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# Ley, periodically reseeded grassland or permanent grassland

With a summary

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# Abstract

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In resowing with mixtures in which *Lolium perenne* was dominant (*L.p.* seed rate 15–20 kg/ha), perennial ryegrass predominated. Under favourable conditions for establishment other species either sown or not sown could hardly develop, when resowing was carried out efficiently. Frequent resowing (every 2 years) decreased the dominance of Lolium *perenne*. *Poa annua* and *Elytrigia repens* increased appreciably.

The dry matter yield was usually somewhat lower after resowing; mainly through losses during resowing, but often even one or more years afterwards. Distribution of the dry matter production throughout the year and drought sensitivity were not markedly affected by resowing.

Maximum rooting depth was attained a few months after resowing and hardly changed afterwards. The root mass continued to increase for some years, especially in the top 5 cm of soil.

The dry matter content of fresh herbage was not distinctly affected by resowing. Crude protein content in dry matter was frequently lower on the resown grassland than on permanent grassland.

Alternating arable crops and grassland resulted in decreased yields of dry matter and crude protein from the grassland sown after the arable crops. This was almost entirely due to insufficient nitrogen supply from the soil. An effect on germination of the grass seed, growth of the young grass plants or on botanical composition was seldom observed. Resowing grassland on permanent arable unaccountably gave very high yields the first year.

# 1 Introduction

A large part of cultivated soil is under grassland; in the Netherlands about 60%. This is partly because arable farming or horticulture are hardly possible in these areas; in addition, however, many soils without limiting conditions are under grass. This grassland is either permanent or it is included in rotations with arable or horticultural crops (temporary or rotational grassland or ley).

Permanent grassland can be kept without reseeding, or it can be (periodically) resown. Sometimes, reseeding is not possible. Reseeding may be done to replace the grass sward by one with a better botanical composition, to eliminate soil deficiencies or to rejuvenate the sward.

Between old grassland, young grassland and leys many differences may occur e.g.:

- Old grassland has a relatively high organic matter content in the top soil. By growing arable or horticultural crops and by soil cultivation this content can be decreased: thus the soil of leys and sometimes of recently resown grassland have a relatively low organic matter content (Hoogerkamp, 1974a).
- Recently (re)sown grassland and leys have a grass sward with a botanical composition mainly determined by the seed mixture and to a much lesser extent by their management and environment. With old grassland the grass sward is in equilibrium with management and environment. If the sown species and varieties are adjusted to the prevailing conditions this difference between old and young grassland will be small (Klapp, 1971).
- In young grassland mechanical soil cultivation carried out before sowing may be evident. Mostly the soil is looser and better aerated, especially in the first period after sowing (Hoogerkamp, 1974a); the subsoil is richer in organic matter and minerals (Hoogerkamp, 1974a); and the roots can penetrate deeper.
- Soil cultivation as well as crop rotation applied before sowing may affect the micro and macro fauna and flora in the soil considerably; for instance, it reduces the number of earthworms and changes the composition of the micro flora and fauna (Müller, 1951 and Voisin, 1960).

For many years there has been much controversy about the relative merits of permanent and temporary grassland, as well as about the intrinsic value of reseeding permanent grassland (Falke, 1920; Schneider, 1927; Stapledon, 1937 and 1946; Davies, 1949 and 1952; Klapp, 1954, 1959 and 1971; OEEC report, 1954 and Clarke, 1959 and 1960). The 4th European Grassland Congress held at Lausanne in 1972 was mainly devoted to this subject.

These controversies partly arise from the differences in agro-ecological and economical situation, but also partly from differences in the evaluation of the different types of grassland farming. The following paragraphs are intended to summarize the essential points in these controversies.

## 1 Temporary grassland

The following points are the main technical advantages of changing from permanent grassland or from permanent arable farming to ley farming often mentioned by its promoters (c.f. Stapledon, 1946; Frankena, 1947a and b; Stapledon & Davies, 1948; Davies, 1949 and 1952; Andreae, 1959; Grootenhuis, 1961; Hoogerkamp, 1961 and Van den Brand, 1962).

### *Changing from permanent grassland to ley farming*

- Permanent grassland locks up fertility, particularly organic matter; this can be used to stimulate the growth of arable crops.
- By alternating grassland with arable crops, old grassland (often synonymous with inferior grassland) is replaced by young (and in principle synonymous with good) grassland; replacing inferior by good grassland as well as old grassland by young is advantageous: the soil is aerated and the complex old sward is replaced by a more simple one, consisting of better species and varieties. Total yield, net as well as gross yield, will be increased, with the additional advantages of a decreased drought sensitivity (Cleveringa, 1941; Goedewaagen, 1942; Jäntti, 1952 and Slaats et al., 1967), a better yield distribution throughout the year (Frankena, 1947a) and a lower incidence of a number of parasitic animal disorders (Anonymous, 1947 and 1949 and Muir, 1948).
- A good rotation of grassland and arable crops will stimulate white clover (or other legumes) in the grassland.
- Grassland included in ley farming is considered as a crop and is therefore better managed than permanent grassland.

### *Changing from permanent arable farming to ley farming*

- Grassland builds up fertility, particular with respect to organic matter and this stimulates the growth of the following arable crops.
- The arable crops profit from the farmyard manure, which is produced by introducing grassland and cattle on the farm.

However opponents state that, apart from the system being only applicable on fields suitable for both arable crops and grassland, permanent grassland is preferable to leys (c.f. Baur, 1930; Czerwinka, 1951; Klapp, 1954 and 1959 and Zürn, 1957):

- Good permanent grassland will yield the same or even better than leys because of a more complex botanical composition of the grass stand better adjusted to the growing

conditions.
- Permanent grassland lacks some of the drawbacks of leys, like costs of reseeding and sowing failure.

Some opponents even doubt whether the introduction of leys in an arable rotation is recommendable (Klapp, 1954).

## 1.2 Reseeding

Changing from old to young grassland is also possible by reseeding without a rotation with arable crops. Without soil cultivation and reseeding, grassland can almost permanently produce plentiful, good forage. Many specialists, however, think that productivity can be increased by soil cultivation or reseeding:
- Soil cultivation aerates the soil and reduces some soil defects (Mulder, 1888; Elema, 1913; Reyntens, 1950; Czerwinka, 1951 and Klapp, 1954). However, it is technically difficult to cultivate the soil without damaging the sward to such an extent that reseeding is not necessary; in experiments in which this was attempted, the results were disappointing (Minderhoud & Woldring, 1953 and Klapp, 1954 and 1971).
- Stapledon, 1939 and 1947; Moore, 1946; Davies, 1949 and 1952; Barker, 1952 and Cooper, 1952, have suggested that a combination of soil cultivation and reseeding has a very good effect. Stapledon (1937 and 1939) stated 'I do not believe, there is a single field that would not benefit from periodic breaking. How often to plough up is a matter of circumstances and condition; once in a 100 years is better than never, once in 20 years better still, and once in 10 years often quite sufficient. Plough more frequently than once in ten years and you begin to be scientific, progressive and a farmer in very truth'.

Recently, more and more people are of the opinion that with the present intensive management of the grassland (heavy nitrogen applications, more frequent cuts, high cattle density), the introduction of new mowers (e.g. cyclomowers) and other methods of manuring (e.g. slurry) the botanical quality of grassland decreases rapidly (Walkowiak, 1969; de Vries, 1971 and Yeo, 1972) and periodical reseeding is required. The increased reduction in the space volume of the soil by cattle and machines will promote this necessity.

The drawbacks connected with reseeding mentioned before and the rather doubtful presumed advantages give rise to a deep controversy on reseeding grassland, whether its botanical composition is good or poor (Müller, 1951; Klapp, 1954 and 1971 and Zürn, 1957).

## 1.3 Aim of the experiments

In a series of trial fields throughout the Netherlands the following disputed aspects of ley farming and of reseeding permanent grassland were studied and are discussed here:

- Effect of reseeding of grassland with a good botanical composition on the botanical composition and gross yield (fresh herbage, dry matter and crude protein).
- The effect of ley farming on grass yield.

Some other aspects were discussed elsewhere:

- Accumulation of organic matter under grassland and its effect on grassland and on arable crops (Hoogerkamp, 1974a).
- Reseeding of poor grassland (Hoogerkamp, 1971b and 1974b).
- The lean years (deterioration of grassland that is some years old) (Hoogerkamp, 1974b).

# 2 Experimental technique

In a number of trial fields, grassland with a good botanical composition (a dense sward with mainly *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *P. trivialis* and other good grasses) was partly resown (E1, E3, E5, E6, E7, E8, E9, E10 and E11), in other trials grassland was established after arable crops as well as after grassland (E4 and E22), on arable land (E18 and E21) and on peat recently covered with sand (E2). One experiment (E22) was carried out in a greenhouse, the other experiments in the field (Table 1).

Reseeding and sowing were usually carried out in summer or late summer, reseeding immediately after destroying the old sward either by digging, ploughing or rotavating. At rotavating the original profile was disturbed as little as possible, since it has been shown that after burying the topsoil rich in organic matter deeply in the profile, as sometimes happens with levelling and deep ploughing, the subsequent yield losses were considerable (Hoogerkamp, 1974a).

A mixture of *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *P. trivialis*, *Festuca pratense* and *Trifolium repens* was usually sown.

During the trial period, the botanical composition was analysed (E1–E22) and fresh-herbage, dry-matter and crude-protein yields determined (E1–E22); in two experiments the root system was examined (E5 and E18) and in three experiments the structure of the soils (E3, E5 and E8). The botanical composition was analysed as dry weight percentages.

Yields were determined by cutting strips before putting the cattle to pasture or by harvesting screened-off parts. The dry matter content of the fresh herbage was analysed by air-drying at 70 °C, followed by drying to constant weight at 105 °C. The crude protein content in the dry matter has been calculated as the N-total content × 6.25; the N-total content was determined by the Kjehldahl method.

The structure of the soil was determined by sampling the layers 2–7 and 7–12 cm. In the sampled soil, water and air content at pF 2.0 were estimated.

The root system was analysed by the pinboard method (0–110 cm) as well as by sampling with an auger; the living roots were separated as far as possible, dried and weighed.

The number of replicates varied from two to six, but was usually four.

Table 1. A brief description of the trial fields.

| Experiment number | Type of activity | Soil type | Soil cultivation at (re) seeding | Approximate N application (kg N/ha per year) |
|---|---|---|---|---|
| E1 | reseeding old grassland | heavy river clay soil | rotavating and digging | 0, 100, 200, 300 |
| E2 | seedling grassland | 18 cm sand on peat soil | | 100, 200, 300 |
| E3 | reseeding old grassland | moderately heavy marine clay | rotavating and digging | 0, 100, 200, 300 |
| E4 | ley farming and reseeding grassland | sandy soil | ploughing | 120, 180, 240, 300 |
| E5 | periodical reseeding grassland | sandy soil | rotavating | 150, 300 |
| E6 | reseeding pold grassland | sandy soil | rotavating and digging | 0, 100, 200, 300, 400, 500 |
| E7 | reseeding old grassland | heavy river clay soil | rotavating and digging | 70 or 70, 220 |
| E8 | reseeding old grassland | moderately heavy river clay soil | digging | 0, 180, 360 |
| E9 | reseeding grassland | sandy soil | rotavating and digging | 100, 200, 300, 400 |
| E10 | reseeding 4-y-old grassland | sandy soil | rotavating | 50, 110, 175 |
| E11 | reseeding 4-y-old grassland | sandy soil | rotavating | 175, 240, 300 |
| E18 | draining young grassland; young grassland sown in: | heavy river clay soil | ploughing | |
| | a. 1957 | | | 0, 70, 220 |
| | b. 1961 | | | 70, 220, 360 |
| | c. 1963 | | | 70, 220, 360 |
| E21 | reseeding grassland | moderately heavy river clay soil | ploughing | 0, 180, 360 |
| E22 | seeding pots with grassland and arable soil | various soils | | 50, 100 |

# 3 Results

## 3.1 Reseeding grassland

### *Establishment*

Apart from the weather, hardly any difficulties were met in resowing the trial fields. Germination of the seed was usually good; lack of water sometimes caused a delay. In practice this lack of water is frequently emphasized by sowing too superficially (0–½ cm) or in a loose seed-bed, so that the capillary rise of water was poor.

The number of seedlings was considerably reduced by competition, sometimes by lack of water and insect damage (e.g. *Tipula* spp., *Melolontha* spp. and *Bibio* spp.). It is not quite clear yet to what degree nematodes and fungi reduce the seedling number; research is still in progress.

The amount of grass seed used in seeding was 25 to 40 kg per ha. It was sufficient to produce a number of seedlings that even after considerable reduction formed a closed sward; in adverse weather, especially drought, this did not form. In the experiment with periodical reseeding (E5) it was increasingly more difficult to obtain a good sward. In August the soil was rotavated twice (5–8 cm deep), with an interval of 10 to 14 days, and then reseeded. Germination of the seed sown was usually good, but growth and competitive ability of the young plants were unsatisfactory, despite the fairly good weather. *Poa annua* and to a less extent *Elytrigia repens* ( = *Agropyron repens*) could therefore become established (Fig. 1).

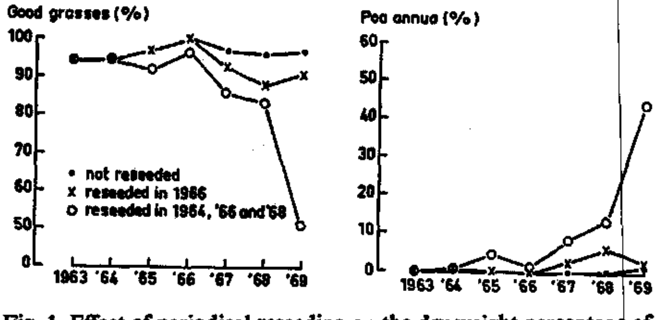


Fig. 1. Effect of periodical reseeding on the dry weight percentage of good grasses (especially *Lolium perenne* and to a less extent *Phleum pratense*) and on the percentage *Poa annua*. Experiment E5.

*Botanical composition*

Since we started from grassland with good swards and reseeding was done with a mixture of the same good grasses, the botanical composition did not change very much. In general the young sward had slightly fewer species, a somewhat higher percentage of good grasses and a predominance of *Lolium perenne*, especially at the beginning (Table 2).

*Lolium perenne* in general developed so rapidly and well that it showed considerable dominance (mostly 15 to 20 kg *Lolium perenne* was sown per hectare). Of the other species sown only *Phleum pratense* and *Poa trivialis* were rather successful in establishing themselves and in persisting; with *Poa trivialis* sometimes the seed present in the soil was more important than that sown. This was also established in a trial series not included here; in those trials sowing of *Lolium perenne* was compared with that of a complex mixture of grass species. *Trifolium repens* could only establish and persist with a low N supply (low organic matter content of the soil and N application).

Dominancy of the species sown was usually lower as the growing conditions of these species became more adverse (drought, water excess and low N application) and as the seed bank in the seed bed became greater. As the top soil was buried deeper into the profile, this seed bank became smaller and the species sown showed more dominance (Table 3).

Table 2. The effect of reseeding on the number of plant species and on the percentage of good grasses (*Lolium perenne*, *Festuca pratensis*, *Phleum pratense*, *Poa pratensis* and *P. trivialis*) and of *Lolium perenne* (dry weight percentages) in five experiments.

| | E1 | | E2 | | E6 | | E7 | | E8 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | o[1] | y | o | y | o | y | o | y | o | y |
| number of plant sp. | 24 | 19 | 24 | 12 | 25 | 21 | 19 | 18 | 18 | 14 |
| good grasses (%) | 79 | 96 | 54 | 91 | 77 | 96 | 55 | 77 | 47 | 96 |
| *Lolium perenne* (%) | 64 | 89 | 16 | 70 | 53 | 88 | 26 | 42 | 33 | 80 |

1. o = old grassland; y = young grassland.

Table 3. The effect of soil cultivation preceding reseeding on the botanical composition (dry weight percentages *Lolium perenne*, good grasses, other grasses and herbs) in five experiments. rot. = rotavating; dig. = digging.

| | E1 | | E3 | | | E6 | | E7 | | E8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | rot. | dig. 20 cm | rot. | dig. 20 cm | dig. 40 cm | rot. | dig. 20 cm | rot. | dig. 20 cm | rot. | dig. 40 cm |
| good grasses | 94 | 98 | 76 | 91 | 91 | 96 | 97 | 80 | 89 | 88 | 96 |
| *Lolium perenne* | 83 | 87 | 32 | 70 | 75 | 59 | 74 | 77 | 85 | 87 | 83 |
| other grasses | 3 | + | 22 | 3 | + | 2 | 1 | 14 | 3 | 12 | 0 |
| herbs | 3 | 2 | 2 | 6 | 9 | 2 | 2 | 6 | 8 | 0 | 4 |

Of the species that were not sown *Poa annua* and *Stellaria media* gave most problems; *Stellaria media*, however, be it at the expense of the white clover, is easy to control with herbicides (MCPP or 2, 4, 5 TP). In the establishment phase of the sown grass seed *Elytrigia repens* may also take advantage of the decreased competition of the good grasses, also because the rhizomes divide during soil cultivation (reduction of the apical dominance, Hoogerkamp, 1971a).

## *Dry matter yields*

Yield determinations were started in the spring following reseeding. In the first production year nearly all the dry matter yields were adversely affected by reseeding (Table 4).

Only in Experiment E1 did the reseeded grassland show a distinctly higher dry matter yield. The yield losses during the breaking and establishment phase were not considered in Table 4; under normal conditions they averaged 1/4 to 1/5th of an annual yield. However, in those cases in which the reseeding failed completely or partly, the yield losses were much greater.

In the following years the yields of the reseeded grassland usually were also somewhat lower (Table 5).

Table 4. Effect of reseeding on the relative dry matter yields in the first year (old grassland = 100).

| Experiment | N-Fertilizer level[1] | | | | | |
|---|---|---|---|---|---|---|
| | 0N | 1N | 2N | 3N | 4N | 5N |
| E1 | 112 | 118 | 120 | 109 | | |
| E5 1st reseeding | | 99 | 94 | | | |
| 2nd reseeding | | 90 | 95 | | | |
| 3rd reseeding | | 95 | 94 | | | |
| E6 | 75 | 94 | 96 | 98 | 104 | 101 |
| E7 | | 100 | | | | |
| E10 | | 88 | 92 | 97 | | |
| E11 | | 99 | 88 | 94 | | |

1. See Table 1.

Table 5. Effect on relative dry matter yield in the years following reseeding (average of the N levels; old grassland = 100). — = No yield determination.

| Experiment | Years after resowing | | | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th | |
| E1 | 87 | 94 | 90 | 106 | 98 | — | — | 97 | 99 | 96 |
| E3 | 88 | 91 | 95 | 109 | 101 | 99 | 97 | — | — | 97 |
| E6 | 101 | 101 | 103 | — | — | — | — | — | — | 102 |
| E7 | 93 | 103 | 90 | 100 | 99 | 99 | — | — | — | 97 |

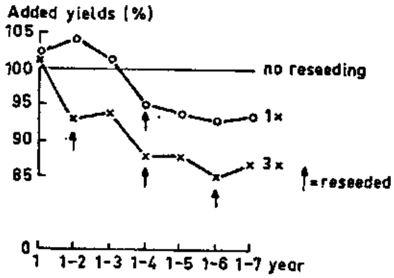


Fig. 2. Effect of periodic reseeding on the relative accumulated dry matter yield (no reseeding = 100). Experiment E5.

In three of the four trial fields the average dry matter yields were in the resown grassland somewhat lower than in the old grassland; in the other the resown grassland yielded somewhat more.

In Experiment E5 where periodic reseeding took place (every 2, 4 or 8 years), the adverse effect of reseeding good grassland was more evident as reseeding was repeated more often (Fig. 2).

In the years reseeding took place the yields of the resown grassland were distincly lower; in the other years there were hardly any differences in dry matter yield.

The yield differences between old and resown grassland in general were not distinctly influenced by the N application; however, in some cases there was an indication that they were smaller with increased nitrogen fertilizing (Table 6).

### *Production distribution*

A distinctly systematic deviation in production distribution did not occur. Sometimes the young grassland started growth somewhat earlier and more rapidly in spring, when very early species occurred in the old sward (e.g. *Alopecurus pratensis*) the

Table 6. Influence of N application (kg N/ha per year) on average dry matter yields (kg/ 100 m$^2$ per year) in old and resown grassland.

| Experiment | Type of grassland | Approximate N application (kg N/ha per year) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 100 | 200 | 300 | 400 | 500 |
| E1 | old grassland | 62 | 76 | 94 | 109 | | |
| | young grassland | 59 | 75 | 92 | 107 | | |
| E3 | old grassland | 75 | 89 | 105 | 117 | | |
| | young grassland | 71 | 83 | 101 | 118 | | |
| E6 | old grassland | 77 | 95 | 104 | 118 | 127 | 130 |
| | young grassland | 81 | 93 | 107 | 117 | 131 | 134 |
| E7 | old grassland | | 86 | 105 | | | |
| | young grassland | | 82 | 105 | | | |

Table 7. Dry matter yield (kg/ha) and botanical composition (percentages *Lolium perenne* and *Agrostis stolonifera*) after mowing a heavy cut in a dry and sunny period (E9).

| | N application (kg N/ha per year) | | | |
|---|---|---|---|---|
| | 100 | 200 | 300 | 400 |
| Yield | | | | |
| 3rd cut | 3050 | 4150 | 4580 | 4720 |
| following two cuts | 2806 | 3063 | 3013 | 2196 |
| Botanical composition | | | | |
| *Lolium perenne* (%) | 80 | 68 | 64 | 41 |
| *Agrostis stolonifera* (%) | 13 | 30 | 33 | 53 |

reverse was the case. The production distribution showed a considerable change when the organic matter content in the topsoil at sub-optimum N levels was decreased; the resulting yield depressions were greatest in spring (Hoogerkamp, 1974a).

### *Drought sensitivity*

Often it has been suggested that young grassland is not particularly sensitive to drought. In this study a difference in drought sensitivity only occurred to a very small extent, even in periods in which drought damage did occur. Moreover, it was not observed in all treatments, but especially in those where the subsoil (20–50 cm) had a greater supply of nutrients due to burying the topsoil with a high organic matter content, due to growing arable crops or to N-injection (Hoogerkamp, 1974a). Young grassland was more sensitive to drought after harvesting a heavy cut in dry and sunny periods. This resulted generally in a lower yield of the following cut(s) and sometimes in a considerable deterioration of the botanical composition, the good grasses being replaced by *Elytrigia repens*, *Poa annua* and other poor grasses (Table 7).

The third cut on this experimental field was relatively heavy, especially at the high N applications; mowing was done under dry and sunny conditions. At the higher yields the sward was damaged to such an extent that regrowth was poor and the botanical composition deteriorated (*Lolium perenne* was replaced by *Agrostis stolonifera*). In other cases frequently, however, *Elytrigia repens* replaced *Lolium perenne*.

Although old grassland can be damaged in the same way, the data suggest that young grassland is more sensitive in this respect.

### *Rooting depth*

In E5 (grassland sown in a sandy arable soil) the maximum rooting depth, attained some months after reseeding, did not change considerably during the trial period. However, the root mass varied considerably and generally increased with the maturing

Table 8. Maximum rooting depth (cm) and absolute root mass in various layers (kg/ha) under young grassland (E5).

| | Maximum rooting depth | Root mass | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0–5 | 5–10 | 10–20 | 20–50 | > 50 cm | total |
| June 1963 | 77 | 628 | 330 | 452 | 444 | 151 | 2005 |
| Sept. 1963 | 80 | 1248 | 273 | 434 | 490 | 156 | 2571 |
| June 1964 | 75 | 1593 | 449 | 517 | 566 | 127 | 3252 |
| Sept. 1964 | 76 | 1580 | 408 | 408 | 545 | 124 | 3065 |
| June 1965 | 68 | 1950 | 631 | 605 | 823 | 182 | 4191 |
| Sept. 1965 | 72 | 2206 | 558 | 496 | 610 | 168 | 4038 |
| June 1966 | 71 | 1931 | 467 | 512 | 779 | 213 | 3902 |
| Sept. 1966 | 67 | 1918 | 377 | 332 | 623 | 110 | 3360 |
| June 1967 | 68 | 2372 | 683 | 431 | 569 | 179 | 4334 |
| Sept. 1967 | 69 | 3195 | 462 | 361 | 623 | 189 | 4830 |
| June 1968 | 65 | 1622 | 555 | 423 | 704 | 104 | 3408 |
| Sept. 1968 | 69 | 1897 | 374 | 364 | 484 | 104 | 3233 |
| June 1969 | 70 | 2279 | 753 | 524 | 945 | 127 | 4628 |

grassland (Table 8).

The increase was highest in the layer 0–5 cm; below 50 cm changes were small.

### *Dry matter content*

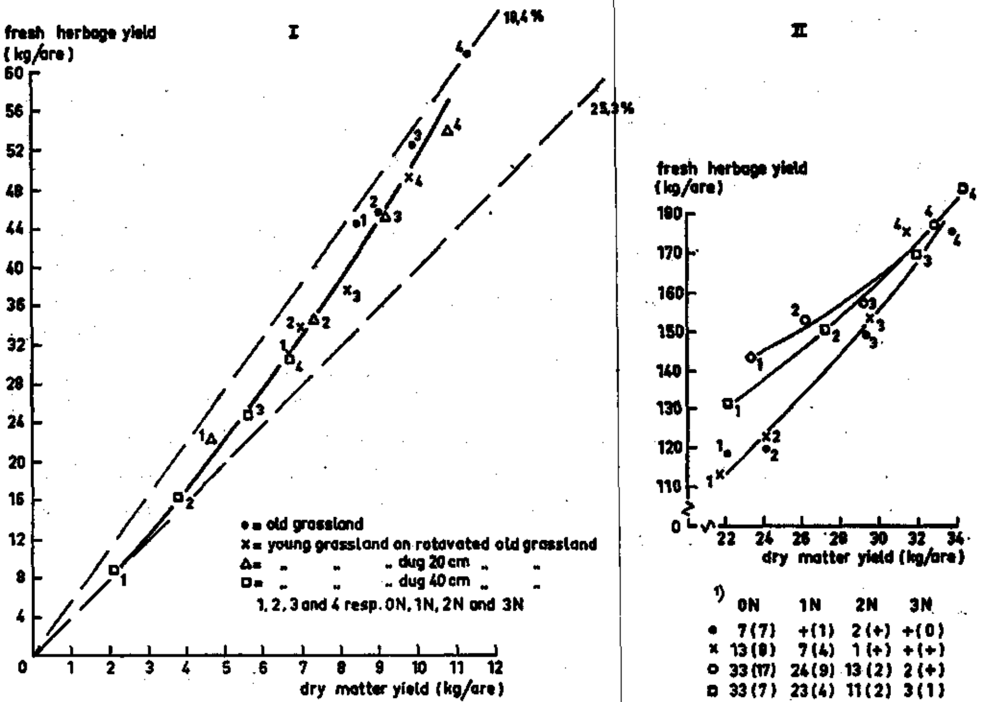
In the harvested fresh herbage, dry matter content varied appreciably according to growing conditions, weather at harvesting, botanical composition, etc. In the same cut it was frequently lower when the yields were higher; generally, there were no distinct differences between the reseeded and not reseeded grasslands (Fig. 3-I).

The dry matter content of white clover or other dicots frequently occurring in the stand, was low; when these species occurred more frequently in the young grassland, such as at lower N applications and at lower organic matter contents in the soil, the dry matter contents in the grass of young grassland were lowest (Fig. 3-II).

### *Crude protein content*

In the dry matter this content also varied appreciably with age of the grass, growing conditions, time of year, botanical composition, etc. At the same percentage of white clover the crude protein content was generally highest in the dry matter of the old grassland, with the same N application and the same dry matter and crude protein yield (Fig. 4).

When distinctly more white clover occurred in the reseeded grassland, the crude protein content of the young grassland was highest.



| 1) | 0N | 1N | 2N | 3N |
|---|---|---|---|---|
| ● | 7 (7) | + (1) | 2 (+) | + (0) |
| × | 13 (8) | 7 (4) | 1 (+) | + (+) |
| ○ | 33 (17) | 24 (9) | 13 (2) | 2 (+) |
| □ | 33 (7) | 23 (4) | 11 (2) | 3 (1) |

Fig. 3. Relation between fresh-herbage and dry-matter yields (kg/100 m²) on old and young grassland. I: First cut, 1964; II: Fourth cut, 1966, Experiment E3.

¹) The percentages of dicots; between brackets white clover.

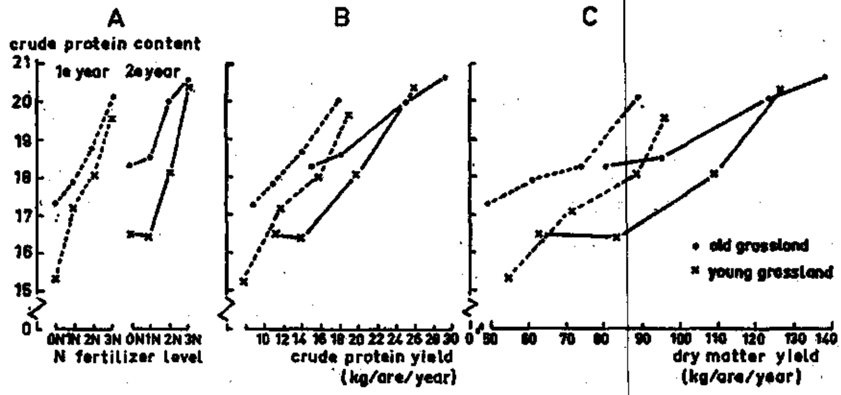


Fig. 4. Relation between crude protein content in the dry matter and nitrogen application (A), crude protein yield (B) and dry matter yield (C). Yield in kg/100 m² per year. Experiment E1.

## 3.2 Ley farming

In our ley-farming experiments, the effect of grassland on arable crops was studied. The increases in arable yields were considerable, especially at the lower nitrogen applications (Hoogerkamp, 1974a). The effect of previous arable cropping on grassland was studied in experiments E4 and E22. In E4 in one treatment potatoes, rye and oats were grown for three years and in the other treatment grassland was grown also for three years. In the autumn of the third year the whole trial field was ploughed and in the succeeding spring sown with a seed mixture in which *Lolium perenne* was dominant. Botanical composition and the gross yields were determined in this test crop for three successive years and at four N application levels. In E22, carried out in the greenhouse, *Lolium perenne* was sown in top soil (0–5 cm) originating from five grassland fields and from four comparable arable fields; there were two N-application levels.

Finally, in three grassland fields, sown on permanent arable fields (E5 and E18) and on peat recently covered with sand (E12), the botanical composition and the gross yields were determined at various N applications.

### *Botanical composition*

In the experiments there was no clear difference between germination on arable and on grassland soil (Table 9).

There was a lower seedling number on the second grassland treatment from sandy soil II because some young plants were killed by insects.

Also no distinct differences in botanical composition of the grass sward on the experimental fields were observed between resown and sown treatments.

In practice, however, the percentage of grass seeds producing young plants is sometimes lower with reseeding than with seeding on arable land. This results in more failures with reseeding than with sowing.

### *Dry matter yields*

In E4 the dry matter yields were distinctly highest on the reseeded grassland (Table 10).

In the first year these differences were highest and in none of the three years were they systematically affected by the N application. The increased yields on the reseeded grassland, absolutely as well as relatively, were highest in spring and autumn.

In the glasshouse experiment (E22) the dry matter yields of the first cut did not differ systematically between sown and resown grassland. In the next cut the *Lolium perenne* sown on the grassland soil always had the highest yield (Table 11).

On the trial fields where grassland was sown on old arable land (E5 and E18) and on sand covered peat (E2) the gross yields were usually relatively high in the first production year (Table 12).

In E18 at the lower N applications the young grassland sown in arable land yielded

Table 9. The percentage *Lolium perenne* seeds which germinated (E22); a = arable soil; b = grassland soil; o = old and y = young.

| Sandy soil I | | Sandy soil II | | | Clay soil I | | Clay soil II | |
|---|---|---|---|---|---|---|---|---|
| a | bo | a | by | bo | a | bo | a | bo |
| 89 | 91 | 85 | 78 | 69 | 84 | 87 | 88 | 85 |

Table 10. The dry matter yields in kg/100 m² per year at 4 N application levels (kg N/ha per year) (E4).

| | Resown grassland | | | | Grassland after arable crops | | | |
|---|---|---|---|---|---|---|---|---|
| | 120 | 180 | 240 | 300 | 120 | 180 | 240 | 300 |
| 1st Year | 101 | 102 | 109 | 115 | 89 | 93 | 98 | 102 |
| 2nd Year | 124 | 131 | 136 | 138 | 116 | 124 | 133 | 128 |
| 3rd Year | 101 | 108 | 117 | 127 | 92 | 102 | 111 | 115 |
| Total | 326 | 341 | 362 | 380 | 297 | 319 | 342 | 345 |

Table 11. The dry matter yield (g per pot) of *Lolium perenne* sown on arable and grassland soil (E22); a = arable soil; b = grassland soil; o = old and y = young.

| N application (kg N/ha) | | Sandy soil I | | Sandy soil II | | | Clay soil I | | Clay soil II | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | a | bo | a | by | bo | a | bo | a | bo |
| 50 | 1st cut | 2,4 | 2,2 | 2,5 | 2,7 | 2,4 | 2,6 | 2,4 | 2,9 | 2,4 |
| | 2nd cut | 1,8 | 2,8 | 1,3 | 2,8 | 4,0 | 3,3 | 4,2 | 2,1 | 2,8 |
| | total | 4,2 | 5,0 | 3,8 | 5,5 | 6,4 | 5,9 | 6,6 | 5,0 | 5,2 |
| 100 | 1st cut | 2,7 | 2,0 | 2,7 | 2,5 | 3,4 | 3,0 | 2,3 | 2,9 | 2,4 |
| | 2nd cut | 2,5 | 2,8 | 2,0 | 3,1 | 4,8 | 3,7 | 4,2 | 2,7 | 3,1 |
| | total | 5,2 | 4,8 | 4,7 | 5,6 | 8,2 | 6,7 | 6,5 | 5,6 | 5,5 |

Table 12. The dry matter yields of experiments E2, E5 and E18 (kg/100 m² per year).

| | Years after resowing | | | | | |
|---|---|---|---|---|---|---|
| | 1[1] | 2 | 3 | 4 | 5 | 6 |
| E2 (averaged over the N applications) | 135 | 125 | 117 | 107 | 110 | |
| E5 (averaged over the N applications) | 136 | 94 | 107 | 99 | 116 | 95 |

| | N application (kg N/ha per year) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 70 | 220 | 360 | 0 | 70 | 220 | 360 |
| | *very old grassland* | | | | *one year old grassland* | | | |
| E18: Field I (1957) | 60 | 76 | 96 | | 36 | 61 | 112 | |
| Field II (1961) | | 107 | 129 | 134 | | 67 | 132 | 164 |
| Field III (1963) | | 89 | 111 | 119 | | 78 | 117 | 135 |

[1] Sown in the preceeding summer.

Table 13. Relative dry matter yields of one-year-old grassland and of very old grassland in dry periods. Nitrogen dressing 40 kg N per ha per cut (E18: 1957, 1961 and 1963).

| | Watertable (cm below the surface) | | | |
|---|---|---|---|---|
| | 40 | 65 | 95 | 140 |
| Old grassland | 100[1] | 86 | 74 | 74 |
| Young grassland | 100[2] | 93 | 92 | 92 |

1. Actual yield 14.2 ton/ha.
2. Actual yield 15.8 ton/ha.

less than the old grassland, but more at the higher N applications.

E2 and E5 could not be compared with corresponding old grassland, so that it could not be clearly established whether the high yield in the first year was accidental (e.g. weather conditions). However, in the reseeding experiments (3.1) the same effect was never found to this extent.

### *Drought sensitivity*

At reseeding lower drought sensitivity was found only when the topsoil rich in organic matter was buried deep into the profile. In the watertable trial (E18), it was found that the grassland sown on arable land was also less drought sensitive (Table 13).

### *Rooting depth*

In E18 a striking difference in distribution of the root mass did not occur (Table 14).

Under the old grassland the total root mass was about twice as much as under the young grassland; the distribution was about the same.

### *Crude protein yields*

Because generally the crude protein content in the dry matter of the resown grassland was higher, the differences in crude protein yield were greater than those in dry matter yield (Table 15).

On E18 too, the crude protein yield at the same dry matter yield was lower on the young grassland than on the old.

Table 14. The absolute (kg/ha) and procentual distribution of the root mass in the soil profile (E18; watertable 140 cm below the surface).

| Layer | Young grassland | Old grassland |
|---|---|---|
| 0– 5 cm | 1386 (56%) | 2715 (54%) |
| 5–10 cm | 327 (13%) | 971 (19%) |
| 10–15 cm | 112 (5%) | 374 (8%) |
| 15–20 cm | 73 (3%) | 223 (5%) |
| 20–30 cm | 145 (6%) | 263 (5%) |
| 30–40 cm | 93 (3%) | 151 (3%) |
| 40–50 cm | 62 (3%) | 104 (2%) |
| > 50 cm | 298 (10%) | 200 (4%) |
| Total | 2496 (100%) | 5001 (100%) |

Table 15. The crude protein yield in kg/100 m² per year (E4).

| | N application (kg N/ha per year) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 120 | 180 | 240 | 300 | 120 | 180 | 240 | 300 |
| | *resown grassland* | | | | *grassland after arable crops* | | | |
| 1st Year | 23 | 24 | 26 | 28 | 21 | 22 | 23 | 24 |
| 2nd Year | 24 | 26 | 26 | 28 | 22 | 23 | 25 | 25 |
| 3rd Year | 14 | 16 | 18 | 22 | 13 | 14 | 16 | 18 |
| Total | 61 | 66 | 70 | 78 | 55 | 59 | 64 | 67 |

# 4 Discussion

## 4.1 Old or young grassland

*Botanical composition*

The top soil (about 0–5 cm) of old grassland contains numerous seeds, usually of undesirable species, e.g. *Poa annua, P. trivialis, Agrostis stolonifera, A. tenuis* and *Stellaria media* (Van Altena & Minderhoud, 1972). When this seed-bank remains near the soil surface, as with rotavating, and germinating or growing conditions for the sown species are adverse (drought, cold), these less desirable species will become established. Sometimes also fungi, nematodes, insects and unfavourable physical or chemical soil properties may be involved; in the periodical reseeding trial (E3) this seemed to be the case.

When the sward is damaged, a superficial seed-bank may also have an adverse effect on the botanical composition (e.g. urine scorching and cutting damage).

Ploughing in the seed bank (> 10 to 15 cm), practically the only solution to eliminate it, can only be successful when the seed bank is concentrated in the upper 5 to 10 cm. Too deep ploughing (> 20 cm), however, may cause yield losses (Hoogerkamp, 1974a). These considerations on the seed bank also apply to rhizomes and stolons; these, however, are easier to destroy (Hoogerkamp, 1971a).

If conditions are favourable for a rapid establishment of the species sown and if reseeding takes place correctly, the grasses preferred, especially *Lolium perenne*, will develop so numerously and rapidly that species present in the seed bank will not have any chance to become established (Table 16).

Contrary to the treatment 'not resown', the species present in the seed bank hardly

Table 16. The effect of sowing a *Lolium perenne* dominant seed mixture on the botanical composition (dry weight percentages) of the young sward (E21).

| | Lp[1] | Phl | Fp | Pp | Pt | Tr | D | As | Ely | Hl | Pa | Weeds |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Resown | 86 | 6 | 5 | 1 | + | + | 0 | 0 | 1 | 0 | 1 | + |
| Not resown | 25 | + | 5 | 1 | 10 | 21 | 11 | 9 | 2 | 2 | 10 | 3 |

1. Lp = *Lolium perenne;* Phl = *Phleum pratensis;* Pp = *Poa pratense;* Pt = *Poa trivialis;* Tr = *Trifolium repens;* D = *Dactylus glomerata;* As = *Agrostis stolonifera;* Ely = *Elytrigia repens;* Hl = *Holcus lanatus;* Pa = *Poa annua;* Weeds = mostly *Ranunculus repens, R. acris, Stellaria media, Taraxacum officinale* and *Leontodon autumnale.*

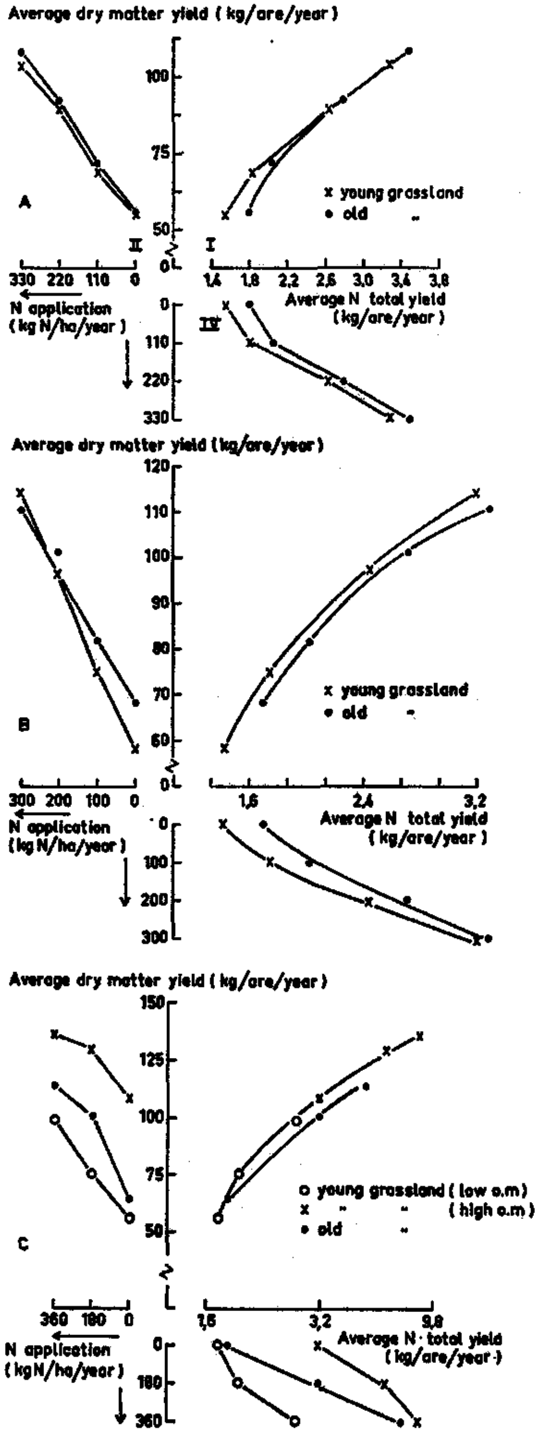


Fig. 5. Relation between dry-matter and nitrogen yield (I), nitrogen application and dry matter yield (II) and nitrogen application and nitrogen yield (IV) of old and young grassland, first three or four year after resowing. Yields expressed as kg/100 m² per year. A: Experiment E1, first 4 years; B: Experiment E3, first 3 years and C: Experiment E8, first 3 years.

contribute to the botanical composition of the young, resown, sward. This is normal.

*Dry matter yield*

The results presented in this paper do not correspond with the opinion, often expressed, that young grassland by definition yields higher than old grassland. This undoubtedly is (mainly) because we used good, old grassland, whereas many other workers (Roberts & Williams, 1940; Currie, 1947; Davies & Williams, 1948; Dallas & Bullen, 1949; 't Hart & Van der Woerdt; 1949; Davies, Milton & Lloyd, 1950; Klapp, 1953; Dijke, 1964; Andries & Van Slijcken, 1965; Davies, 1967) compared inferior old grassland to good young grassland, with in the latter case generally much better growing conditions. Our results correspond to those of other experiments in which these inaccuracies were also avoided; here, young grassland did not show higher gross yields either (Illingworth, 1960; Mudd & Mair, 1961; Davies, 1971 and Mudd, 1971).

As to the impression gained in practice of young grassland often giving higher yields, it should be realized that the yields of grassland are difficult to estimate and that those of young grassland, due to the more erect habit of the grass, are often overestimated.

If the effect of the changing N supply of the soil is separated from the other effects, the young grass sward in most cases gives at the same N yield a somewhat higher dry matter yield (Fig. 5; Quadrant I).

These higher yields may be due to sowing other species or varieties but also to the young age of the grass sward (e.g. to a more erect growth habit of the young grass plants, resulting in harvesting more N-deficient stubbles). In Experiment E5, where the botanical composition showed only a relatively small change by reseeding, the same effect occurred (Fig. 6).

In 1964 and 1966 the grassland was resown and in 1965 and 1967 the dry matter production was at the same N yield distinctly higher on the young grassland than on the older grassland; by 1968, however, the difference had completely disappeared.

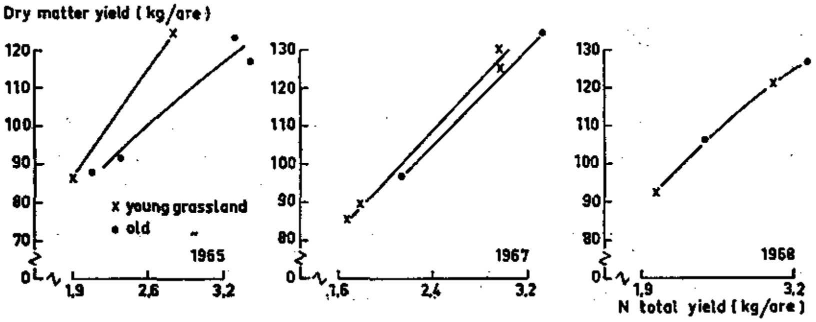


Fig. 6. Relation between dry-matter and nitrogen yield, in kg/100 m² per year, on the resown and not resown treatments of Experiment E5.

Net N mineralization here, was not stimulated at reseeding. The nitrogen yield was frequently even decreased, whereas it was increased by N application (Fig. 5; IV). These results differ from those of others (e.g. Rice & Penfound, 1954; Klapp, 1971 and Zürn, 1971). This is probably because these workers attributed the frequent yield increases obtained by reseeding inferior grassland to the mentioned stimulation, whereas they were really caused by replacing an inferior grass sward by a good one. Occasionally, however, an abnormally large accumulation of not or incompletely decomposed plant parts may occur at or near the soil surface; this 'mat formation' or 'sod or root-bound conditions' is usually caused by the joint occurrence of many rhizomatous or stoloniferous grass species and adverse mineralization conditions (Bates, 1948; Van Rhee, 1963; Kirkwood, 1964; Kleinig, 1966 and Nielson, 1969). This accumulation may lead after reseeding and improving the growing conditions to increased net N mineralization. After reseeding increased mineralization may also occur due to liming (Müller, 1951; Frecks & Puffe, 1958; Konova, 1958; Hasler, 1961; and others).

Since soil cultivation of a bare soil usually does stimulate net N mineralization (Rippel-Balder, 1952; Frecks & Puffe, 1958; Winsor, 1958; Ivanov, 1959 and Woldendorp, 1963), the lack of stimulated N mineralization after direct reseeding evidently lies in the presence of the grass. The grass apparently counteracts the stimulation obtained by cultivation. Huntjens' (1971) findings correspond with this. When the period between cultivation and reseeding is longer or when arable crops are grown increased mineralization will occur (Barrow, 1957 and Hoogerkamp, 1974a).

The decreased nitrogen yields on the reseeded treatments (Fig. 5; IV) are caused by the organic matter in the topsoil being distributed over a thicker soil layer and by the negative effect of decreasing the organic matter content in the topsoil being greater than the positive effect of increasing it in the subsoil (Hoogerkamp, 1974a).

As the negative effect of lowering the N supply from the soil dominated the higher dry matter production at the same N yield, the dry matter yield of the reseeded grassland mostly was somewhat lower. These yield differences increased by yield losses brought about directly by reseeding; under adverse growing conditions even for many weeks the young sward has a more open canopy than the old one.

### *Production distribution*

There was no distinct variation between old and resown grassland. Currie (1946) and Hunt (1966) came to the same results, whereas Moore's (1946) and Mudd & Meadowcroft's (1964) results were different. The species (e.g. *Lolium multiflorum* in young grassland, *Alopecurus pratensis* in old) or the variety combination (e.g. early varieties of *Lolium perenne*) and the growing conditions (e.g. drainage, organic matter content and fertilizer status) are essential for the production distribution.

### *Drought sensitivity*

Normally there was no difference in drought sensitivity between old and recently reseeded grassland. The mentioned observations that young grassland is more drought resistant are mainly based on comparisons of inferior old grassland, often consisting of many drought sensitive species (*Poa annua, Poa trivialis*, etc.) and in more drought sensitive conditions (poor fertilizer status, periodic water excess), with young grassland, which is better in these respects. Other precise comparisons between good old grassland and good young grassland are not known to the author.

In this research only young grassland reseeded on grassland whose grass sward was previously deeply buried, appeared to be somewhat more drought resistant; mainly due to better nitrogen supply (Hoogerkamp, 1974a). In dry periods the moisture tension often decreases with increasing depth in the profile (Wind, 1955 and Rijtema, 1965); root growth and activity will therefore gradually move to lower layers (Garwood & Williams, 1967). This results in the best growth on treatments were besides water the most available N amounts are present.

The withdrawal of the root system to more superficial layers, which is often put forward as a cause of the greater drought sensitivity of old grassland (Könnecke, 1951; Goedewaagen & Schuurman, 1956 and Alberda et al., 1966), hardly occurred in the present experiments. Like in the experiments of Salonen (1949) and of Goedewaagen & Schuurman (1956), there was indeed an appreciable accumulation of roots in the topsoil, but in absolute terms, there was no distinct decrease in the subsoil. Three zones could easily be distinguished:

a. very dense root mass in the layer 0–(3–5) cm;
b. rather dense root mass in the layer (3–5)–30 cm;
c. much thinner root mass in the subsoil 30–80 cm.

However, the chemical or mechanical structure of the profile may change this rooting picture considerably. In how far root mass corresponds with root activity is not known; often there is no apparent correlation (Kraus, 1911; Van Lieshout, 1956; Troughton, 1957; Hanus, 1962; Wiersum, 1967). Wind's (1955) findings showed on heavy clay considerably more water depletion in the subsoil of young grassland than of old grassland. Hoogerkamp & Woldring (1967) stated that in drought periods young grassland on deeper drained soils, mainly struggled with nitrogen deficiency, whereas with old grassland another factor, like water, was also limiting.

The drought sensitivity of young grassland that occurred especially after harvesting a heavy cut, is probably due to the smaller amount of green parts remaining in the field, due to more erect growth of the grass. Removal of most of the aerial parts generally reduces water and mineral uptake, this effect being greater when fewer green leaves remain (Jäntti, 1952; Jäntti & Kramer, 1956; Garwood & Williams, 1967). Also other factors may be important:

- removal of more growing points due to the more erect growth,
- low sward density resulting in more drying and greater temperature fluctuations,
- one-sideness of the sward (more plants in the same development stage).

### *Dry matter content*

The dry matter content in the fresh herbage at the same dry matter yield was the same on old and young grassland. This is contrary to the findings of Mudd & Meadowcroft (1964) and to what is frequently stated by farmers. Only when the clover percentage varied did the dry matter content at the same dry matter yield vary as well; it was lowest on the grassland with the highest clover percentage. Other differences in botanical composition (e.g. tetraploid varieties or *Lolium multiflorum* in young grassland) may give such differences.

### *Crude protein content*

The often lower crude protein content in the dry matter of the young grassland at the same nitrogen application and at the same clover content of the herbage, orginated partly from a lower nitrogen supply (e.g. by a lower organic matter content of the soil). The cause of the remaining differences was not clearly established, as mentioned before.

These observations may also be important with respect to nitrate poisoning, especially occurring recently on young grassland in the Netherlands (Thomas & Willemsen, 1971). Usually the nitrate content is reasonably well correlated with the crude protein content of the grass (Kleyburg, 1971). This might indicate that the origin of the high nitrate content of young grassland is an indirect rather than a direct result of reseeding; e.g. due to excessive organic or inorganic dressing.

### *Net yields*

Net yields were not determined; the results obtained in the botanical and chemical analysis and the way in which the experimental fields were grazed do not make differences plausible. However, animal experiments would be required to establish whether there are differences or not.

## 4.2 Temporary grassland

### *Establishment*

The inclusion of arable crops between ploughing up and reseeding did not have a positive effect on germination and growing. In the greenhouse experiment, on one of the sandy soils there was some damage by insects to the grassland treatment. Seeding results were better or arable land than on ploughed-up grassland mainly because the seed bed was mostly firmer and thus the capillary rise of water was better. On arable land, this firmer seed-bed is achieved by a better soil cultivation (e.g. ploughing up instead of rotavating) heavier rolling of the seed bed and lower organic matter content.

It is not clear to what degree insects, nematodes and fungi cause difference in damage between grassland sown on arable land and resown grassland.

### *Botanical composition*

Differences in botanical composition between grassland sown on arable land and reseeded grassland were not clear; at most there were in reseeding more problems with undesired species (e.g. *Elytrigia repens*, *Poa annua* and *Stellaria media*), while white clover sometimes showed poorer development as the result of a higher organic matter content of the soil and more diseases and pests).

### *Dry matter yields*

The favourable effect of grassland on the yield of the following arable crops (Hoogerkamp, 1974a) is offset by a lower yield of the grassland sown after arable crops compared with direct reseeding. This also results from a difference in the nitrogen supply from the soil (Fig. 7).

The lower dry matter yield in grass on the arable treatment in nearly all cases was due to a decreased N supply in the soil. The dry matter yield at the same nitrogen yield was the same for both treatments. Here too the same organic matter effect occurred as is discussed elsewhere (Hoogerkamp, 1974a).

To what extent the amount of nitrogen economized during the arable crop phase differ from that additionally applied during the grassland phase could not be estimated exactly. The impression was, however, that the latter amount of nitrogen was greater.

Striking and as yet inexplanable is the relatively high yield immediately after sowing grass on permanent arable land; perhaps this is due to the absence of rotation diseases and pests (Eissa, 1972 and Oostenbrink, 1973).

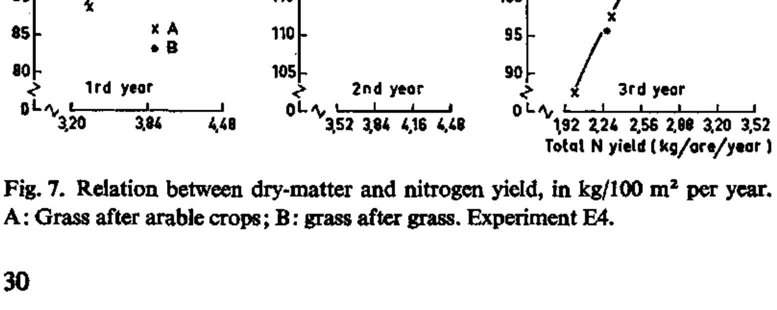


Fig. 7. Relation between dry-matter and nitrogen yield, in kg/100 m$^2$ per year.
A: Grass after arable crops; B: grass after grass. Experiment E4.

# Summary

There is a considerable controversy on the relative value of temporary and permanent grassland and on the effect of resowing old grassland. This study was on the resowing of permanent grassland of a good botanical composition and on including arable crops in a grassland rotation.

In resowing with mixtures in which *Lolium perenne* was dominant (15 to 20 kg/ha *L.p.*), perennial ryegrass dominated considerably. Under favourable weather conditions the other species either sown or not sown could hardly develop, when resowing was carried out efficiently. Frequent resowing (every two years) decreased the dominance of *Lolium perenne*. *Poa annua* and *Elytrigia repens* increased appreciably.

The dry matter yield was usually somewhat lower after resowing; mainly through losses during the resowing phase, but often one or more years afterwards as well. Distribution of the dry matter production throughout the year and drought sensitivity were not markedly affected by resowing.

Maximum rooting depth was attained a few months after resowing and hardly changed afterwards. The root mass continued to increase for some years, especially in the top 5 cm of the profile.

The dry matter content of the fresh herbage was not distinctly affected by resowing. The crude protein content of the dry matter was frequently lower on the resown grassland than on the permanent grassland.

Alternating arable crops and grassland resulted in decreased yields of dry matter and crude protein from the grassland sown after the arable crops. This was almost entirely due to insufficient N supply from the soil. An effect on germination of the grass seed, growth of the young grass plants or on the botanical composition was seldom observed. Resowing grassland on permanent arable land unaccountably gave very high yields the first year.

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

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

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# Abstract

Middelkoop, J. H. van (1973) Physiological and genetical aspects of egg production in White Plymouth Rock pullets. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 813, ISBN 90 220 0495 3, (viii) + 76 p., 27 tables, 12 figs, summary.
Also: Doctoral thesis, Wageningen (available also as Commun. Spelderholt Inst. Poultry Res. 205).

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.

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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.

# Contents

**Physiological aspects**

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

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# 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.

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# 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

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## 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

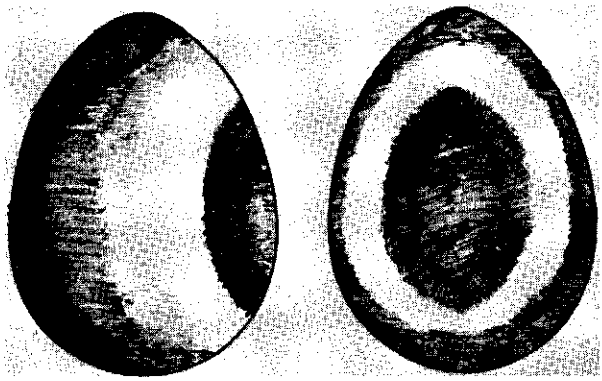

Fig. 1. Diagrammatic representation of the additional calcification shown as a light band over the surface of the egg.

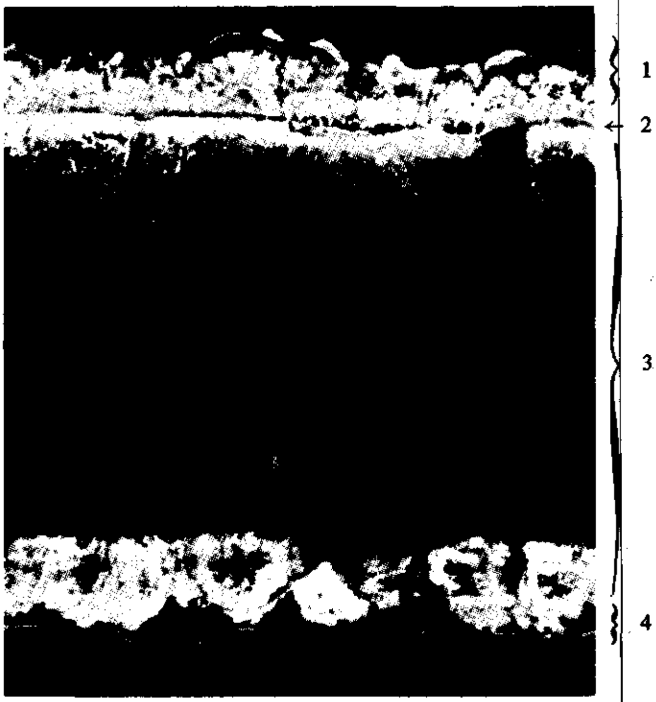

Fig. 2. Radical section of the shell of the first egg of a pair at a point with additional shell deposition. 1 = extra shell deposition, 2 = true cuticle, 3 = 'normal' shell, 4 = shell membranes.

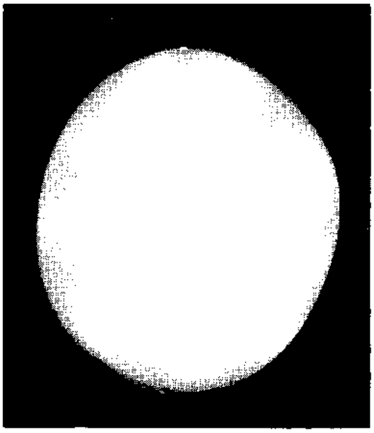

Fig. 6. Photograph of an egg with a characteristic bulge.

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

The authors thanks Dr. Ir. P. C. M. Simons for his critical advice and useful help.

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

Already published in Archiv für Geflügelkunde 36:63–70 (1972)

## 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 | Fertilized | | Hatched | |
|---|---|---|---|---|---|
| | number | 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 ($\chi^2$-test).

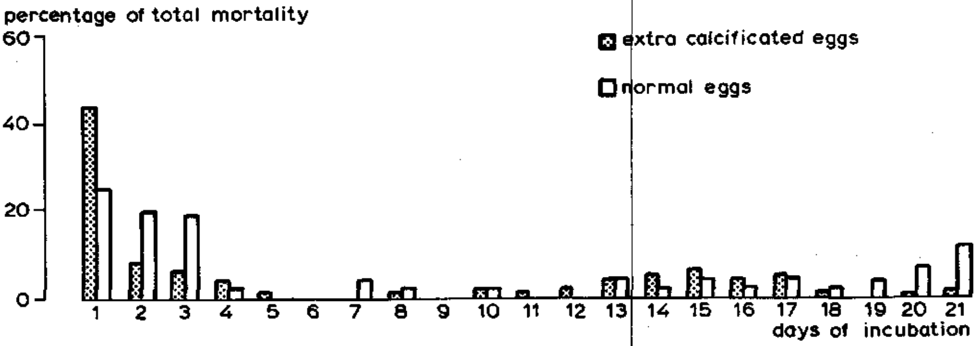

**Fig. 1.** Distribution of embryonic death.

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

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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

## 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

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; Tardatjian, 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.

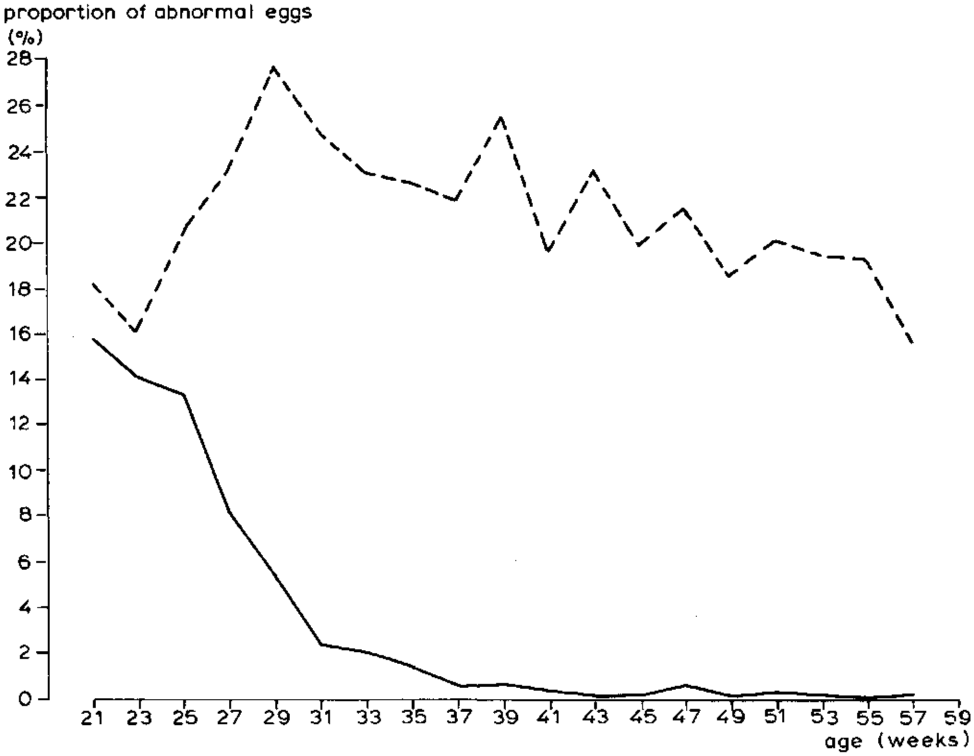

Fig. 1. The proportion of abnormal eggs/total egg production (%) during the laying period. Double-yolked eggs laid in the DY line of 1972 hatch (——); 'first' eggs laid in the TE/D line of 1972 hatch (– – –).

## 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 |

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

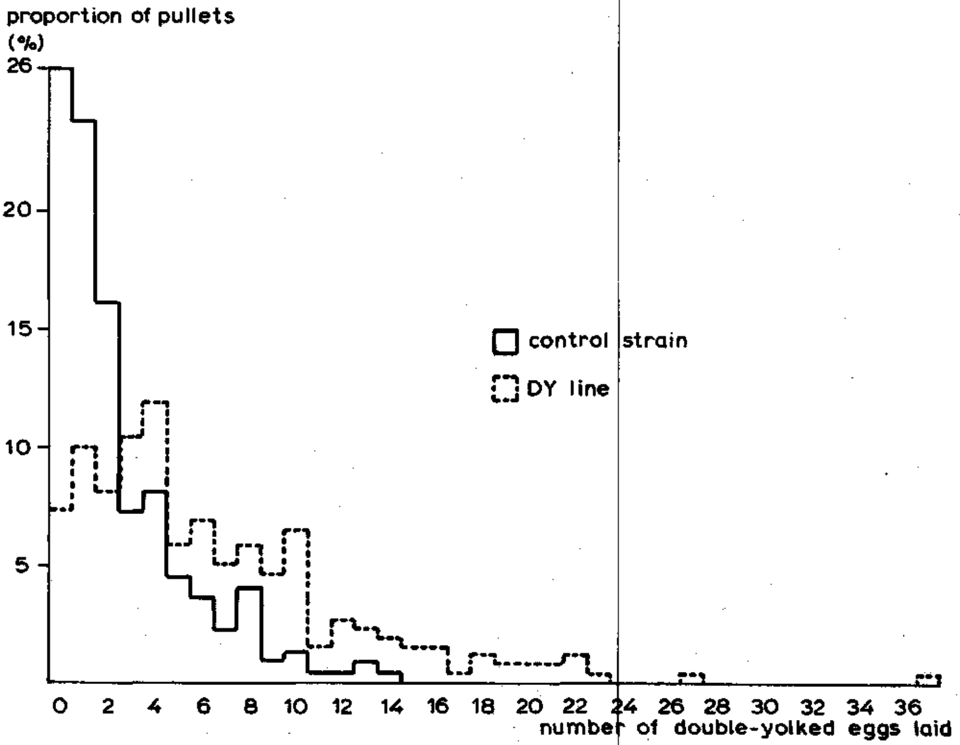

**Fig. 2. Frequency distribution of hens laying different numbers of double-yolked eggs in 200 ~~days of~~ production from the 1972 hatch.**

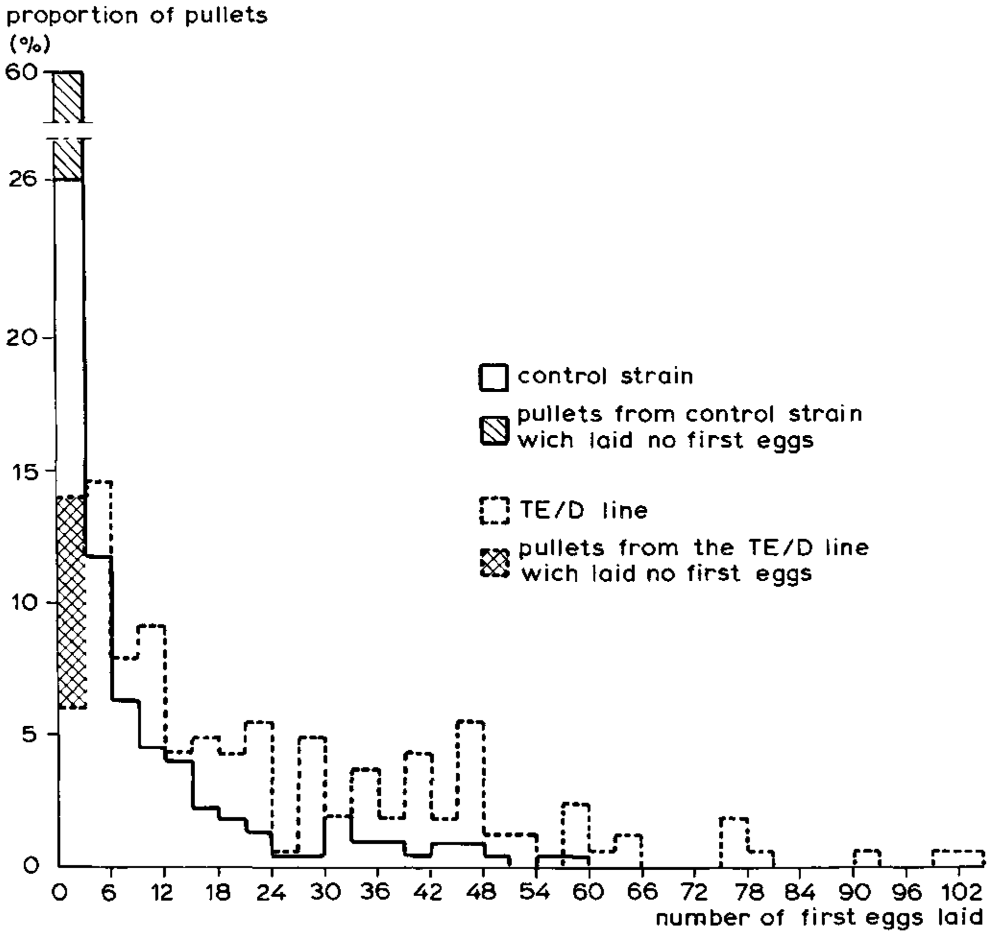

Fig. 3. Frequency distribution of hens laying different numbers of 'first' eggs in 200 days of production from the 1972 hatch.

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).

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

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

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

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

5 ± 0.17 0.45 ± 0.18 0.55 ± 0.23 0.52 ± 0.21 0.45 ± 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

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

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 mea |
| *Total number of yolks* | | | | | | | | |

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* × *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* × *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* × *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* × *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* × *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* × *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 |

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

# 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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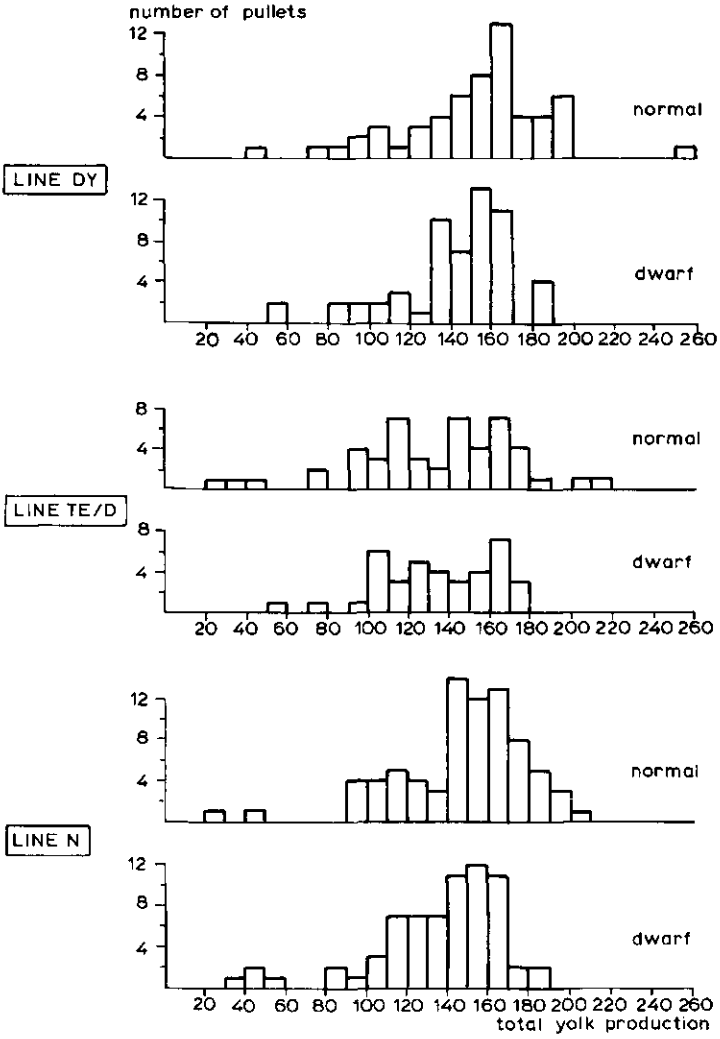

Fig. 1. Frequency distribution of total yolk production in 224 days per line and per genotype.

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

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.

## References

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