, one shortly after the other;

– hard-shelled eggs with additional shell deposition represent the laying of two eggs a day (van Middelkoop, 1971, 1972a) as a result of two ovulations occurring in separate ovulation periods, but still a few hours closer to each other than the time needed for the formation of a normal egg;

– entirely normal eggs being laid when the ovulation rate does not exceed the limit of one yolk a day (van Middelkoop, 1972b).

The period during which egg-laying is recorded is the same for each pullet, viz. a fixed period after the bird laid her first egg.

Results and discussion

As several authors have also found (Hutt, 1953, 1959; Bernier & Arscott, 1960, 1972; Mérat, 1969, 1972; Rapp, 1970; Selvarajah et al., 1970; French, 1971; Mohammadian, 1971), normal pullets matured earlier than their dwarf sisters. On an average the dwarf birds in this experiment started laying at 160.7 d, and their normal sisters at 146.2 d. The difference was significant ($P < 0.01$). It is noticeable that the dwarf daughters of the mothers selected for the production of two eggs a day started laying more than five days later than the dwarfs originating from the other lines (Table 1).

Table 1. Mean age (d) when first egg is laid in each group.

	Dw-	dw-
Line DY	144.7^{a}	159.0^{b}
Line TE/D	147.2^{a}	164.3^{c}
Line N	146.9^{a}	158.8^{b}

Numbers with the same superscript do not differ significantly ($P > 0.05$).

To eliminate a part of the influence of the difference in precocity between and within the experimental groups, egg production was always taken during a fixed period after the hen had laid her first egg. No account was taken of the fact that the dwarf pullets started laying under conditions somewhat different from those of the normal-sized pullets, because they differed about a fortnight in age at first egg.

In calculating the data obtained, no results are included relating to birds which matured later than 180 d and did not finish a 224 d laying period. Thus the results refer to a total of 353 hens. Table 2 shows their distribution over the different groups and the average bodyweight at 55 d and 34 w respectively.

Although in all three lines birds were sired by the same fathers, the laying patterns of the normal-sized pullets, as characterized by the above criteria, differed from one another according to the selection of their mother (Table 3). No difference existed between the dwarf hens of the three different lines; practically none laid any abnormal eggs.

When studying the influence of the dwarfing gene on the egg laying pattern it is important not to confuse this effect with the influence of aging of the hens. It can be inferred from Lacassagne (1957, 1960) that the rate at which follicles in the ovary reach maturity declines with increasing age. For this reason the laying period was divided into four parts of 56 d each. This makes it possible to study the influence of the dwarfing gene in lines with a different surplus in yolk production, and to distinguish periods of excessive and more reduced yolk formation within these lines.

Table 2. Number of birds per group and their average bodyweight (g) at 55 d and 34 w.

	Dw-			dw-		
	number	weight 55 d	weight 34 w	number	weight 55 d	weight 34 w
Line DY	58	1263	3730	58	809	2401
Line TE/D	49	1261	3780	38	796	2444
Line N	78	1231	3622	72	784	2381

Table 3. Laying pattern for each line and genotype (mean numbers per bird in 224 d of lay).

	Dw-			dw-		
	Line DY	Line TE/D	Line N	Line DY	Line TE/D	Line N
Yolks lost in formation of double-yolked eggs[1]	9.4	4.3[a]	4.8[a]	0.8[b]	0.7[b]	0.5[b]
Yolks lost in laying two eggs a day[1]	2.7	4.6	0.7	0.3[a]	0.2[a]	0.2[a]
Yolks found in formation of normal eggs	122.5	110.9	133.2[a]	138.4	131.4[b]	131.6[ab]

[1] One double-yolked egg or one case of two eggs a day means two yolks lost for normal egg formation. Numbers with the same superscript in each row do not differ significantly ($P > 0.05$).

Table 4. Average production per bird for each group and for each period during laying.

	Period	Total number of yolks (A)		Normal eggs (B)	
		Dw-	dw-	Dw-	dw-
Line DY	1– 56 d	47.1	42.3^{b}	26.8^{b}	40.5^{a}
	57–112 d	41.9^{cf}	39.6^{ce}	34.7^{e}	39.1^{d}
	113–168 d	36.6^{i}	33.8	34.3^{if}	33.4^{f}
	169–224 d	28.1^{n}	25.5^{l}	26.7^{nk}	25.4^{k}
	Total	153.7	141.2	122.5	138.4
Line TE/D	1– 56 d	41.0^{a}	41.0^{ab}	28.0^{b}	39.2^{a}
	57–112 d	37.9^{d}	38.8^{de}	32.9^{e}	38.3^{d}
	113–168 d	31.0^{g}	31.2^{gh}	28.9^{g}	31.0^{gh}
	169–224 d	22.7^{k}	23.1^{km}	21.1^{l}	22.9^{lm}
	Total	132.6^{p}	134.1^{pq}	110.9	131.4^{q}
Line N	1– 56 d	43.7	41.2^{b}	32.8	39.9^{a}
	57–112 d	40.7^{f}	38.1^{e}	38.2^{c}	37.8^{cd}
	113–168 d	34.9^{i}	30.7^{h}	34.4^{i}	37.8^{cd}
	169–224 d	28.2^{n}	24.0^{lm}	27.8^{n}	23.5^{m}
	Total	147.5	133.9^{q}	133.2^{p}	131.6^{pq}

The same superscript means that the numbers do not differ significantly from the other numbers of the same character, for each line, respectively for each genotype; $P > 0.05$.

It was established from the eggs laid that except in the TE/D line (Table 4A) the dwarfed hens invariably produced a lower average number of yolks than their normal sisters. It was only in the second part of the laying period that no difference could be demonstrated in the DY line. It is not known why the yolk production of *dw-* hens in the TE/D line was about the same as that of the *Dw-* pullets, but two explanations are possible. Since yolk production was established from the eggs laid and an increase did actually occur in the normal egg production of the dwarfed hens, it can be assumed that in the heavy pullets of the TE/D line more yolks are lost than in the other groups through atrophy of the follicles in the ovary and by failure of the oviduct to pick up the yolk after its ovulation. It should also be noted that the *Dw-* hens of this line include some birds with an extremely low yolk production (Fig. 1). The whole group being small in number, the average will be considerably depressed by these individuals. When we omit these extreme hens from the experiment, it can be seen that in the TE/D line the remaining *Dw-* pullets also produce more yolks than their dwarf sisters and that the difference in normal egg production between the two types decreases to the same amount as in the DY line (Table 5). In general good agreement exists between this reduction in yolk production of the dwarf broiler mothers and the lowering in egg production of specific laying hens carrying *dw* (Hutt, 1953, 1959; Bernier & Arscott, 1960, 1972; Mérat, 1969, 1972; Rapp, 1970; Selvarajah et al., 1970; Mohammadian, 1971; Rajaratnam et al., 1971; Quisenberry, 1972). Since the normal egg production of egg-type pullets is almost identical with their yolk production, this agreement supports the assumption

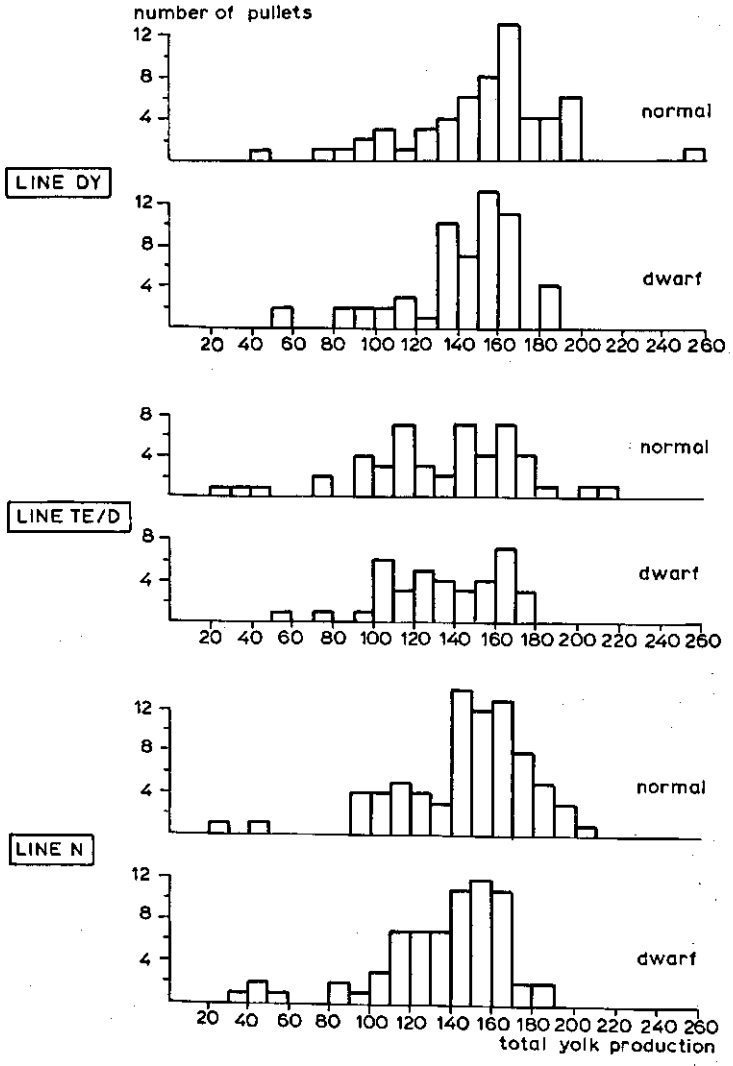

Fig. 1. Frequency distribution of total yolk production in 224 days per line and per genotype.

that the dwarfing gene, *dw*, has the same effect on yolk production of meat-type pullets as of egg-type birds. It has been shown in other experiments (Prod'homme & Mérat, 1969; Jaap & Mohammadian, 1969; Mohammadian, 1971; Ricard & Cochez, 1972) that the total production of dwarfs of meat-type strains was not less but at least equal to that of their normal sisters. It can however be seen from Table 4B that the dwarfing gene sometimes has a detrimental effect on normal egg production. During the second half of the laying period the dwarfs of line N produced fewer normal eggs than their *Dw-* sisters ($P < 0.01$). Had we only considered the results of the laying period as a whole, this fact would not have attracted attention because the dwarf birds produced far more normal eggs during the first 56 d of lay.

Although the dwarfing gene has the same effect on yolk production of both meat-type

Table 5. Average production per bird during 224 d of lay, omitting hens which produced less than 50 yolks.

	Total yolk production		Normal eggs	
	Dw-	dw-	Dw-	dw-
Line DY	155.6	141.2	123.9	138.4
Line TE/D	139.0	134.1	116.6	131.4
Line N	150.5	139.1	135.7	135.4

and egg-type pullets, increased normal egg laying during the first part of lay had been anticipated in line DY and TE/D. The yolk production of broiler mothers, and the associated ovulation rate, is sometimes so great that the oviduct has insufficient time to work up the engulfed yolk to a normal egg (van Middelkoop, 1972b). For this reason the gene *dw* may be expected to cause a shift from abnormal to normal egg laying owing to reduced yolk formation. Hence it is not surprising that during the first half of lay, the dwarf broiler mothers laid far more normal and far fewer abnormal eggs than their *Dw-* sisters. The fact that the *dw-* hens in the N line did not produce a greater number of normal eggs can be ascribed to the genotype of their mothers, being selected for a normal egg production as is the case with egg-type pullets. As a result these birds did not have enough surplus yolk formation to level out the influence of the dwarfing gene.

Conclusions

The following general conclusions may be drawn from this experiment:

1. The dwarfing gene, *dw*, has the same effect on yolk production of meat-type as of egg-type pullets.
2. Normal egg production will be reduced by the influence of gene *dw* in hens which have an optimal ovulation rate with respect to the time needed by the oviduct to form a normal egg.
3. The dwarfing gene can be used to increase the normal egg production of birds whose ovulation rate exceeds the limit of one ovulation per egg formation period.
4. The effect of the dwarfing gene on normal egg production can be predicted from the laying pattern, since the ovulation rate can be inferred from the latter.

Summary

This experiment was conducted with normal and dwarf broiler mothers which were kept in individual laying cages. These hens were the progeny of the mating of *Dwdw* cocks with normal hens selected from three different experimental White Plymouth Rock lines. These lines differed in egg-laying pattern as a result of selection for the laying of double-yolked eggs, two eggs a day, and the production of normal eggs only.

The egg production and laying pattern were ascertained by making a daily record of the performance of each bird and establishing to which type the eggs belonged. In this way it was found that the normal pullets matured earlier and laid more yolks than their

dwarf sisters. Except for the pullets of the line selected for normal egg laying, it was demonstrated that the reduced yolk production in the dwarfed hens resulted in increased normal egg laying. The results support the theory that the influence of the dwarfing gene is to increase normal egg production in birds with an excessive yolk formation in the ovary.

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8 Summary of chapters 3–7

Physiological aspects

Chapter 3

The phenomenon of the laying of two eggs a day, which are usually both abnormal, was studied in a White Plymouth Rock experimental strain. The most common case of two shell eggs laid on the same day by one bird is a hard-shelled egg, which has partly additional shell deposition, followed by a more or less soft-shelled egg with a flattened area. The time of oviposition was noted and the shell thickness measured at different points of the eggs laid by the same hen within 24 hours. Radiographs were taken of hens with two eggs together in the shell gland and were examined post mortem. The results of these investigations confirmed the hypothesis that the laying of two abnormal shell-eggs on one day has to be attributed to the presence of two eggs in the shell gland and an explanation could be given of the cause of the formation of the abnormal shells. When two eggs are together in the shell gland, they usually exhibit the same pattern. In contrast to normal, these eggs are oriented more perpendicular to the direction of movement through the oviduct. The second egg is misshapen soon after it arrives in the shell gland, where it is compressed by the first. Under such abnormal conditions, the membraneous egg grows by 'plumping'. The first egg, of which the shell usually is completely formed, is very often partly pushed into the vagina. Owing to the position of these two eggs, part of the second egg is deprived of shell formation during this period and the first egg receives an additional shell deposition, sometimes on the whole surface but usually as a band over a part of it. This band covers the egg in an almost longitudinal direction. For some reason the first egg is held in the shell gland for several hours longer than normal and the second one is expelled prematurely or laid at the 'normal time'. As a result both eggs are laid within 24 hours and the phenomenon is called 'laying of two eggs a day'.

Chapter 4

Shell eggs, which can easily be recognized by their appearance as the first of a pair laid as two eggs a day, are unsuitable for hatching. Of the fertilized eggs of this type, only 13–26 % give a viable chicken. The influence of the longer stay of these eggs in the oviduct on the hatching results was investigated by holding normal eggs for 10–11 hours at body temperature immediately after laying and by preventing the escape of carbon-dioxide during this period. The hatching results of those eggs, however, do not prove that either the delayed escape of carbondioxide or the prolonged stay of the egg at body temperature have any adverse effect on hatchability. To study the effect of the extra shell deposition on the hatchability of 'first' eggs, the shell pores were counted and the

porosity was measured. The porosity of the shell of 'first' eggs was less than half that of normal eggs of the same hens. This lower porosity can be explained by pore blockage caused by extra shell deposition. The effect of the pore blockage was investigated by coating whole or half the surface of the shell of normal eggs with paraffin. This coating was done immediately after laying and just before placing the eggs in the incubator. The hatching results of the eggs of which half the shell surface had been coated, confirmed the assumption that the blocking of the pores by the extra shell deposition is the main reason for the high embryonic mortality in 'first' eggs.

Chapter 5

The research on the laying of two eggs a day was extended to the cause of the formation of the most important types of abnormal eggs and to their relationship to the laying of normal eggs. In that trial egg laying and the type of egg laid were recorded hourly for each hen over a single period of 4½ days. The part of the day during which the oviposition of normal eggs could be expected was inferred from the one in which ovulation would theoretically be expected and the time needed by the oviduct to form a normal egg. This theoretical period was compared with the one during which normal eggs were actually laid. In this way I checked whether the theoretically expected ovulation period tallied with reality. The period when membraneous and soft-shelled eggs were expected to be laid was then derived from the established ovulation period and the process of normal egg formation. Comparison of this period with the time the eggs were actually laid showed that the laying of this type of eggs did, in fact, result from premature expulsion, but this was not the only cause. After the time in the oviduct had been established for the different types of abnormal eggs, the ovulations in question were inferred from the oviposition times. Together with the ovulations connected with normal egg laying it could thus be deduced which eggs were associated with different ovulations in the same period and which eggs resulted from ovulations occurring at different periods. In this way it was found that a large proportion of the yolks lost in abnormal eggs originated from different ovulations occurring in the same ovulation period. For other yolks going into abnormal eggs, the ovulations did occur in two separate periods but were still some hours closer together than the time needed for the oviduct to form a normal egg. The laying of normal eggs results from ovulations occurring at least 21 hours before or after another ovulation.

Genetical aspects

Chapter 6

In 1969, a selection trial was started by the development from the Institute's White Plymouth Rock strain of one line selected for double-yolked egg laying, another for the production of normal eggs. Simultaneously a control strain was developed and kept together with the selected lines. In 1970, a third line was initiated by selection for the laying of two eggs a day.

The laying performance was measured by taking fixed periods for each bird of 80 and 200 days, respectively, after laying the first egg. From the results obtained with those

selection lines, realized heritability of the selected traits were calculated after correction for annual influences with the help of the control strain. Besides this, heritability of total yolk production and of normal egg laying were estimated, both from the regression of daughter on dam and from an analysis of variance. The estimates of these traits agreed well and the laying of double-yolked eggs and of two eggs a day were highly heritable. Therefore specific genes are expected to play an important role. This is confirmed by the fact that it appeared to be possible to select separately for double-yolked eggs or for the laying of two eggs a day.

Phenotypic (r_p) and genetic correlations (r_g) have been calculated between the traits normal egg production, double-yolked eggs, laying of two eggs a day, total yolk production and 8-week bodyweight. Both the r_g and r_p between total yolk production and the other laying traits was positive, though the correlation with the laying of two eggs a day was much lower than the others. The relation between 8-week bodyweight and the laying traits showed no distinct pattern, because both negative and positive values were found. Normal egg production could be increased significantly without a difference in growth rate from the control strain. Selection for double-yolked eggs and the laying of two eggs a day, however, resulted in a simultaneous higher 8-week bodyweight.

Chapter 7

The influence of the sex-linked dwarfing gene on egg production has been studied with the progeny of *Dwdw* cocks with (normal-sized) hens selected from the three different selection lines of the 1971 hatch. The normal and the dwarf hens were kept in individual laying cages. Both the egg laying and the type of the eggs laid were recorded daily. In this way, it was found that the normal pullets matured earlier and laid more yolks than their dwarf sisters. Except for the pullets of the line selected for the laying of normal eggs, the reduced yolk production in dwarf hens resulted in an increased production of normal eggs. The results support the assumption that the influence of the dwarfing gene is to increase normal egg production in hens with excessive yolk formation in the ovary.

9 General discussion and conclusions

Chapter 3

White Plymouth Rock pullets selected for a high growth rate lay a larger proportion of abnormal eggs. These abnormal eggs can be distinguished into several physical classes, according to the amount of extra shell deposition, shape, number of yolks present and whether or not they are soiled with yolk material. More difficult, however, is division according to etiology, which is here needed. Research into the cause of the occurrence of hard-shelled eggs with additional shell deposition was a first step toward the solution of this problem. The laying of this type of egg was followed by the oviposition of another abnormal type of egg within about 20 hours ('two eggs a day'). The second egg of the pair is usually abnormal in two ways: compressed-sided and with incomplete shell deposition. The cause of the abnormality of these eggs could be ascribed to a temporary stay of both eggs in the shell gland at the same time. The shell formation of the first egg was already finished when the second one entered the shell gland. Hence a second yolk was released from the ovary before the foregoing one had been laid as a normal egg. Also the time between the ovulations involved can be assumed to be some hours shorter than the time needed to form a normal egg. The importance of the study lies in the fact that it is proven that the phenomenon of the laying of two eggs a day is primarily the result of a shorter interval between two successive ovulations. In this situation the normal rhythm of the oviduct is disturbed, causing formation of abnormal eggs. After my research on the laying of two eggs a day was published, Ivy et al. (1972) published a paper on the same subject, but their results did not differ from mine.

Chapter 4

The hard-shelled eggs with the typical additional shell deposition of the first egg of a pair were shown to be unsuitable for hatching. Most of the embryos died because of the larger number of the pores of the shell proper being blocked by the extra shell deposition. Thus it was shown that these eggs have indeed to be seen as abnormal, while in addition it was confirmed that the shell formation of the egg which was still in the shell gland stopped and the shell secretion started again after the entrance of the second one.

Chapter 5

The abnormality of the eggs not clearly involved in the 'laying of two eggs a day' was shown to be caused by premature expulsion from the oviduct, although this seemed not to be the only cause. For most of those eggs it was deduced that they were associated with the occurrence of ovulations succeeding each other in the same ovulation period.

The laying of normal eggs results from ovulations occurring at least 21 hours before or after another ovulation. In general, the laying of abnormal eggs in healthy hens should not be ascribed primarily to a disfunction of the oviduct, but to the function of the ovary. Most of the abnormal eggs can be distinguished into two main etiological classes according to the ovulation pattern: (1) abnormal eggs owing to ovulations occurring in the same ovulation period; (2) abnormal eggs related to ovulations occurring in separate periods, but still some hours closer together than the time needed for the oviduct to form a normal egg. Typical representatives of these classes are double-yolked eggs and hard-shelled eggs with additional shell deposition, respectively. Membraneous and soft-shelled eggs are found in both classes, so that the number of abnormal eggs in both groups cannot be derived from the classification according to a clinical division.

Chapter 6

After it was shown that the eggs laid can be distinguished into three categories according to the ovulation pattern, the next step was to study the genetical aspects of typical representatives of these categories. On account of the length of the ovulation interval, it could be imagined that the transition from normal egg laying through two eggs a day to double-yolked eggs may be closely related to a forced yolk formation in the ovary resulting in a decreasing interval between two successive ovulations. Although a positive genetic correlation between total yolk production and abnormal egg laying does seem to exist, specific genes are suspected to play a major role, as indicated by the fact that the laying of double-yolked eggs must be distinguished genetically from the laying of two eggs a day, because selection for each of these traits separately lead to distinct results. A significant increase in the laying of normal eggs by selection was not accompanied by a difference in 8-week bodyweight from a control strain. Selection for double-yolked eggs and for the laying of two eggs a day, however, resulted not only in an increase in the trait selected, but also in a higher 8-week bodyweight. These results can be of high practical value, because they may provide a key for the improvement of the normal egg production of broiler mothers while maintaining a high growth rate. It may even be permissible to speculate that this end can best be achieved by selection for double-yolked egg laying, combined with a suppression of this trait by means of negative environmental factors. Perhaps the same could be achieved by selection for the laying of two eggs a day.

Chapter 7

Because specific genes are expected to play an important role in the laying of abnormal eggs, this in itself does not need to exclude the possibility of a surplus in yolk production: the total yolk production was established on the basis of the number of eggs laid, thus neglecting the ovulated yolks which were not engulfed by the infundibulum. With the dwarfing gene, it proved possible to increase, genetically, the normal egg production of birds whose ovulation rate exceeds the limits of one ovulation per normal egg formation period. Thus the laying of abnormal eggs is still genetically correlated with excessive yolk production in the ovary. The normal egg production of broiler mothers can be increased to a higher level by the use of the dwarfing gene than would perhaps be possible by direct selection.

General comments

That neither the real number of ovulations nor the exact time of ovulation could be established in the living bird was felt as a serious handicap. Through lack of this information, the number of yolks released had to be established from the number of eggs laid and therefore has to be seen as an approximation. A drawback was that the time of ovulation could not be measured continuously, especially for the laying of two eggs a day. Here a knowledge of the time of ovulation is needed in order to establish exactly the interval between the two ovulations. Only so can it be deduced what stage the foregoing egg had reached at the moment of the second ovulation. Supposedly the second ovulation occurs about 5–6 hours before the egg still present in the oviduct would be laid. This implies that in these hens the oviduct requires more time for normal egg formation than in hens able to lay a normal egg each day. Further research on this subject is needed to try to answer the question whether important differences exist in the time taken by the oviduct to form a normal egg between individual birds. This is an important point, because the more time required for normal egg formation, the greater the chance of the laying of two eggs a day. Then it must be investigated whether a positive correlation exists between rate of bodygrowth and the oviduct term of normal eggs. If important differences between birds in the time required to form a normal egg are found to exist, this would imply that selection for the laying of two eggs a day is in fact the same as selection for a slower working oviduct and that selection for normal egg laying does have a correlated effect in the direction of a shorter oviduct term. This can also explain why the laying of two eggs a day has to be distinguished genetically from double-yolked eggs, which are assumed to be in no way correlated to the time needed for normal egg formation. The only similarity between both traits is the fact that a relative short ovulation interval is needed to express the ability.

Secondly it should be remarked that insufficient knowledge exists about the agents controlling the follicles in the ovary. It is especially important to know why follicles enter the rapid-growing phase and the sequence in which this occurs. As long as this information is lacking, it is hard to perform purposeful research on how to improve the hatching egg production of broiler mothers and to avoid unnecessary work.

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