Divergent selection for ω3:ω6 polyunsaturated fatty acid ratio in quail eggs

Dedicated to Prof. Dr. Peter Horst on the occasion of his 75th birthday

Abstract
It is well known, that the ω6:ω3 PUFA (polyunsaturated fatty acids) ratio of the egg yolk can be changed by modifying the hen’s diet. Information on genetic effects on PUFA in egg yolks is limited. Therefore four generations of divergent selection of high and low ω6:ω3 PUFA ratio were performed with quails as model animals to estimate genetic effects on the fatty acid profile of eggs. Heritability and correlated response to selection were analysed. Generation 4 consisted of 125 hens in the HIGH and 114 hens in the LOW line and 40 sires per line. Fatty acid profiles of the eggs were measured on pools of 3 yolks per hen, two times per hen. After four generations of selection ω6:ω3 PUFA ratio was significantly different in the LOW (12.4) and HIGH line (14.9), i.e. a difference of 1.6 phenotypic standard units. Heritability for ω6:ω3 PUFA ratio was estimated at 0.45 (SE 0.05). Selection tended to be asymmetric in the way that response to selection in the LOW line was higher. Fertility and hatchability of fertile, age at first egg, laying intensity, egg weight and fat percentage were not significantly different between selected lines, but yolk weight, yolk proportion and fat content were significantly higher in the Low line. Moderate heritability of ω6:ω3 PUFA ratio in egg yolks and lack of correlated responses to selection in major production and reproduction traits indicate that breeding for a lower ω6:ω3 PUFA ratio in eggs is promising.

Key Words: selection, ω3 fatty acids, product quality, functional food, heritability

Zusammenfassung
Titel der Arbeit: Divergente Selektion auf das Verhältnis von ω6 und ω3 mehrfach ungesättigten Fettsäuren in Wachteleien

Schlüsselwörter: Selektion, ω3 Fettsäuren, Produktqualität, funktionales Nahrungsmittel, Heritabilität
Introduction
Fatty acids play important metabolic, structural and functional roles in physiology. In human nutrition the uptake of polyunsaturated fatty acids (PUFA) of the ω3 type is too low compared to the uptake of the ω6 type PUFA, with a ratio of around 25:1. This is far away from the recommended ratio of 5:1. This unsatisfactory ratio in human nutrition leads to a down regulation of ω3 PUFA derived endogenous synthesis of docosahexaenoic acid (DHA, C22:6, ω3) and eicosapentaenoic acid (EPA, C20:5, ω3), which are known to be important players in the prevention of cardiovascular diseases in humans. A lower ratio of ω6:ω3 in food should contribute to maintenance and improvement of human health (PFEUFFER, 2001). There are several attempts to increase the content of ω3 fatty acids in meat and eggs in sense of producing functional food by feeding specific ω3 enriched diets to the animals (FARRELL, 1995; EDER et al., 1998; KOUBA et al., 2003; DANNENBERGER et al., 2004; BOURRE, 2005). However breeding, the most sustainable mode to improve product quality, has not been widely addressed in terms of modifications of fatty acid composition of foodstuff derived from farm animals. Poultry species are able to synthesize EPA and DHA by fatty acid elongation and desaturation from short feed ω3 fatty acids and to deposit these substances in the yolk. Up to now minor knowledge is available on the genetic variation of ω6 and ω3 PUFA in egg yolk and to which extent ω3 fatty acid absorption, endogenous biosynthesis or deposition in the egg yolk contribute to variation. In order to address these questions and to evaluate the feasibility to breed for reduced ω6:ω3-ratio and/or increased ω3 PUFA yolk content a divergent selection experiment was conducted with quail as a model animal for other poultry species.

Materials and Methods
Animals
Japanese quails (coturnix coturnix japonica) of four flocks kept as close population at the Institute of Animal Science, University of Bonn, since 1966, with three representing lines divergently selected for dustbathing activity and a control line (GERKEN and PETERSEN, 1992) and one representing an independent line were used (generation S0). Within each of the four flocks divergent selection was performed first by selecting six hens with highest and six hens with lowest ω6:ω3-ratio in egg yolk out of 30 hens by mass selection. These hens were mated to randomly chosen cocks of the same flock to produce two sub lines within each flock, which in total represent the S1 (n=240 hens) consisting of the HIGH and LOW line, respectively. The following mass selection was done within each of the eight sub lines leading to S2 (n=240 hens). Starting with individually signed hens of the S2 a pedigree structure was established with 48 fullsib families (six per sub line). The S4 generation finally consisted of 125 hens in the HIGH and 114 hens in the LOW line and 40 sires per line. An unselected control line was maintained at all time. Hens were kept in individual cages and fed a commercial layer diet that was analysed for the fatty acid profile (Table 1).

Phenotype records
Fatty acid profiles of the eggs were measured on pools of three yolks per hen, two times per hen after chloroform-methanol extraction (FOLCH et al., 1957) at room
temperature followed by transmethylation with trimethylsulphoniumhydroxide (TMSH) (SCHULTE and WEBER, 1989) and quantification by gas chromatography (MENNICKEN et al., 2000). Moreover, age at first egg, laying intensity, egg weight, yolk weight and fat percentage as well as fertility and hatchability of fertile were recorded.

Statistical analysis
Analyses of variance were examined with SAS for Windows version 8.2, using the following model:

\[ y_{ijkl} = \mu + \text{hatch}_i + \text{flock}_j + \text{selection line}_k + e_{ijkl} \]

where:

- \( y_{ijkl} \) is the proportion of respective fatty acids and \( \omega_6: \omega_3 \) ratio of egg yolk
- \( \mu \) is the overall mean
- hatch (fixed effect) \( i = 1–2 \)
- flock (fixed effect) \( j = 1–4 \)
- selection line (fixed effect) \( k = 1–2 \) (high, low)
- \( e_{ijkl} \) is the residual error

Heritabilities were estimated using an animal model with the program VCE4 (GROENEVELT, 1998). Records of the S2 to S4 were analyzed in a one-trait model with repeated measures. Estimates are based on records of 264 animals (401 measures) of the LOW line and 301 animals (479 measures) of the HIGH line. Breeding value estimation was performed using the program PEST (GROENEVELT, 1993).

Results
Compared to the feed egg yolk had a higher proportion of SFA and MUFA (Table 1). These fatty acids, mainly palmitic acid stearic acid as well as oleic acid and palmitoleic acid, are synthesized endogenously and preferentially deposited in the egg yolk. The proportion of linoleic acid and \( \alpha \)-linolenic acid was higher in the diet than in the egg yolk. Arachidonic acid and DHA were not detected in the feed but were present in the egg yolk (Table 1). Comparison of the fatty acid profiles between the selection lines shows that after four generations of selection for \( \omega_6: \omega_3 \) PUFA ratio the LOW line (12.4) was significantly different from the HIGH line (14.9), i.e. a difference of 1.6 phenotypic standard units (\( s_p = 1.57 \)) and four genetic standard units (\( s_g = 0.64 \)) (Table 1). Moreover the selection lines differed significantly for the proportion of myristic, palmitic palmitoleic and linoleic acid, that were higher while the proportion of oleic and docosahexaenoic acid were lower in the HIGH line (Table 1). The selected lines differed significantly in SFA, MUFA, PUFA \( \omega_3 \), PUFA \( \omega_6 \) and the \( \omega_6 \) and \( \omega_3 \) PUFA ratio (Table 1).
Heritability for ω6:ω3 PUFA ratio was estimated at 0.45 (SE 0.05). Selection tended to be asymmetric in the way that response to selection in the LOW line (the desired direction) was higher.

Table 1
Fatty acid profiles of feed and egg yolk of quails after four generations of divergent selection for high (HIGH line) and low (LOW line) ω6:ω3 PUFA ratio and of the unselected CONTROL (% of total fatty acids, least square means) (Fettsäuremuster im Futter und in Wachteleidottern nach vier Generationen divergenter Selektion auf hohes (HIGH) oder niedriges (LOW) ω6:ω3 PUFA-Verhältnis und der unselektierten Kontrolle (% Gesamtfettsäuren))

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>feed</th>
<th>LOW Line</th>
<th>HIGH line</th>
<th>pooled</th>
<th>F-Test</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>(percentage [%])</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristic acid</td>
<td>C14:0</td>
<td>1.36</td>
<td>0.51</td>
<td>0.55</td>
<td>&lt;0.01</td>
<td>***</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>C16:0</td>
<td>14.42</td>
<td>26.28</td>
<td>26.61</td>
<td>0.09</td>
<td>*</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>C16:1ω7</td>
<td>0.30</td>
<td>3.65</td>
<td>3.83</td>
<td>0.06</td>
<td>*</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>C18:0</td>
<td>4.96</td>
<td>9.52</td>
<td>9.56</td>
<td>0.08</td>
<td>ns</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>C18:1ω9</td>
<td>29.48</td>
<td>42.69</td>
<td>40.66</td>
<td>0.17</td>
<td>***</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>C18:2ω6</td>
<td>45.97</td>
<td>13.28</td>
<td>14.80</td>
<td>0.14</td>
<td>***</td>
</tr>
<tr>
<td>ω-Linolenic acid</td>
<td>C18:3ω3</td>
<td>3.53</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>C20:4ω6</td>
<td>-</td>
<td>2.78</td>
<td>2.81</td>
<td>0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>C22:6ω3</td>
<td>-</td>
<td>1.30</td>
<td>1.18</td>
<td>0.02</td>
<td>***</td>
</tr>
<tr>
<td>SFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.74</td>
<td>36.31</td>
<td>36.72</td>
<td>0.11</td>
<td>*</td>
<td>37.14</td>
</tr>
<tr>
<td>MUFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29.78</td>
<td>46.34</td>
<td>44.49</td>
<td>0.17</td>
<td>***</td>
<td>44.79</td>
</tr>
<tr>
<td>PUFA ω3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.53</td>
<td>1.30</td>
<td>1.18</td>
<td>0.18</td>
<td>0.02</td>
<td>***</td>
</tr>
<tr>
<td>PUFA ω6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.97</td>
<td>16.06</td>
<td>17.61</td>
<td>0.14</td>
<td>***</td>
<td>16.88</td>
</tr>
<tr>
<td>ω6:ω3-PUFA ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.02</td>
<td>12.35</td>
<td>14.93</td>
<td>0.16</td>
<td>***</td>
<td>14.23</td>
</tr>
</tbody>
</table>

SFA: saturated fatty acids (gesättigte Fettsäuren), MUFA: mono unsaturated fatty acids (einfach ungesättigte Fettsäuren), PUFA: poly unsaturated fatty acids (mehrfach ungesättigte Fettsäuren); only cis-fatty acids were addressed; na: not analysed; γ-linolenic acid and eicosapentaenoic acid proportions were < 0.01 % in egg yolk fatty acids and were not analysed; pooled SEM: standard error of mean; F-Test p: significance of “line” effect.

Fertility and hatchability was high in both selected lines (Table 2). Higher fertility of the selected lines than the CONTROL is due to the fact that selected animals were tested for fertility (2 cocks and 1 hen where replaced due to infertility) while control animals were not. Differences between the selected lines were not significant but reproduction traits tended to be superior in the LOW line. Age at first egg, laying intensity, egg weight and yolk total fat percentage were not significantly different between selected lines, but yolk weight and proportion as well as the absolute fat content per egg were significantly higher in the LOW compared to the HIGH line (Table 2). Given the differences in ω6:ω3 PUFA ratio and yolk weights, eggs of the LOW line hens contained significantly lower amounts of ω6 PUFA (-8 mg/egg) but highly significant larger amounts of ω3 PUFA (+2 mg/egg) than the HIGH. Correspondingly, phenotypic correlations between ω6:ω3 PUFA ratio and ω3 PUFA proportion and content were high (-0.62 to -0.78); correlation to the ω6 proportion was low (0.18) but also significant, while there was no significant correlation to ω6 absolute content. Phenotypic correlations between ω6:ω3 PUFA ratio and egg weight,
yolk weight and proportion, fat content/egg as well as age at first egg and laying intensity were in general low and only partly significant.

Table 2
Reproduction and laying performance traits of quails after four generations of divergent selection for high (HIGH line) and low (LOW line) ω6:ω3 PUFA ratio and of the unselected CONTROL (least square means ± standard error) (Reproduktions- und Legeleistungsmerkmale bei Wachteln nach vier Generationen divergenter Selektion auf hohes (HIGH) oder niedriges (LOW) ω6:ω3 PUFA-Verhältnis und der unselektierten Kontrolle)

<table>
<thead>
<tr>
<th>Traits</th>
<th>HIGH</th>
<th>LOW</th>
<th>F-Test</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of eggs set</td>
<td>509</td>
<td>501</td>
<td></td>
<td>180</td>
</tr>
<tr>
<td>fertility [%]</td>
<td>91</td>
<td>95</td>
<td>ns¹</td>
<td>80</td>
</tr>
<tr>
<td>hatchability [%]</td>
<td>80</td>
<td>82</td>
<td>ns¹</td>
<td>81</td>
</tr>
<tr>
<td>age at first egg [d]</td>
<td>51.01±0.52</td>
<td>50.49±0.52</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>laying intensity [%]</td>
<td>86.79±0.86</td>
<td>86.35±0.86</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>egg weight [g]</td>
<td>10.0±0.08</td>
<td>10.0±0.08</td>
<td>ns</td>
<td>10.73</td>
</tr>
<tr>
<td>yolk weight [g]</td>
<td>3.1±0.03</td>
<td>3.3±0.03</td>
<td>***</td>
<td>3.42</td>
</tr>
<tr>
<td>yolk proportion [%]</td>
<td>30.8±0.16</td>
<td>32.7±0.17</td>
<td>***</td>
<td>31.9</td>
</tr>
<tr>
<td>yolk total fat [%]</td>
<td>31.6±0.08</td>
<td>31.4±0.08</td>
<td>ns</td>
<td>31.8</td>
</tr>
<tr>
<td>fat content per egg [mg]</td>
<td>980±10</td>
<td>1026±10</td>
<td>**</td>
<td>1092</td>
</tr>
</tbody>
</table>

¹chi-square test;

Discussion
Quality of foodstuff produced by agricultural animals becomes increasingly important with growing consumer awareness for healthy aspects of food. These aspects do not only include pathogens or traces of contaminants but also the composition of the food itself is an important characteristic of its nutritional value. We aim to evaluate the feasibility to sustainable improve the nutritional value of eggs by breeding for low ω6:ω3 ratio and/or increased ω3 PUFA yolk content and thereby create a “functional food”.

Fat content and composition of diets affect human health for example the risk of cancer, diabetes, and cardiovascular diseases. With this regard PUFA are of major interest. Linoleic acid and α-linolenic acid are essential precursors of longer ω6 and ω3 PUFA. However their synthesis via elongation, desaturation, and β-oxidation is inefficient in human and thus they need to be delivered by the diet. Since synthesis of ω6 and ω3 PUFA from shorter precursors is catalysed by the same enzyme complexes, including Δ5- and Δ6-desaturases and elongases (VOSS et al., 1991), high ω6:ω3 ratios of the diet prevent sufficient synthesis of ω3-PUFA, exhibiting most favourable effects on health. The recommended ω6:ω3 PUFA ratio of human diets is 5:1 while it actually is mostly 25:1 in typical western diets (FARRELL, 1995). Besides fish products, eggs with their high proportion of fat, with ca. 18% unsaturated fatty acids, variability of composition of this fraction could serve as a source of ω3 fatty acids. Modification of the fatty acid composition of egg yolk by ω3 fatty acid enriched diets is well established and is commercially used (LESKANICH and NOBLE, 1997). However, little attention was dedicated so far to the natural genetic variability of the poultry regarding absorption, biosynthesis and deposition of ω3-PUFA in egg yolk and thus the possibility of the improvement of the nutritional value of the egg by breeding. Variability of the content of ω3-PUFA in egg yolk has been shown in different poultry species, like chicken, turkey, duck, goose and quail (LESKANICH and NOBLE, 1997; SURAI et al, 1999). Within the species chicken differences between different origins
are described regarding fatty acid profiles (AHN et al., 1995; SCHEIDELER et al., 1998). Age effects as well as interactions between lines and age of the hens have been reported. Also the body fat content of the hens affects the fatty acid profile in the egg (WASHBURN, 1990; SCHEIDELER et al., 1998). The absorption rate for linoleic acid and to a higher extend of \( \alpha \)-linolenic acid increases with the age of the hens. Also the biosynthesis rate of the long derivatives from the appropriate precursors correlates with the age of the hens (SCHEIDELER et al., 1998). Moreover, there is clear indication that there is genetic variability enabling to increase the \( \omega_3 \)-PUFA content or decrease the \( \omega_6: \omega_3 \) PUFA ratio in the egg yolk (MENNIKEN et al., 2000). It is not so far well-known, to which extent this is based on variance in the absorption rate or biosynthesis rate or deposition rate of fatty acids in the yolk. It is also not well-known, to what extent unwanted genetic correlations exist to important capability characteristics e.g. to the reproduction ability. In this study phenotypic variation in the yolk fatty acid profile observed was used to divergently select on the \( \omega_6: \omega_3 \) PUFA ratio. Our data clearly indicate that quail are able do enrich arachidonic acid and DHA in the egg yolk. Linoleic and \( \alpha \)-linolenic acid are precursors of long polyunsaturated fatty acids that were available to the bird via the diet. Obviously, since affinity of delta-6-desaturase to \( \alpha \)-linolenic acid is high the later is metabolised to DHA. Significant differences between HIGH and LOW in \( \omega_6: \omega_3 \) PUFA ratio are due to differences in the percentage of DHA on the one hand and linoleic acid on the other hand. Animals of the LOW line deposit a higher percentage of DHA but a lower percentage of linoleic acid in the egg yolk. These differences might be due to differences in endogenous utilisation of \( \omega_6 \) and \( \omega_3 \) precursors. Biosynthesis rate of DHA is higher in the LOW line. Feed linoleic acid is used to a higher degree in the LOW line but not to produce arachidonic acid. This may indicate a higher affinity to \( \omega_3 \) fatty acids of the desaturases and elongases involved in this metabolic pathway. After four generations of selection moderate heritability of the trait \( \omega_6: \omega_3 \) PUFA ratio was observed, essentially reaching coefficients that were estimated for body weight and carcass traits in quail and exceeding those estimated of reproduction traits (SCHUELER et al., 1996).

Divergent selection had no effect on fertility and hatchability. Since \( \omega_3 \) fatty acids are of importance for prenatal brain development such effects might be expected due to impact on vitality of embryos. It is of interest to further analyse this in following generations of divergent selection. Age at first egg, laying intensity and egg weight were also not different between the selected lines. However HIGH and LOW lines differed significantly in yolk weight and yolk proportion but not in total yolk fat percentage. Thus differences in the fatty acid profile also represent differences in total content of the fatty acids.

This selection experiment demonstrated for the first time that selection for high and low \( \omega_6: \omega_3 \) PUFA ratio is feasible in the quail. Moderate heritability, considerable genetic variability, and the lack of negative impact on other traits promote breeding as the most sustainable way to improve egg quality in terms of nutritional value. However, though the selection response was already remarkable after four generations, further evaluation of the selection response to approach possible limits of selection for \( \omega_6: \omega_3 \) PUFA ratio will be necessary. Moreover, the selection lines represent a valuable experimental resource to identify the genes and their alleles controlling the
ω6 and ω3 PUFA metabolic pathways by genomic approaches including candidate genes analyses, in particular focusing on fatty acid elongases and desaturases, as well as QTL mapping and expression studies. Finally, it will be of interest to evaluate the feasibility of ω6:ω3 PUFA ratio selection in chicken, the main egg producing species. Since chickens are able to enrich a higher amount of DHA in the yolk than other poultry species including quail (SURAI et al., 1999) this perspective is promising.

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