Fatty acid composition of meat and genetic mapping of quantitative trait loci in 3 generations of Japanese quail populations

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Abstract

The present study was carried out to evaluate the effect of different lines and generations of adult males and females of Japanese quail (*Coturnix japonica*) on total lipid, fatty acid (FA) composition and cholesterol content of breast muscle, as well as the identification of quantitative trait loci (QTLs) controlling the above mentioned meat quality traits. Forty-four quails (generation F_0), 22 Pharaoh (F-33) meat-type males and 22 Standard (S-22) laying-type females, were crossed to produce the F_1 hybrids generation. F_2 generation was created by mating one F_1 male with one F_1 female, full siblings. The birds, randomly chosen from F_0 (22 males and 22 females), F_1 (22 males and 22 females) and F_2 (84 males and 152 females), were raised to 20 weeks of age in collective cages. After slaughter the carcasses were dissected and the breast muscle was removed for the chemical analysis. Genomic DNA was extracted from the whole blood and 30 microsatellite markers located on two quail chromosomes were genotyped. S-22 quails exhibited higher amounts of total saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), while F-33 quails had a greater content of total monounsaturated fatty acids (MUFA). F-33 quails supplied meat with lower atherogenic and thrombogenic indexes. A partial effect of sex on the FA composition of quail meat was found in the F_1 generation; while in the F_2 generation a sex-effect was more evident. F_2 females exhibited a higher PUFA content and PUFA/SFA ratio, and a lower muscle cholesterol content compared to F_2 males. In conclusion, total lipid, FA composition and cholesterol content were affected by first- and second-generation crosses. For the cholesterol content a QTL showing additive effect has been detected on CJA02 at 85 cM; no QTLs were found for total SFA, MUFA and PUFA. To current knowledge, this is the first study of a QTL associated with muscle cholesterol in quails.

Keywords: cholesterol, cross-breed, fatty acid, Japanese quail, quantitative trait loci

Introduction

The consumption of chicken meat is growing worldwide and it will continue to grow in the future, particularly in the developing countries (Alexandratos and Bruinsma, 2012). In this scenario, the consumption of game meat, such as quail, pheasant, partridge and pigeon, has gained increasing interest among consumers, who appreciate its texture and flavour as well as the low content of fat and cholesterol. Nowadays, Japanese quail (*Coturnix japonica*) has attained economic importance as an agricultural species producing eggs and meat that are enjoyed for their unique flavor. Several lines, breeds and varieties of Japanese quail have been developed for different production purposes (Genchev et al., 2008; Maiorano et al., 2011). Quail is one of the leanest type of poultry (Boni et al., 2010 cited in Gecgel et al., 2015) and its meat is characterized by high protein and low fat contents, with a high degree of unsaturated fatty acids (FA) and low sodium and cholesterol levels (Gecgel et al., 2015). Effects of factors such as breed, age, sex and nutritional condition on fat deposition and FA profile of Japanese quail are not widely studied. In fact, very few studies on the FA composition and cholesterol content of Japanese quail meat have been published (Botsoglou et al., 2004; Genchev et al., 2008; Boni et al., 2010; Sartowska et al., 2014; Gecgel et al., 2015; Yalcin et al., 2017), and available results are not always directly comparable. In addition, far too little attention has been paid to the determination of meat quality traits of adult or spent quails at the end of their productive life (Santhi and Kalaikannan, 2017). In fact, even if research shows that quail meat production is economically most effective when performed at the age of 35 days, there may also be markets for larger quail (300 g) to be sold as broiler or processed, and for older and slower growing quails (Minvielle, 2004). On the other hand, the old breeding birds are slaughtered and sold on commercial market without any distinction being made on age (Shanaway, 1994). Therefore, in the present research, the choice to slaughter quails at 20 weeks of age was first of all subordinated to developing 3 generation populations of Japanese quail for the quantitative trait loci (QTL) study, but also to extend the knowledge, still very scarce, on meat quality traits of adult/spent quails. The idea of QTL study is to identify linkage between the genotypic and phenotypic data. This can be done on the basis of interval mapping which identifies QTL region located between 2 flanking molecular markers. In this QTL analysis method, it is assumed the existence of the reference population derived by crossing 2 lines (meat line males and laying line females) which differ only with respect to the analyzed QTL. The mapping of QTLs is performed using regression analysis within F_2 generation. Cross of meat type and laying type Japanese quail, created for this experiment, fulfills all of these requirements. Few publications can be found in literature reporting QTLs responsible for some productive traits in Japanese quail (Tavaniello et al., 2014); QTL studies for meat quality traits as muscle cholesterol content or FA composition have not been performed in poultry species. The only results reported in AnimalQTLdb concern pigs (Malek et al., 2001; Thomsen et al., 2004; Quintanilla et al., 2011). In light of this, the present study was undertaken to evaluate the effect of different lines (meat-type and egg-type quails), cross (meat-type x egg-type quails; F_0 x F_1) and sex (males and females) on total lipid, FA composition and cholesterol content of meat from adult Japanese quails. Second goal of this study was to detect QTL for the above mentioned traits in an experimental cross of meat-type and egg-type quails.

Materials and methods

Experimental population

The experiment was performed with 2 Japanese quail (*Coturnix japonica*) populations (meat type and egg type) reared at the didactic experimental station of the University of Life Sciences in Lublin (Poland). The F_2 generation originates from a cross of Standard (S-22) laying-type females and Pharaoh (F-33) meat-type males. Forty-four quails (generation F_0), 22 Pharaoh (F-33) meat type males and 22 Standard (S-22) laying type females, were crossed to produce the F_1 hybrids generation. The F_2 generation was created by mating one F_1 male with one F_1 female, full siblings. A total 236 F_2 individuals were obtained (84 males, 152 females) in 2 hatches. The age of F_0 at the time of hatching offspring was 16 weeks and that of F_1 was 16 weeks (1^{st} hatch) and 18 weeks (2^{nd} hatch). The birds from F_2 were not parents of next generations. The birds from F_0 (22 males and 22 females), F_1 (22 males and 22 females) and F_2 (84 males and 152 females) were raised to 20 weeks of age in collective cages (F_0 and F_1: 6 birds in each 6 cages and 4 birds in each 2 cages; F_2 hatch 1: 6 birds in each 16 cages; F_2 hatch 2: 6 birds in each 22 cages and 4 birds in each 2 cages) under continuous lighting (natural and artificial). The rearing temperature was gradually decreased, 38 to 34 °C in the first week, 33 to 28 °C in the second week, and 27 to 22 °C in the third week. Afterwards, it was maintained between 18–20 °C. The quail were fed ad libitum commercial diets according to age. The diet containing 24% crude protein and 2,900 kcal of ME*kg^{-1} was used for the first 28 days; the finisher ration had 20% crude protein and 2,800 kcal of ME*kg^{-1}. Birds had free access to water during the experiment.

Slaughter surveys

At slaughter (20 weeks of age), all birds were individually weighed (after a fasting period of 12 hours), stunned, decapitated, and blood was collected for DNA analyses (F_2 generation only). Stunning was performed by a percussive blow to the back part of the head (occiput) and decapitation was performed with scissors between the cervical vertebrae and the base of the skull according to the European regulations on the protection of animals at the time of killing (European Communities, 2009). After the refrigeration period (24 hours at 4 °C), the left pectoral muscle was removed from 22 males and 22 females for each sex and generation, vacuum packaged, and stored frozen (−20 °C) for analyses of total lipid, FA composition and cholesterol content of muscle.

Total lipid and fatty acid composition of muscle

Lipid extraction from breast muscle samples was performed by Folch et al. (1957) method. FA were quantified as methyl esters (FAME) using a gas chromatograph an HRGC 5300 Fisons (Rodano, Milan, Italy), equipped with a flame ionization detector and a fused silica capillary Column (CP-Sil RTX 2330), 30 m x 0.25 mm x 0.5 µm film thickness (Restek, Bellefonte, PA, USA). Helium was used as carrier gas. The oven temperature program was 120 °C for one min then increasing at 5 °C*min^{-1} up to

230 °C where it was maintained for 20 min. The individual FA peaks were identified by comparison of retention times with mixtures of standard fatty acids (FAME, Sigma, St. Louis, MO) run under the same operating conditions. Quantification of individual FA was based on the internal standard method using tridecanoic acid methyl ester (C13:0). Results were expressed as percentage of the total FA analyzed. To assess the nutritional implications, the n-6/n-3 FA ratio and the PUFA/SFA (P/S) ratio were calculated. Moreover, in order to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, respectively the atherogenic index (AI) and the thrombogenic index (TI) were calculated, according to the formulas suggested by Ulbricht and Southgate (1991).

Measurement of muscle cholesterol

Cholesterol was extracted using the method of Maraschiello et al. (1996) and then quantified by high performance liquid chromatography (HPLC). A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Kinetex 5µ C18 reverse-phase column (150 x 4.6 mm x 5 µm; Phenomenex, Torrance, CA), was used. The HPLC mobile phase consisted of acetonitrile: 2-propanol (55:45, vol*vol^{-1}) at a flow rate of 1 ml*min^{-1}. The detection wavelength was 210 nm. The quantitation of muscle cholesterol content was based on the external standard method using a pure cholesterol standard (Sigma, St. Louis, MO).

DNA extraction and microsatellite genotyping

Genomic DNA has been extracted from blood with use QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to the protocol recommended by the manufacturer. All together 30 microsatellite markers located on 2 quail chromosomes (quail chromosome 1 and 2) were genotyped. The list of the microsatellites has been already reported in Tavaniello et al. (2014). The PCR reaction was performed in an MJ Research PTC-225 Tetrad thermocycler (MJ Research, San Diego, CA), using fluorescently labelled primers: 6-FAM, VIC, NED (Applied Biosystems, Foster City, CA). The PCR mixture was as follows: 100 mM Tris-HCl (pH 8.9), 20 mM $MgCl_2$, 500 mM KCl, deoxynucleoside triphosphate, 20 ng of DNA, 0.25 U of AmpliTaq GOLD 360 DNA Polymerase (Applied Biosystems), primers (0.2 – 0.75 pmol). The PCR reaction profile was as follows: denature cycle at 95 °C (600 s), then 30 cycles of the following: 95 °C (30 s); optimized annealing temperature (as per locus: 48 °C/52 °C/55 °C/60 °C) for 30 s; elongation 72 °C (30 s); followed by final extension at 72 °C (1,200 s).

Microsatellite scoring

After amplification, PCR products were subjected to electrophoresis on 4% polyacrylamide gel (POP4) using ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems). Fragment length analysis was based on 2 types of software: 3100-Avant Abi Prism Data Collection and Gene Mapper v. 3.5. Allele length was analyzed based on internal size standard Gene-Scan-350 ROX with nucleotide sizes 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340 and 350 bp.

Statistical analyses

Since a preliminary ANOVA showed that the 2 hatches did not differ significantly, hatch was not included in the statistical model. One-way ANOVA was performed for total lipid, FA composition and cholesterol content (SPSS, 2010). Scheffé's test was applied to compare the mean values among the 3 generations.

Quantitative trait loci analysis

Regression interval mapping was used for QTL detection. Analyses were performed with web-based GRID QTL software (http://www.gridqtl.org.uk/index.htm; Seaton et al., 2006) and line-cross analysis model (F_2 inbred portlets). In the line-cross model, the power of QTL detection depends on the assumption of fixation of QTL alleles for the trait of interest in the founder lines. In this model the alternative alleles at the QTL are traced back to the founder line. In the line cross (LC) model, a simple regression analysis was used to calculate the test statistics (F) for the presence or absence of a single QTL at each centimorgan. Significance thresholds (5% and 1%) were calculated for each chromosome and each trait individually, by performing 500 chromosome-wide permutations. Confidence intervals of the QTL were calculated using bootstrap with resampling. The hatch and sex of animals were considered as fixed effects. The LC model included additionally additive and dominant genetic effects.

Results and discussion

Total lipid, cholesterol contents and fatty acid composition

Total lipid, FA composition and cholesterol content of breast muscle of meat line and egg line quails (F_0 generation) are shown in Table 1. As for the total lipid content, no significant difference was found between the 2 quail lines. Conversely, Genchev et al. (2005) observed differences in the total lipid content between Pharaoh breed (specialized for meat production, 3.27%) and White English breed (good for meat and egg production, 1.58%). In addition, the lipid content observed in the current study were higher than those reported by Genchev et al. (2005) what might be related to different slaughter age of the birds (31 day old) and different diet. In fact, Boni et al. (2010) reported that meat from spent quail (8 months) had higher lipid content compared to young quail (8 weeks), (12.91% and 9.21% for spent and young quails, respectively). Nevertheless, the values found by Boni et al. (2010) are slightly higher than the present results, mainly due to the different anatomical part analyzed consisting in a bulk samples mechanically deboned. Also for the muscle cholesterol content no significant difference was found between the 2 quail lines. Likewise, other studies did not find any statistically significant difference among the birds of egg line and meat line (Maiorano et al., 2009) and between different genetic groups (Maiorano et al., 2011).

Table 1. Total lipid, cholesterol content and fatty acid profiles of breast muscle from Japanese quails of F_0 generation

Item[b]	Group[a]		
	F-33 (male)	S-22 (female)	P-value
Total lipid, g*100 g^{-1}	8.47±0.31	8.88±0.38	0.404
Cholesterol, mg*100 g^{-1}	75.33±2.47	80.26±2.18	0.142
Fatty acids, % total FA			
C12:0	0.25±0.02	0.59±0.05	0.001
C14:0	0.62±0.03	0.93±0.06	0.001
C14:1	0.12±0.01	0.08±0.01	0.036
C16:0	17.95±0.22	20.86±0.41	0.001
C16:1n-9	6.21±0.32	3.09±0.32	0.001
C17:0	0.1±0.01	0.17±0.01	0.001
C18:0	9.34±0.32	12.75±0.79	0.001
C18:1n-9	44.91±1.08	36.52±1.93	0.001
C18:2n-6	14.24±0.55	17.11±0.8	0.008
C18:3n-3	0.31±0.02	0.4±0.04	0.051
C20:1n-9	0.18±0.01	0.23±0.01	0.004
C20:4n-6	4.16±0.42	5.26±0.9	0.281
C20:5n-3	0.18±0.02	0.26±0.04	0.096
C22:1	0.1±0.01	0.09±0.01	0.693
C22:4n-6	0.17±0.02	0.35±0.06	0.013
C22:5n-3	0.16±0.02	0.12±0.02	0.167
C22:6n-3	1.01±0.11	1.21±0.24	0.472
Partial sum			
∑SFA	28.26±0.37	35.28±0.71	0.001
∑MUFA	51.51±1.16	40.01±2.13	0.001
∑PUFA	20.24±0.99	24.7±1.76	0.039
∑n-6	18.57±0.87	22.72±1.5	0.027
∑n-3	1.67±0.14	1.99±0.31	0.352
Nutritional ratios			
n-6/n-3	11.15±0.61	11.43±0.99	0.334
P/S	0.72±0.03	0.7±0.04	0.771
Atherogenic index	0.29±0.01	0.39±0.01	0.001
Thrombogenic index	0.7±0.01	0.93±0.03	0.001

[a]F-33 = meat-line males; S-22 = egg-line females; [b]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = PUFA/SFA ratio.

Egg-type quails supplied meat with a higher (+ 7 p.p.) amount of total SFA compared to meat line quails (P<0.01), and particularly, a higher (P<0.01) proportions of lauric (C12:0; 2-fold higher), myristic (C14:0), palmitic (C16:0), heptadecanoic (C17:0) and stearic (C18:0) acids. The proportions of the single SFA found in the present study were consistent with those reported in literature on quails (Botsoglou et al., 2004; Genchev et al., 2008; Boni et al., 2010; Gecgel et al., 2015) and on chicken (Rule et al., 2002; Poureslami et al., 2010). The total monounsaturated fatty acids (MUFA)

content, which in chickens is related either to the endogenous synthesis or to the gut absorption from the diet, was highest in meat-type quails (+ 11.5 p.p.; P<0.01), with a greater (P<0.01) proportion of palmitoleic (C16:1n-9; 2-fold higher), oleic (C18:1n-9; + 8.39 p.p.) and C14:1 (P<0.05) acids; while, only the C20:1n-9 was higher (P<0.01) in egg line quails, even if contained at low weight percentages. The amount of total MUFA detected in meat line quails (51.5%) was similar to that reported by Gecgel et al. (2015) for 56 day old male quails (48.72±3.27%); whereas, it was higher than that reported in Pharaoh breed (specialized for meat production) of 35 days old- 40.7% (Genchev et al., 2008) and in spent quails of 8 months old- 42.8% (Boni et al., 2010). Meat from egg-type quails had higher content of total PUFA (+4.5 p.p.; P<0.05), with a higher proportion of linoleic (C18:2n-6; P<0.01), linolenic (C18:3n-3; P<0.06) and docosatetraenoic (C22:4n-6; P<0.05) acids, and a higher (P<0.05) content of total n-6 FA. No differences, between the 2 quail lines, were found for the amount of n-3 long chain PUFA: eicosapentaenoic fatty acid (EPA, C20:5n-3), docosapentaenoic fatty acid (DPA, C22:5n-3) and docosahexaenoic fatty acid (DHA, C22:6n-3). In general, the total PUFA content detected in quails of F_0 generation (ranging from 20.2% to 24.7%) was similar to the results reported by Genchev et al. (2008) and Botsoglou et al. (2004), but lower than that reported by Boni et al. (2010) (32.6% and 28.4% for young and spent quail, respectively). These latter results could be due to the presence of skin in the bulk samples (rich in PUFA) or to the different nutritional regime of quails. In fact, the PUFA content in chicken tissues depends more on the variation in dietary FA content than the SFA and MUFA contents in the tissues (López-Ferrer et al., 2001). Neither the P/S nor the n-6/n-3 ratios were significantly different between the parental lines. Differently, the AI and TI were lower (P<0.01) in meat-type quails compared to those of the egg-type quails. The AI and TI represent criteria for evaluating the level and interrelation through which some FA may have atherogenic or thrombogenic properties, respectively. The low AI and TI values found in the current study revealed the good nutritional quality of quail meat.

Although marked genotype-related differences were found in the F_0 generation, in the F_1 generation the only effect of sex on the FA composition of quail meat was observed for the proportion of lauric acid, higher (P<0.05) in F_1 females (Table 2). Also the total lipid amount and the muscle cholesterol content were not significantly affected by sex. The probable explanation of such a differences in FA composition and total lipid amount in F_0 could be the origin of lines that created F_0 generation. The parental generation (F_0) consisted of 2 genetically different lines: F-33 and S-22. The unselected meat-type line (F-33) was maintained in closed population for over 50 generations whereas the egg-type line (S-22) was previously selected for 18 generations for high yolk cholesterol concentration. The F_1 generation is most genetically and phenotypically homogenous (first generation of hybrids) as it was created by mating 2 distinct lines and should presented higher heterozygosity comparing to F_0 and F_2 generation. That could be the reason why in this generation most of analyzed traits were not affected by sex. But even if not significant, once could perceived the same trends i.e. higher amount cholesterol, PUFA and total n-6 in females than in males.

Table 2. Total lipid, cholesterol content and fatty acid profiles of breast muscle from Japanese quails of F_1 generation

Item[a]	Group		
	Male	Female	P-value
Total lipid, g*100 g^{-1}	5.86±0.36	5.14±0.4	0.187
Cholesterol, mg*100 g^{-1}	64.97±2.42	67.06±2.46	0.547
Fatty acids, % total FA			
C12:0	0.14±0.03	0.23±0.02	0.019
C14:0	0.61±0.05	0.67±0.04	0.329
C14:1	0.13±0.02	0.13±0.04	1
C16:0	20.15±0.27	19.79±0.51	0.54
C16:1n-9	6.32±0.49	6.09±0.41	0.73
C17:0	0.11±0.01	0.13±0.01	0.223
C18:0	8.52±0.54	7.75±0.59	0.346
C18:1n-9	46.85±1.34	46.94±1.07	0.96
C18:2n-6	15.04±0.88	16.32±0.86	0.311
C18:3n-3	0.36±0.05	0.38±0.03	0.769
C20:1n-9	0.19±0.01	0.23±0.02	0.068
C20:4n-6	0.98±0.17	0.81±0.1	0.389
C20:5n-3	0.21±0.02	0.18±0.02	0.397
C22:1	0.07±0.01	0.06±0.01	0.352
C22:4n-6	0.06±0.01	0.07±0.01	0.629
C22:5n-3	0.07±0.01	0.05±0.01	0.197
C22:6n-3	0.19±0.03	0.17±0.02	0.564
Partial sum			
∑SFA	29.53±0.73	28.57±1.04	0.458
∑MUFA	53.56±1.33	53.45±1.2	0.951
∑PUFA	16.91±1.01	17.98±0.9	0.438
∑n-6	16.08±0.95	17.20±0.88	0.397
∑n-3	0.83±0.07	0.78±0.05	0.551
Nutritional ratios			
n-6/n-3	19.4±1.23	22.1±1.25	0.16
P/S	0.58±0.03	0.64±0.04	0.281
Atherogenic index	0.32±0.01	0.32±0.01	0.821
Thrombogenic index	0.79±0.03	0.75±0.04	0.461

[a]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = PUFA/SFA ratio.

On the contrary, in the F_2 generation a sex-effect was more evident (Table 3). Even if total lipid content was similar between sexes, muscle cholesterol content was lower in females (- 6.2 p.p.; $P<0.05$). In general, the obtained values from the 3 generations, regarding the muscle cholesterol content, are consistent with those reported by Genchev et al. (2008) in 42 days old Japanese quail (ranging from 68 to 75 mg*100 g^{-1}). On the contrary, in previous studies (Maiorano et al., 2009, 2011) it

was found a lower cholesterol level (ranging from 23.57 to 43.38 mg*100 g^{-1}) in breast muscle of quail slaughtered at 35 days of age of different line.

Table 3. Total lipid, cholesterol content and fatty acid profiles of breast muscle from Japanese quails of F_2 generation

Item[a]	Group		
	Male	Female	P-value
Total lipid, g*100 g^{-1}	4.87±0.57	4.33±0.44	0.46
Cholesterol, mg*100 g^{-1}	72.71±2.17	66.51±1.75	0.032
Fatty acids, % total FA			
C12:0	0.01±0	0.02±0	0.008
C14:0	0.4±0.01	0.44±0.02	0.058
C14:1	0.06±0.01	0.06±0.01	0.755
C16:0	19.65±0.47	18.97±0.41	0.278
C16:1n-9	4.71±0.32	4.28±0.23	0.279
C17:0	0.13±0.01	0.15±0.01	0.055
C18:0	9.78±0.37	8.82±0.34	0.063
C18:1n-9	42.77±1.12	40.72±1.29	0.237
C18:2n-6	17.81±0.73	21.90±0.81	0.001
C18:3n-3	0.46±0.03	0.69±0.04	0.001
C20:1n-9	0.19±0	0.2±0.01	0.39
C20:4n-6	2.87±0.35	2.58±0.28	0.533
C20:5n-3	0.14±0.01	0.16±0.01	0.084
C22:1	0.09±0.01	0.08±0.01	0.157
C22:4n-6	0.1±0.02	0.12±0.01	0.407
C22:5n-3	0.18±0.02	0.1±0.01	0.012
C22:6n-3	0.65±0.1	0.7±0.09	0.7
Partial sums			
∑SFA	29.97±0.73	28.40±0.5	0.085
∑MUFA	47.83±1.30	45.34±1.28	0.18
∑PUFA	22.20±1.13	26.26±1.13	0.015
∑n-6	20.78±1	24.6±1.02	0.011
∑n-3	1.43±0.15	1.66±0.12	0.24
Nutritional ratios			
n-6/n-3	14.85±1.1	14.58±0.72	0.61
P/S	0.75±0.04	0.93±0.04	0.004
Atherogenic index	0.31±0.01	0.29±0.01	0.255
Thrombogenic index	0.78±0.03	0.71±0.02	0.033

[a]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = PUFA/SFA ratio.

As for the FA composition, a slightly higher (P<0.09) amount of total SFA was found in males, mainly represented by stearic acid (P<0.07). Similarly, Gecgel et al. (2015) found a significantly lower SFA levels in female quail meat compare to males (33.22% vs 34.65%, respectively). No significant sex-effect was found for total MUFA

content; while the total PUFA amount was higher in females (+ 4 p.p.; P<0.05), with a higher (P<0.01) proportion of linoleic and linolenic acids. Sartowska et al. (2014) found that oleic, linoleic and α-linolenic acids were significantly higher in female Japanese quail meat, while palmitic, stearic and arachidonic acids were significantly higher in meat from males, indicating that sex had a marked influence on the fatty acid profile. Females had higher (P<0.05) amount of total n-6 FA and a lower (P<0.05) TI compared to males. The P/S ratio was lower (P<0.01) in males. Similarly, Gecgel et al. (2015) found that meat from males had lower P/S ratio than that of females (0.4 vs 0.43, respectively). The comparison of total lipid and cholesterol contents and total FA composition, of quail breast muscle among the 3 generations, according to the sex, are shown in the Table 4 (females) and 5 (males).

Table 4. Total lipid, cholesterol content and fatty acid profiles of breast muscle from Japanese quails females of F_0, F_1 and F_2 generations

Item[a]	Group			
	F_0 Females	F_1 Females	F_2 Females	P-value
Total lipid, g*100 g^{-1}	8.88±0.38^{A}	5.14±0.4^{B}	4.33±0.44^{B}	0.001
Cholesterol, mg*100 g^{-1}	80.26±2.18^{A}	67.06±2.46^{B}	66.51±1.75^{B}	0.001
∑SFA, %	35.28±0.71^{A}	28.57±1.04^{B}	28.4±0.5^{B}	0.001
∑MUFA, %	40.01±2.13^{Bb}	53.45±1.2^{A}	45.34±1.28^{aB}	0.001
∑PUFA, %	24.7±1.76^{a}	17.98±0.9^{Bb}	26.26±1.13^{A}	0.001
∑n-6, %	22.72±1.5^{a}	17.2±0.88^{bB}	24.6±1.02^{A}	0.001
∑n-3, %	1.99±0.31^{a}	0.78±0.05^{b}	1.66±0.13^{a}	0.031
Nutritional ratios				
n-6/n-3	11.43±0.99^{B}	22.1±1.25^{A}	14.58±0.72^{B}	0.001
P/S	0.7±0.04^{B}	0.64±0.04^{B}	0.93±0.04^{A}	0.001
Atherogenic index	0.39±0.01^{A}	0.32±0.01^{B}	0.29±0.01^{B}	0.001
Thrombogenic index	0.93±0.03^{A}	0.75±0.04^{B}	0.71±0.02^{B}	0.001

[a]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = PUFA/SFA ratio; [a-b]Means within a row lacking a common superscript differ (P<0.05); [A-B]Means within a row lacking a common superscript differ (P<0.01).

A significant reduction (P<0.01) of total lipid content was found in both male and female hybrids compared to parental lines. Due to the lack of information available from current literature, it is not easy to explain the decrease of total lipid content found in the hybrid generations. However, Wyatt et al. (1982) found an heterotic effect for the carcass fat percentage with hybrids characterized by lower fat content compared to parental lines. The comparison among the 3 generations within sex, evidenced also a significant reduction (P<0.01) of muscle cholesterol in both hybrid females (Table 4), while in males this reduction (P<0.05) was observed only in the F_1 generation (Table 5). S-22 females belonged to the line selected for high yolk cholesterol content. Maiorano et al. (2009) revealed that higher amount of cholesterol occurred also in breast muscles of that line. Mating S-22 females with F-33 males leaded to create F_1 and F_2 hybrids in which 50% and 25% of genes came from unselected F-33 line which might cause decline of cholesterol content in muscles.

In fact, in females total lipid and cholesterol content showed the same trend but, contrary to expectations, in males the decrease of total lipid content, observed in both hybrid generations, was not followed by cholesterol content. This discrepancy could be attributed to other factors such as the level of heritability of muscle cholesterol or transgression which might occurred in the second generation of hybrids and caused appearance of individuals with higher or lower amount of cholesterol than parents and grandparents. The transgression is a result of the split of genotypes and phenotypes which should occurred in the second generation of hybrids (F_2) and is necessary for QTL mapping. It also explain the highest value of some traits in F_2 comparing to F_0 and F_1 i.e. PUFA, n-6 or P/S.

Table 5. Total lipid, cholesterol content and fatty acid profiles of breast muscle from Japanese quails males of F_0, F_1 and F_2 generations

Item[a]	Group			
	F_0 Males	F_1 Males	F_2 Males	P-value
Total lipid, g*100 g^{-1}	8.47±0.31^{A}	5.87±0.36^{B}	4.87±0.57^{B}	0.001
Cholesterol, mg*100 g^{-1}	75.33±2.47^{a}	64.97±2.42^{b}	72.71±2.17^{a}	0.007
∑SFA, %	28.26±0.37	29.53±0.73	29.97±0.73	0.26
∑MUFA, %	51.51±1.16	53.56±1.33^{a}	47.83±1.3^{b}	0.013
∑PUFA, %	20.24±0.99	16.91±1.01^{b}	22.20±1.13^{a}	0.01
∑n-6, %	18.57±0.87	16.08±0.95^{b}	20.78±1^{a}	0.009
∑n-3, %	1.67±0.14^{a}	0.83±0.07^{b}	1.43±0.15^{a}	0.003
Nutritional ratios				
n-6/n-3	11.15±0.61^{Bb}	19.4±1.23^{A}	14.85±1.1^{Ba}	0.001
P/S	0.72±0.03	0.58±0.03^{b}	0.75±0.04^{a}	0.014
Atherogenic index	0.29±0.01	0.32±0.01	0.31±0.01	0.121
Thrombogenic index	0.7±0.01	0.79±0.03	0.78±0.03	0.084

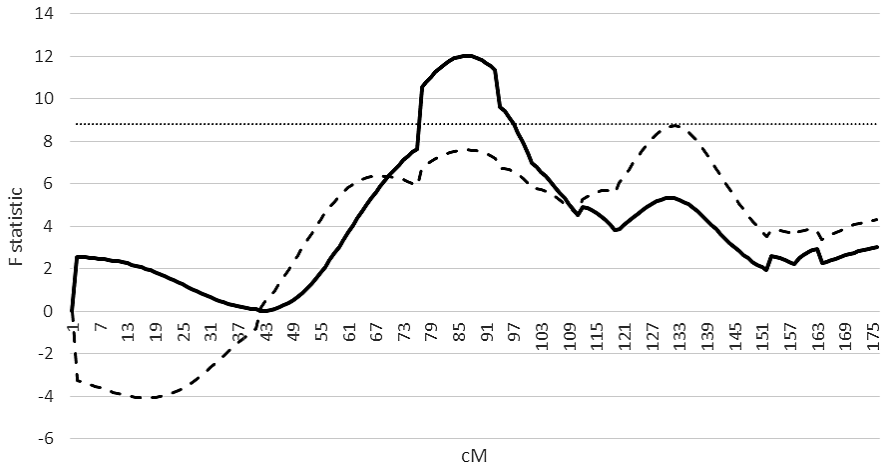
[a]SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; P/S = PUFA/SFA ratio; [a-b]Means within a row lacking a common superscript differ (P<0.05); [A-B]Means within a row lacking a common superscript differ (P<0.01).

Regarding the total FA composition, breast muscle from hybrid females had a lower (P<0.01) amount of total SFA (- 7 p.p. in F_1 and F_2) compared to F_0 females; this reduction in the SFA content is desirable, from the nutritional point of view. On the contrary, no significant impact of first- and second- generation crosses was observed for the total SFA content in males (Table 5). The total MUFA content (Table 4) showed the highest levels (P<0.01) in F_1 females compared to F_0 and F_2 ones, and F_2>F_0 (P<0.05), with oleic and palmitoleic acids higher (P<0.05 and P<0.01) in hybrid females compared to parental line (data not shown). Likewise, F_1 males had higher total MUFA content (P<0.05) compared to F_2 but similar to parental line (Table 5). The obtained results suggest a positive heterosis in the F_1 generation, even if the influence of the crosses was more evident in females. The structure of the experimental population was not a reciprocal cross, which would allow for definite answer. Using current data it might be only speculate that these results could be

related to the genetic structure of the birds as regards mitochondrial DNA (mtDNA) and the sex chromosomes. In fact, as suggested by Sohrabi et al. (2012), if the same effect is estimated in both males and females, it is likely to imply a maternal effect or genetic differences in mtDNA; while, an effect only observed in females is likely to reflect differences in the sex chromosome. Hybrid combinations increased the total PUFA amount in F_2 compared to F_1 quails ($P<0.01$ and $P<0.05$ for females and males, respectively), with intermediate value for F_0 ones, due to the significant contribution of linoleic and α-linolenic acids (data not shown). The total amount of n-3 and n-6 FA was also significantly affected by the crosses. Both sexes of F_2 generation showed higher amount of total n-6 FA compared to F_1 ($P<0.01$ and $P<0.05$ for females and males, respectively), and only F_1 females differed by F_0 ones ($F_1 < F_0$; $P<0.05$). In light of this, also the n-6/n-3 and P/S ratios, commonly used criterion to describe the dietetic value of fat, were affected by first- and second-generation crosses. The n-6/n-3 ratio was higher ($P<0.01$) in both F_1 males and females due to the lower ($P<0.05$) amount of total n-3 FA in both sexes, even if in males also the F_2 birds had higher n-6/n-3 ratio compared to parental line ($P<0.05$). In general, the observed values were similar to that reported for chicken breast meat by Rule et al. (2002). The P/S ratio was higher in F_2 generation compared to F_1 ($P<0.01$ and $P<0.05$ for females and males, respectively), with intermediate value for F_0, that differed ($P<0.01$) only from F_2 females. From nutritional point of view, a higher P/S ratio is recommended, indeed it should be increased to above 0.4 (Wood et al., 2003). In spite of the high n-6/n-3 ratio found in Japanese quails, the AI and TI were lower compared to the findings of Castellini et al. (2006) in chicken meat, confirming that quail meat could be an interesting meat also from the nutritional point of view. Indices markedly lower were found in hybrid females (F_1 and F_2) compared to parental line ($P<0.01$).

Quantitative trait loci

QTL analysis has been done for major traits of interest: lipid, FA composition and muscle cholesterol content. For the muscle cholesterol content a QTL has been detected on CJA02 at 85cM. F statistic profile for this QTL is presented in Figure 1. It was not found any QTL for PUFA, MUFA or SFA. As it has been mentioned in the introduction, knowledge on QTL regions linked to cholesterol content in meat is scarce. The cellular control of cholesterol metabolism is mediated by lipoproteins, LDL receptor which inhibits hydroxymethylglutaryl CoA reductase (HMG-CoA) (Brown and Goldstein, 1974). Study reported on Beijing-you chickens proves that mutation in UTR of HMGCR gene influences cholesterol content in muscles (Cui et al., 2010). The HMBCR gene is located on GGAZ which shows synteny with quail chromosome Z. The QTL region linked with cholesterol muscle content in this quail experimental population shows synteny with chicken chromosome 2 (GGA2:42.4-46.4cM) (Kayang et al., 2006). None of the genes located in this specific region of the chicken genome seems an obvious biological candidate for the cellular control of cholesterol metabolism. Also, QTL analysis done in pigs confirm that considerable amount of genetic variance determining cholesterol content exists, but more powerful tools of high – throughput genotyping are needed to detect candidate genes (Casellas et al., 2013).



The solid curve describes the test statistic. The dotted curve indicates QTL additive effect. The dotted line presents F statistic threshold for P<0.05.

Figure 1. Test statistic for CJA02 with regard to cholesterol content in meat under line cross analysis model F2 cross between Pharaoh (F-33) meat type males and Standard (S-22) laying-type females population

Conclusions

The present study describes new data regarding 3 generation cross of 2 types (meat line and egg line) of Japanese quail with respect to muscle lipid composition and the relative QTL study. An evident sex-effect was found in all the 3 generations on total lipid, FA composition and cholesterol content. In addition, the comparison among the 3 generations within sex showed a marked influence of first- and second-generation crosses on the studied meat quality traits. One of the more significant findings to emerge from this study is the marked reduction of muscle cholesterol content in hybrid females. A QTL with additive effect for the muscle cholesterol content has been detected on quail chromosome 2.

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