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Effect of cross-breed of meat and egg line on productive performance and meat quality in Japanese quail (*Coturnix japonica*) from different generations

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To all the people I have in my hearth

and to all those who believe in

the extraordinary richness of knowledge

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ABSTRACT

In recent years, quail meat has gained popularity among consumers and several lines, breed and varieties have been developed for different production purposes. Anyway, the available information in literature are still very scarce. In light of this, the aim of the current study was 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 gender (males and females) on growth performance, carcass and meat quality traits in Japanese quail (*Coturnix japonica*). Current research was a part of a larger research project whose main aim was the detection of quantitative trait loci (QTLs) and their linkage with phenotipical traits (meat and egg quality traits), in order to study the genetic structure at the basis of the variability of the quantitative traits. Also, verify and demonstrate, on the basis of information on the QTL, if quail could be considered as an "Animal model" for chicken with interesting economic implications. The experiment was performed with two Japanese quail (*Coturnix japonica*) population (meat type and egg type) reared at the Didactic Experimental Station of the University of Life Sciences in Lublin (Poland). 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 has been 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 wk 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: 6 birds in each 38 cages, 4 birds in each two cages) under continuous lighting (natural and artificial). Quail were fed ad libitum commercial diets according to age. The diet containing 28 % CP and 3,000 kcal of ME/kg for the first week, then till 28 days it containing 24 % CP and 2,900 kcal of ME/kg; the finisher ration had 20 % CP and 2,800 kcal of ME/kg. Birds had free access to water during the experiment.

At slaughter (20 weeks of age), all birds were individually weighed (after a fasting period of 12 h), stunned and decapitated, according to the EU regulations on the protection of animals at the time of killing (European Communities, 2009). After plucking and eviscerating, carcasses were weighted and dissected (leg, breast, giblets, abdominal fat; theirs percentages were calculated based on hot carcass weight) and, in addition, dressing percentage (without giblets) was calculated. After the refrigeration period (24 h at 4°C), the right Pectoral muscle (PM) pH (pH_{24}) was recorded and the left PM was removed, vacuum packaged, and stored frozen (− 40°C) for analyses of intramuscular collagen (IMC) properties, cholesterol content and fatty acid composition. Data were evaluated by one way analysis of variance (ANOVA; SPSS Inc., 2010) and Scheffé's test was applied to compare the mean values among the three generations. Quails of meat line (F-33) were significantly heavier than those of the egg line (S-22) and they had higher carcass weight, carcass yield and abdominal fat percentage; differently, giblets percentage and meat pH were higher in egg type quails. The IMC amount did not differ significantly between the two lines; however, meat of the egg line had a slower collagen maturation (hydroxylysylpyridinoline crosslink/collagen). Breast meat of S-22 quails had higher total saturated fatty acid (SFA) amount, but also higher total polyunsaturated fatty acid (PUFA) content compared with F-33 quails; on the contrary, the latter had higher (+ 11.5 %) total monounsaturated fatty acid (MUFA) amount. The ratio between polyunsaturated to saturate fatty acids (P/S) and muscle cholesterol were similar between lines, even if meat line quails supplied meat with lower atherogenic index (AI) and thrombogenic index (TI). The F_1 and F_2 generations showed an evident sexual dimorphism and an additional effect could be due to hybrid heterosis. Both females of F_1 and F_2 generations were heavier than males and had higher giblets percentage, while males showed a higher carcass yield and abdominal fat percentage. The IMC amount was not influenced by gender in both generations, even if meat from F_1 females had higher degree of collagen maturation. In the F_2 generation, a significant hatch-effect was found for the IMC amount and the degree of collagen maturation. In the F_1 generation the fatty acid composition and the relative ratios, as

well as the muscle cholesterol content, were similar between sexes. On the contrary, in the F_2 generation females were characterized by higher total PUFA content and consequently higher P/S ratio, but lower muscle cholesterol content compared to males. The comparison of performance traits among the three generations showed an evident phenotypic variation. The cross between two genetically distant lines as well as the cross between full siblings hybrids did not influence the body weight of hybrid males but had a negative effect on their carcass weight. Instead, hybrid females were heavier than parental line (S-22): F_1 hybrid had an increase of body weight of 23.9 %, while F_2 showed an increase of 31.4 %. The total content of SFA was lower in muscle hybrid females (F_1 and F_2) compared to female of parental line, while the SFA amount between males was similar among the three generations. The total MUFA amount was higher in both F_1 males and females, suggesting a positive heterosis in the F_1 generation, especially for females. On the contrary, the total PUFA content, as well as the total n-6 fatty acids amount were higher in F_2 hybrids in both sexes. The P/S ratio was highest in F_2 quails, while both F_1 males and females were characterized by the highest n-6/n-3 ratio. Hybrid females (F_1 and F_2) showed a significant lower AI and TI compared to parental line. Interestingly, both F_1 and F_2 females and only F_1 males showed a considerably lower muscle cholesterol content compared to parental lines.

Overall, the results obtained at the end of this study have provided information regarding three generation cross of two types (meat line and egg line) of Japanese quail which could be used in the future in poultry breeding industry.

RIASSUNTO

Negli ultimi decenni, si è assistito ad un cambio graduale delle preferenze del consumatore, il quale richiede prodotti alimentari che siano in grado di soddisfare non solo le caratteristiche organolettiche ma anche quelle nutrizionali e salutistiche, con particolare attenzione ai prodotti emergenti ed innovativi. Queste nuove tendenze hanno orientato i consumi anche verso le cosiddette carni alternative o non convenzionali come quelle di quaglia, che negli ultimi anni stanno riscuotendo un crescente interesse. In molti paesi, la quaglia è altamente apprezzata per le peculiari caratteristiche organolettiche delle sue carni e delle uova, utilizzate per la preparazione di piatti sofisticati; al tempo stesso, la carne di quaglia, grazie alle sua accessibilità economica, è in grado di incontrare anche l'interesse di consumatori meno esigenti (Minvielle, 2004; Genchev et al, 2008a). Inoltre, molteplici sono gli aspetti che rendono l'allevamento di questa specie molto interessante, primo fra tutti quello economico.

Nonostante, il crescente interesse verso questo tipo di carne, ancora limitate sono le ricerche riguardanti gli aspetti qualitativi e nutrizionali della carne di quaglia, mentre non vi sono studi che confrontano gli aspetti qualitativi della carne tra diverse linee e generazioni di quaglie giapponesi *(Coturnix japonica).*

Allo scopo di ampliare le conoscenze di base sulla carne di quaglia, l'obiettivo del presente studio è stato quello di valutare l'effetto dell'incrocio di due linee (linea carne e linea uova) sulle performance di crescita e post-mortem e sulla qualità della carne di quaglie giapponesi (*Coturnix japonica*) di tre diverse generazioni. Questo studio era parte di un progetto più ampio il cui obiettivo principale era quello di identificare i Quantitative Traits Loci (QTLs) associati ai caratteri quantitativi in esame (performance produttive, contenuto di colesterolo, proprietà del collagene intramuscolare della carne, ecc.), al fine di comprendere la struttura genetica che sta alla base della variabilità delle caratteristiche produttive stesse. Inoltre, verificare e dimostrare, sulla base delle informazioni relative ai QTL, se le quaglie possono essere considerate come "animal model" per i polli. Ciò porterebbe ad interessanti risvolti economici, in quanto le quaglie potrebbero essere utilizzate come modello nei programmi di selezione per incrementare la qualità delle produzioni in campo avicolo.

La sperimentazione è stata condotta presso un'azienda didattica sperimentale dell'Università di Lublin (Polonia). Allo scopo, 44 quaglie (generazione F_0), di cui 22 quaglie Pharaoh linea carne (F-33) e 22 quaglie linea uova (S-22), sono state incrociate tra loro al fine di ottenere una generazione ibrida (F_1). Gli ibridi F_1, a loro volta, sono stati incrociati tra di loro per ottenere una seconda generazione ibrida (F_2), ottenuta da 2 schiuse consecutive. Le quaglie, scelte a random, dalle generazioni F_0 (22 maschi e 22 femmine), F_1 (22 maschi e 22 femmine) e F_2 (84 maschi e 152 femmine), sono state allevate fino a 20 settimane di età in gabbie collettive (F_0 e F_1: 6 quaglie in ciascuna delle 6 gabbie e 4 quaglie in ciascuna delle 2 gabbie; F_2: 6 quaglie in ciascuna delle 38 gabbie, 4 quaglie in ciascuna delle 2 gabbie). Le quaglie sono state alimentate ad libitum con diete commerciali, formulate in funzione della loro età, usufruendo ad libitum di acqua fresca. La razione alimentare per la prima settimana aveva un contenuto proteico del 28 % e 3,000 kcal/kg di energia metabolizzabile (EM), successivamente fino al ventottesimo giorno un contenuto proteico del 24 % e 2,900 kcal/kg; quindi, fino alla ventesima settimana, un contenuto proteico del 20 % e 2,800 kcal/kg. A 20 settimane di età, le quaglie sono state pesate individualmente (dopo un periodo di digiuno di 12 ore), stordite elettricamente e macellate. Le carcasse sono state pesate, calcolata la rispettiva resa a caldo (senza frattaglie) e sezionate. Il peso delle singole parti (cosce, petto, frattaglie e grasso addominale) è stato registrato e successivamente calcolata l'incidenza delle singole parti sul peso della carcassa a caldo. Le carcasse sono state refrigerate a 4°C per 24 ore, e successivamente è stato misurato il pH del muscolo pettorale destro. Il muscolo pettorale sinistro è stato poi messo sottovuoto e congelato (- 40°C) fino al momento delle analisi del collagene intramuscolare (IMC), del contenuto di colesterolo e del profilo acidico. I dati ottenuti sono stati sottoposti ad analisi della varianza (ANOVA) ad una via, utilizzando il pacchetto statistico SPSS (2010). Le differenze tra le medie sono state valutate mediante il test di Scheffé.

Le quaglie della linea carne (F-33) sono risultate più pesanti rispetto a quelle della linea uova, come anche il peso della carcassa, la resa alla macellazione e la percentuale di grasso addominale; al contrario, l'incidenza delle frattaglie ed i valori del pH_{24} sono risultati maggiori nelle quaglie della linea uova. Il contenuto di collagene intramuscolare (CIM) è risultato simile tra le due linee, anche se le quaglie della linea uova hanno mostrato un livello di stabilizzazione/maturità delle fibre di collagene più

basso rispetto alle quaglie della linea carne. Il muscolo pettorale delle quaglie della linea S-22 ha mostrato un maggior contenuto di acidi grassi saturi (SFA), nonché un maggior contenuto di acidi grassi polinsaturi (PUFA) rispetto alle quaglie della linea carne, che, invece, hanno mostrato un maggior contenuto di acidi grassi monoinsaturi (MUFA). Il rapporto tra acidi grassi polinsaturi e saturi (P/S) ed il contenuto di colesterolo nel muscolo pettorale sono risultati simile tra i due gruppi sperimentali, anche se, nella carne delle quaglie F-33 sono stati riscontrati migliori (più bassi) indici aterogenici (IA) e trombogenici (IT). Le quaglie delle generazioni F_1 e F_2 hanno mostrato un evidente dimorfismo sessuale, probabilmente amplificato dall'effetto dell'eterosi ibrida. Infatti, le femmine ibride di entrambe le generazioni F_1 e F_2 sono risultate più pesanti rispetto ai maschi ibridi e con una maggiore incidenza delle frattaglie; mentre, i maschi hanno mostrato una maggiore resa alla macellazione nonché una maggiore incidenza del grasso addominale. Il contenuto di collagene intramuscolare (CIM) del muscolo pettorale è risultato simile tra i sessi in entrambe le generazioni (F_1 e F_2), anche se le femmine F_1 hanno mostrato un livello di stabilizzazione/maturità delle fibre di collagene più elevato. Nella generazione F_2 le proprietà del CIM sono risultate significativamente differenti tra le quaglie delle due schiuse. Nella generazione F_1 la composizione acidica ed i relativi rapporti, così come il contenuto di colesterolo muscolare, sono risultati simili tra i sessi. Al contrario, nella generazione F_2 le femmine hanno mostrato un maggior contenuto di PUFA e conseguentemente un più alto rapporto P/S, ma un minor contenuto di colesterolo muscolare rispetto ai maschi F_2. Il confronto delle performance in vivo e post-mortem tra le diverse generazioni, ha evidenziato una significativa variazione dei caratteri studiati. L'incrocio di prima e seconda generazione non ha influenzato il peso vivo dei maschi ma ha avuto un effetto negativo sulla loro resa alla macellazione. Al contrario, le femmine ibride sono risultate più pesanti rispetto alle femmine della linea parentale (S-22): le femmine F_1 hanno mostrato un incremento di peso del 23.9 %, che è risultato ancora più marcato nelle femmine F_2 (+ 31.4 %). Le femmine ibride (F_1 e F_2) hanno mostrato un minor contenuto totale di SFA rispetto alle femmine S-22, mentre nessuna differenza significativa è stata riscontrata nei maschi delle tre generazioni. Il contenuto totale di MUFA è risultato più alto sia nei maschi che nelle femmine F_1, indicando un'eterosi positiva nella generazione F_1, soprattutto nelle femmine. Gli ibridi F_2 (maschi e femmine), invece, hanno mostrato un contenuto totale di PUFA e di acidi grassi n-6 simile tra loro. Il

rapporto P/S è risultato maggiore negli ibridi F_2, mentre entrambi i sessi della generazione F_1 hanno mostrato un maggior rapporto tra acidi grassi n-6 e acidi grassi n-3. Le femmine ibride (F_1 e F_2) sono state caratterizzate da indici aterogenici e trombogenici significativamente bassi. Particolarmente interessante, è la significativa diminuzione del contenuto di colesterolo nel muscolo pettorale sia delle femmine ibride (F_1 e F_2) che nei maschi F1 rispetto alle linee parentali.

Nel complesso, i risultati ottenuti dal presente studio hanno fornito importanti informazioni di base riguardanti le performance produttive e le caratteristiche qualitative della carne di quaglie giapponesi appartenenti a diverse linee e generazioni; evidenziando, altresì, una significativa e positiva eterosi negli ibridi di seconda generazione. Inoltre, i risultati ottenuti hanno fornito una base importante per l'individuazione dei QTL associati ai caratteri quantitativi oggetto di studio.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

List of abbreviation

AI	atherogenic index
ANOVA	analysis of variance
bp	base pairs
BW	body weight
BW	body weight
CLA	conjugated linoleic acid
CP	crude protein
CW	carcass weight
CY	carcass yield
d	day
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acids
g	gram
h	hour
HLP	hydroxylysilpyridinoline
HPLC	high-performance (high-pressure) liquid chromatography
IMC	intramuscular collagen
IMCT	intramuscular connective tissue
IMF	intramuscular fat
kb	kilobase pairs
L:D	hours light:hours darkness in a photoperiod (e.g., 23L:1D)
m	meter
ME	metabolizable energy
min	minute
MUFA	monounsaturated fatty acids
n	number of observations
PS	Pectoralis Superficialis
PUFA	polyunsaturated fatty acids
QTL	quantitative trait loci
r	correlation coefficient
RH	relative humidity
s	second
SE	standard error
SFA	saturated fatty acids
TI	thrombogenic index
USDA	United States Department of Agriculture
UV	ultraviolet
WHC	water-holding capacity
µ	micro

PART 1. INTRODUCTION (Literature review)

Chapter 1

QUAIL: ORIGIN, BREEDS AND DISTRIBUTION

Quail have been farmed since ancient times. The earliest known representation of the quail can be found in the Egyptian hieroglyphics (2000 B.C., Figure 1.1), where the quail represents the letter "W" in the alphabet (Shanaway, 1994). Quail meat has been known for centuries and there are even Biblical quotations of their use as a meat source (Boni et al., 2010).

Figure 1.1. Quails in the Egyptian hieroglyphics

source: http://www.landofpyramids.org/hieroglyphs-and-hieroglyphics.htm

During the last decade, quail has attained economic importance as an agricultural species producing eggs and meat that are enjoyed for their unique flavour (Kayang et al. 2004). In fact, quail are found in all continents. Several lines, breeds and varieties have been developed for different production purposes (Maiorano et al., 2012). Around the world, there are 20 types of wild and about 70 domestic quail breeds or strains, including laboratory and commercial quail. Although all domestic quails derive from wild strains, many obvious differences are evident today. However, how these differences occurred and which wild population was the first to be domesticated, remains unclear (Chang et al., 2005).

Quail belong to the order *Galliformes* and the family *Phasianidae* (Table 1.1), which is the largest and most varied of the gallinaceous birds. The family *Phasianidae* is so diverse that it is difficult to divide into natural groups, but three subfamilies are generally recognized: the *Odontophorinae* (the New World quail), the *Perdicinae* (the

Old World quail) and the *Phasianinae* (the true pheasants and peafowls) (Shanaway, 1994).

Table 1.1. Taxonomic classification of quail in the Animal Kingdom

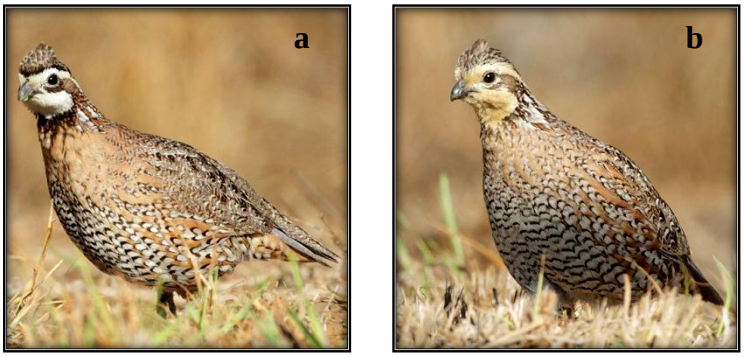
Phylum:	*Chordata*
Subphylum:	*Vertebrata*
Class:	*Avis*
Order:	*Galliformes*
Family:	*Phasianidae*
Subfamily:	*Phasianinae*
	Perdicinae (Old World quail)
	Odontophorinae (New World quail)

1.1 The New World quail

The New World quail are a fairly homogeneous group of small-to medium-sized birds, there are 32 species in 9 genera. None of the New World quail is migratory and all of them tend to be resident wherever they are found. Typical of this subfamily, and certainly the best known, are the Bobwhite quail and the California quail. (Shanaway, 1994).

The Northern Bobwhite (*Colinus virginianus*), commonly referred to as bobwhite quail, is a ground-dwelling bird native to the United States, Mexico, and the Caribbean. The name "bobwhite" derives from its characteristic whistling call. The Northern Bobwhite is one of the most familiar quail in eastern North America because it is frequently the only quail in its range (http://en.wikipedia.org/wiki/Northern_Bobwhite). The bobwhite is an attractive bird. The back, tail and crown in both sexes are brown; while the chest, abdomen and flanks are lighter, with black and white striations (Figure 1.2). A white stripe covers the eyes and, in the male, there is a white patch under the mandible. In some female this patch is absent or reduced and is normally replaced by buff markings. The beak is greyish-brown, the legs yellowish-brown and the eyes are dark brown (Shanaway, 1994). The Common Bobwhite is bigger than the *Coturnix* quail, with the male reaching 24 cm in length and the female over 26 cm. For this reason it is more usually a meat birds than an egg producer (Shanaway, 1994).

Figure 1.2. Northern Bobwhite quails: a) adult male; b) adult female



source: http://www.seattleaudubon.org/birdweb/bird/northern_bobwhite

Next to the Bobwhite quails, there is one of the most widely distributed quail in the America continent. The genus *Callipepla* contains approximately 12 species, the best known of which are the California Valley quail (*Callipepla californica*) and the Gambel's quail (*Callipepla gambelii*).

The California Valley quail is similar in size to the Common Bobwhite quail. The male is about 23.5 cm and the female can reach a maximum of 27.5 cm. The California Valley quail (Figure 1.3) resembles the Gambel's quail (Figure 1.4) so far as both sexes have forward-tilting blackish crests which enlarge terminally into a "comma" or "teardrop" shape. Both sexes also have clear bluish-grey to grey chest which became buff towards the abdomen with darker markings, reminiscent of reptilian scales. The flanks brownish-grey with lighter shaft-streaks, and upper parts are generally grey to brownish grey, intricately marked with darker scale-like markings. Males have black throats and a chestnut-tinged abdomen and are chocolate brown behind the plume; while the area in front of the eyes and above the bill is white. Females have dark brown rather than black crests and lack in the black throats (Shanaway, 1994).

Figure 1.3. California Valley quail: a) adult male; b) adult female

source: http://www.seattleaudubon.org/birdweb/bird/california_quail

The Gambel's quail may be confuse with the California Valley quail, but is slightly larger and has a different colour pattern on its sides and breast. It completely lacks the scale pattern found on the Valley quail. The males have a distinctive red chest and a black throat lined with a white border. The hens area a drab greyish colour and lack the red or black in the head area. In captivity, the California quail is one of the easier quail to breed although they are very nervous by nature and do not do as well in small cages as the *Coturnix*. Gambel's quails are very hardy and, when fully mature, seem to be more resistant to diseases (Shanaway, 1994).

Figure 1.4. Gambel's quail: a) adult male; b) adult female

source: http://www.birdphotography.com/index.html

1.2 The Old World quail

Old World quail are so varied a group that is difficult to characterize them. For the most part they are relatively plain-coloured, small to medium-sized birds, shorter and stouter. Widespread over most of Eurasia, Africa and the Australo-Malayan region. (Shanaway, 1994). The most widely distributed quail of the Old World quail is the *Coturnix* genus that is considered to be the most common type in captivity worldwide and is the only truly migratory members of the order *Galliformes*.

The *Coturnix* genus contains several species, the best known of which are:

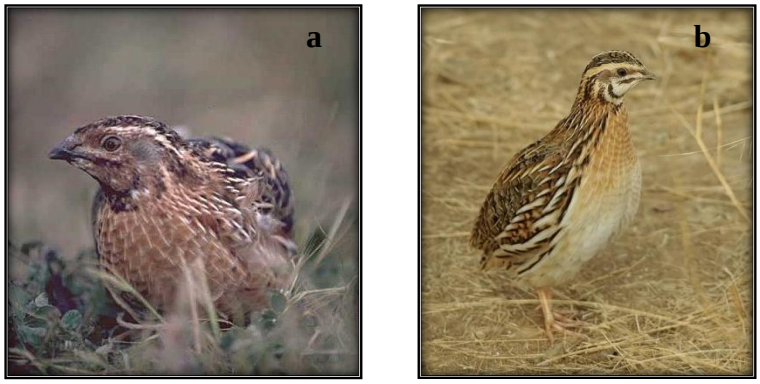
- Common Quail, *Coturnix coturnix;*
- Japanese Quail, *Coturnix japonica;*
- King Quail or Button quail or Chinese quail, *Coturnix chinensis;*
- Stubble Quail, *Coturnix pectoralis;*
- Rain Quail, *Coturnix coromandelica;*
- Harlequin Quail, *Coturnix delegorguei;*
- Brown Quail, *Coturnix ypsilophora;*
- Blue Quail, *Coturnix adansonii.*

There has been considerable confusion and controversy concerning the taxonomic status of the Japanese quail and a variety of vernacular names persist even in the current literature. Several authors considered the Japanese quail as a subspecies of the common quail *(Coturnix coturnix)* giving the scientific designation of *Coturnix coturnix japonica*. However, subsequent taxonomic evidence concerning differences in vocalizations, sympatry, sexual isolation and hybrid inviability suggests that the common quail and the Japanese quail are distinct species (reviewed in Mills et al., 1997). Hence, throughout this thesis the scientific designation used for the Japanese quail is *Coturnix japonica.*

Typical of the *Coturnix* genus, and certainly the best recognized, are the Common quail and the Japanese quail.

The Common quail (*Coturnix coturnix*) is a small (Figure 1.5), rotund bird, essentially streaked brown with a white eye stripe, and, in the male, a white chin. As befits its migratory nature, it has long wings, unlike the typically short-winged game birds. It measures roughly 18 – 21.9 cm and weighs 91 – 131 g.

Figure 1.5. Common quail a) adult male; b) adult female



source: http://ibc.lynxeds.com

The Japanese quail (*Coturnix japonica*) is also a small rotund bird (Figure 1.6) with evident sexual dimorphism. Males tend to be smaller than females. Wild adults weigh between 90 and 100 g while, their domesticated counterparts typically weigh between 100 and 140 g; females are slightly heavier, weighing from 120 to 160 g. However, weight among domesticated lines varies considerably, as commercial strains bred for meat production can weigh up to 300 g (Shanaway, 1994; Minvielle, 2004). The plumage of the Japanese quail is sexually dimorphic, allowing for differing sexes to be distinguished from one another. The plumage color of the wild type is predominantly dark cinnamon brown. Adult females have pale breast feathers which are speckled with

dark colored spots, while, males have uniform dark rust-red breast feathers and similarly colored pigmentation on the cheeks, which is absent in the female. During winter, males develop a white collar and have generally lighter plumage overall than during summer. This plumage change is testosterone independent, as castrated males develop normal summer plumage. The two sexes can be distinguished outwardly at about 3 weeks of age. (Woodard et al., 1973; Mizutani, 2003). Domestication and selective breeding of Japanese quail has resulted in numerous different strains exhibiting a variety of plumage colors and patterns.

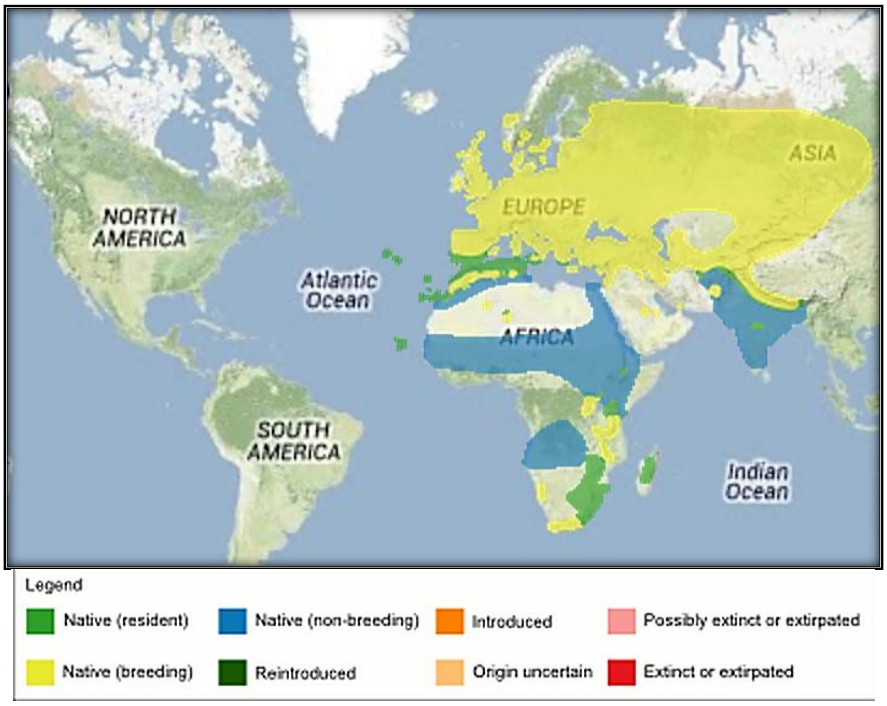
Figure 1.6. Japanese quail: adult female on the left; adult male on the right (*personal photo*)

1.2.1 Natural distribution of Common quail and Japanese quail

The common quail (*Coturnix coturnix*) is undomesticated, migratory bird with an area of distribution (Figure 1.7) that extends from central Siberia to Africa, encompassing Western and southern Europe. Breeding takes place around the Mediterranean Sea, in Europe and in western Asia, up to central Siberia (Chazara et al., 2010). This species has an annual migratory cycle between breeding and wintering zones, located in sub-Saharan Africa and in the south of Eurasia (from Ukraine to India) (Chazara et al., 2010). Common quail is a widespread summer visitor to much of Europe, which account for less than a quarter of its global breeding range. Its European breeding population is very large (> 2,800,000 pairs) and fluctuates, but underwent a large decline during 1970 – 1990, especially in central and eastern Europe. Although the species increased in northern and central Europe during 1990 – 2000, declines continued in south-eastern Europe, and the total population size probably remains below the level that preceded its decline. As a result, it is currently evaluated as "depleted" in Europe, rather than "vulnerable" as it was a decade ago (http://www.birdlife.org). Even

though, common quail is considered as a migratory bird, some reports about sporadic winter resident quail populations in Western Europe have been published. In contrast, some populations seem to be partially resident in the southern part of the breeding area: northern Africa and southern Portugal and Spain or Israel; probably this sedentary behaviour could be related to the climate warming and development of irrigated perimeters (Guyomarc'h, 2003).

Figure 1.7. Distribution of Common quail in the world



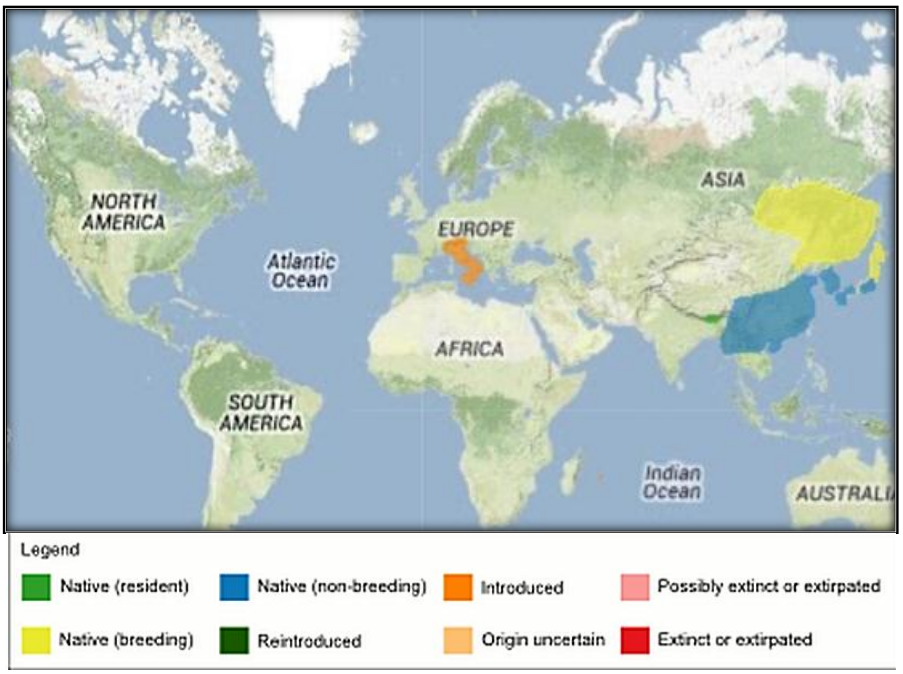
source: http://www.birdlife.org/datazone/speciesfactsheet.php?id=194 (retrieved September 12,2013)

The Japanese quail is native to the eastern Palaearctic (Japan, Eastern Siberia, Mongolia, north China, Korea) and migrates each year to the south of China (del Hoyo et al., 1994). The domestic Japanese quail, however, has lost all migratory behaviour, in contrast to its wild form. Japanese quails have been reproduced in captivity for centuries and domesticated.

Wild Japanese quails are mainly distributed throughout East Asia including northern Mongolia, Sakhalin Island and the Baikal and Vitim regions of Russia, north-eastern China, Japan, North Korea and South Korea (Figure 1.8). Some populations in Japan are resident, but most birds migrate south, wintering in southern China, Laos, Vietnam, Cambodia, Myanmar, Bhutan and north-eastern India (del Hoyo et al., 1994). There are also introduced populations in Italy and Hawaii (USA). No reliable population estimate

exists, and although the species was previously considered to be fairly common in China, declines appear to have occurred in Laos and Japan, and there are fears that the species has undergone a significant decline overall (http://www.birdlife.org).

Figure 1.8 Distribution of Japanese quail in the world

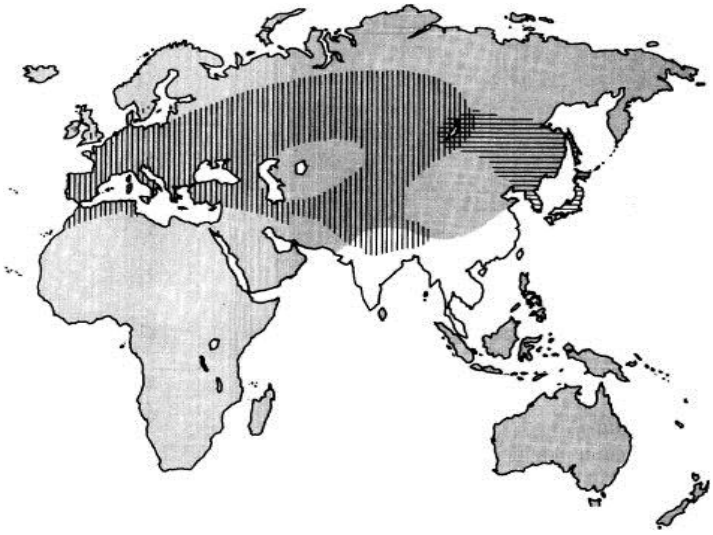


source: http://www.birdlife.org/datazone/speciesfactsheet.php?id=195 (retrieved September 12,2013)

The global population size has not been quantified, but the species was has been reported to be fairly common (del Hoyo et al., 1994; Fuller et al., 2000). However, owing to recent suspected declines, the species is likely to be less common than previously thought. National population estimates include: c.10,000 - 100,000 breeding pairs and c.1,000 - 10,000 individuals on migration in China; c.100 - 10,000 breeding pairs, c.50 - 1,000 individuals on migration and < c.50 wintering individuals in Japan and c.10,000 - 100,000 breeding pairs in Russia. This species may have undergone a decline of over 80 % between 1973 and 2002 (http://www.birdlife.org). Egg-laying occurs from late April to early August in Russia, and late May to August in Japan. Clutch size is varied, with larger clutches in Russia (nine to ten) than in Japan (five to eight). The female is the sole incubator of the eggs (del Hoyo et al., 1994). Little is known about the preferred habitat of this species, although it is thought to prefer open habitats such as meadows, steppes, and dry mountain slopes near water. It has also been recorded in grassland and cultivated land.

The evolutionary relationships and taxonomic status of European and East Asian populations of quail (*Coturnix coturnix*) are controversial. European and Far Eastern Japanese quails have been considered as distinct, albeit closely related allospecies, or as two subspecies, namely the Common quail *Coturnix coturnix coturnix* and the Japanese quail *Coturnix coturnix japonica* (Barilani et al., 2005). Japanese quail and Common quail have allopatric distributions in Europe, Maghreb and western Asia (Common quail), and in eastern Asia (Japanese quail), except for sympatric breeding areas in the Baikal (Russia) and Kentei (Mongolia) regions, where they could hybridize (Barilani et al. 2005; Puigcerver et al., 2007) (Figure 1.9).

Figure 1.9 Breeding areas of the common quail (vertical lines) and the Japanese quail (horizontal lines), showing the area of populations' overlap (*source: Puigcerver et al., 2007*)



Despite some older reports to the contrary, it has been shown recently that Japanese quail (*Coturnix japonica*) and Common quail (*Coturnix coturnix*) of similar size may interbreed in captivity and produce fertile F_1 crossbreds (Derégnaucourt et al., 2002), but these sympatric sub-species do not seem to hybridize on their natural breeding grounds, near the Lake Baikal (Crawford, 1990). In Europe, the two taxa come into contact, artificially, mainly through the release of domestic Japanese quails, or hybrids, for hunting (Guyomarc'h 2003; Puigcerver et al. 2007), raising the issue of the introgressive hybridization of common quail populations by Japanese quail (Chazara et al., 2010) and threatening the genetic background of the common quail that occurs naturally in Europe (Barilani et al. 2005; Amaral et al. 2007; Chazara et al. 2010). This

potentially hybridization could affect the genetic structure and functional traits of natural populations. In particular, gene introgression could change the behaviour of the partially migrant Common quail (Guyomarc'h et al., 1998). Domestic Japanese quails or hybrids do not show migratory behaviour, and gene introgression could accelerate the ongoing sedentarization process in wild Common quail populations (Deregnaucourt, 2000; Barilani et al., 2005).

The in-depth comparison of the two species (including commercial Japanese lines) has an interest which goes beyond checking their taxonomic status. It could have a significant impact on game and wildlife management of Common quail in Europe and on knowledge on domestication and conservation of *Coturnix*, both for its own sake and as a pilot animal. The genetic load of Japanese quail is apparently high (possibly because most present time lines were founded from a few breeders), since early quail populations were reported to be quite sensitive to inbreeding (Woodard et al., 1973). Modern screening of genetic variability in Japanese quail lines and comparisons with wild populations of Japanese and Common quail would help understand better, and possibly overcome, this feature of *Coturnix japonica* (Minvielle, 2004).

1.2.2 Domestication of Japanese quail

Domesticated Japanese quail, deriving from wild Japanese quail (*Coturnix japonica*) as laying, meat, and laboratory animals have produced a flourishing industry. The main differences between wild and domesticated Japanese quail are that the latter are heavier, show earlier sexual maturity, greater egg production with higher hatching and survival rate. In fact, wild Japanese quail lay 7 to 14 eggs per year, whereas domestic quail can lay about 280 eggs per year under normal feeding conditions (Mills et al., 1997; Chang et al., 2009). The reason why common quail are not bred in farms is that Japanese quail and hybrids are better adapted to captivity, and are more productive (Puigcerver et al., 2007).

Several authors (reviewed in Mills et al., 1997) reported the history of domestication of Japanese quail, but the available information appears to be fragmentary. The first record of wild Japanese quail appeared in the eighth century in Japan. Thereafter, several records of wild Japanese quail were found in several eras in Japan. The first written records of domesticated quail date from twelfth century in Japan. Quails were raised primarily for the enjoyment of their rhythmic call (as singing birds) and in the

feudal age raising song quail was particularly popular among Samurai warriors, who used to hold contests to identify the most beautiful song; birds with the best songs were interbred in closed colonies. The modern domesticated form of Japanese quail is thought to have been derived, at least in part, from lines selected for song. By the early twentieth century quail had become widely used for egg and meat production and, between 1910 and 1941, the population of *Coturnix* quail increased rapidly in Japan and by 1940 a thriving industry existed. However, all lines of song-type quail and the majority of commercial lines were lost during the Second World War. By this time singing *Coturnix* were also becoming less popular in central Europe where they had been independently selected for the same purpose (Shanaway, 1994; Mills et al., 1997; Mizutani, 2003). Between 1945 and 1955 the value of quails as a research animal was first exploited, and now their use for research purposes has become widely accepted (Shanaway, 1994; Minvielle, 2004). After the war, the Japanese quail egg industry was rebuilt from the few remaining domesticated birds, possibly with the addition of domesticated lines from Korea, China, Taiwan and quails captured in the wild. (Mills et al., 1997; Mizutani, 2003). All present day lines of domesticated Japanese quail in the United States and Europe appear to have been derived from this post-war population (reviewed in Mills et al., 1997).

1.2.2.1 Japanese quail as an experimental and model animal

The Japanese quail was first reported as a useful research model in 1959 by Padgett and Ivey, who noted its practicality as a laboratory animal for avian developmental studies (Huss, 2008). The low maintenance cost associated with its small body size (80 – 300 g) coupled with its early sexual maturity, short generation interval and high egg production, render it an excellent laboratory animal (Kayang et al., 2004). It has thus been used extensively in many studies including behavioural (Mills and Faure, 1991), developmental (Le Douarin et al., 1997), physiological (Balthazart et al,. 2003), genetic (Jones et al., 1991) and biomedical (Ratnamohan, 1985) researches. For example, both embryos and adult Japanese quail are widely used in studies of vertebrate physiology and diseases that affect human health. Knowledge on myogenesis, vasculogenesis, angiogenesis, skeletogenesis, wound healing, virology and teratology has progressed substantially as a result of studies on avian embryos (Huss et al., 2008). In addition, quail was used as a model to investigate age-related disease. The Japanese quail's short

lifespan (females live 2.5 - 3 years, whereas males live 3-5 years; Ottinger, 2001) combined with its physiological similarity to humans have made this bird an ideal model for studies addressing senescence in immunology, endocrinology and reproductive biology. The Japanese quail also serves in studies of the reproductive toxicology of chemical compounds and the effects of environmental endocrine disruptors (Huss et al., 2008).

Quails are increasingly being used as a comparator organism in cell-based investigations and are still heavily utilized in quail-chick chimeric studies for elucidating cell fates during development (reviewed in Ainsworth et al., 2010). Recently, the quail has proven to be a successful model for the production of a transgenic avian with several advantages. The hardy nature of its embryo limits mortality during introduction of the transgene into the blastoderm. The quail's short embryonic development period of 16 days, rapid advancement to sexual maturity and prodigious egg production all combine to substantially shorten the time needed to produce a stable line of transgenic avians when compared with the chicken (Huss et al., 2008).

The Japanese quail has also been recommended as a model species for poultry (Wilson, 1961; Baumgartner, 1994; Mills et al., 1997). Research work in poultry is often handicapped by limits in budget, time and space. Some of these problems might be alleviated by using Japanese quail as a pilot animal for the more expensive experiments on chickens or turkeys. In fact, Japanese quail is phylogenetically closely related to the chicken (Stock and Bunch, 1982). Both species have a karyotype of 2n = 78 chromosomes and a similar genome length of $1.2x10^9$ bp, consisting of morphologically distinct macrochromosomes (1–8 and the ZW sex chromosomes) and cytologically indistinguishable microchromosomes. Indeed, recent cytogenetic studies have confirmed the highly conserved chromosome homology between Japanese quail and chicken, revealing only very few chromosome rearrangements after divergence of the two species (reviewed in Kayang et al., 2004).

In the recent years, the development of the quail genetic maps with amplified fragment length polymorphism (Roussot et al., 2003) and microsatellite markers (Kayang et al., 2004) have opened the way to more comparative genetic studies with chickens, and to detect quantitative trait loci (QTL) for a variety of traits (Esmailizadeh et al., 2012). Anyway, despite many efforts to construct linkage maps and identification

of QTL in chicken genome, very little information is available in mapping genomic regions underlying quantitative traits in Japanese quail. In the available literature on Japanese quail, only few publications can be found, reporting QTL responsible for laying traits, the quality of the egg and the shell (Minvielle et al., 2005), daily weight gains in certain weeks of life (Esmailizadeh et al., 2012; Sohrabi et al., 2012), the shape of the laying curve (Minvielle et al., 2006) or behavioural traits associated with fearfulness (tonic immobility) (Beaumont et al., 2005). Further works should enhance the interest of the quail in biological research and open the way to more comparative genetic studies with chickens in order to better estimate and understand the genetic similarities and differences of these two *Phasianidae* species.

Over the last 50 years, the Japanese quail has proven to be a truly diverse and efficient animal model. The value of *Coturnix japonica* as a laboratory species seems limited only by two undesirable characteristics. First, the Japanese quail genome has not been sequenced to date. This may limit the usefulness of the quail for laboratory requiring the full complement of online genomic resources. Several studies established, however, that the quail genome is highly homologous to that of the chicken (Kayang et al., 2006; Sasazaki et al., 2006). Second, it has long been known that *Coturnix japonica* do not tolerate extensive inbreeding. Repeated sibling mating will decrease viability, hatchability and egg production. New sources of genetic variability must be introduced regularly to maintain flock fitness (Woodward et al., 1973; Mills et al., 1997; Huss et al., 2008).

In the future, it is likely that the Japanese quail will continue to occupy a small yet prominent place in research laboratories around the world (Minvielle, 2004).

1.2.2.2 Japanese quail as production bird

Although the amount of research involving the Japanese quail has been considerable, little attention has been paid to the bird itself. In literature, there are only few studies carried out on meat quality traits and its genetic components in Japanese quail. However, the situation has changed in recent years with a renewed interest in quail meat, subsequently to the growing consumers' demand towards alternative meats such as meat from ostrich, pheasant, partridges and other non commercial bird species, reared under industrial conditions. The Japanese quail is fast becoming recognized within the commercial poultry industry as a source of uniquely flavoured meat for consumers, at

an affordable price compared with most poultry species (Minvielle, 2004; Genchev et al., 2008a). Even tough, quail meat production is negligible when compared with that of broiler chickens, it represents a good source of meat and occupies a relevant place in poultry breeding and contributes to the global poultry industry (reviewed in Maiorano et al., 2009, 2011). The valuable taste and dietary properties of quail meat are pivotal in determining the growing interest of consumers to this product. Therefore, quail meat could be an interesting niche business (Genchev et al., 2008a; Maiorano et al., 2011).

In the last decades, much research was conducted to improve growth in poultry. Growth is a trait of prime interest to the animal industry for the potential to increase the economic value of domesticated species. In particular, quail has been intensively selected for high growth rate and this trait will continue to be one of the most important economic traits in the quail breeding programs. In fact, Marks (1978) and Nestor et al. (1982) have demonstrated that Japanese quail show a marked response to selection for increased body weight. Marks (1990) has also shown that genetic parameters for growth traits in Japanese quail are similar to those of chickens and turkeys. In addition, the availability of molecular tools for the Japanese quail (Kayang et al., 2004) makes possible to unravel the genetic bases of the growth trait. Knowledge on position and effects of QTL underlying variation in live weight of quail would be useful for marker-assisted selection as well as understanding of genetic background of growth (Esmailizadeh et al., 2012).

According to heritability of carcass traits (ranging from 0.08 and 0.55) estimated in many studies, carcass composition in Japanese quail can be significantly improved through selection (reviewed in Lotfi et al., 2011). Infact, poultry breeders have predominantly focused on selection for increased breast muscle yield in response to the consumers' demand for processed poultry products and correspondingly lees for whole ready to roast carcasses (Zerehdaran et al., 2012). Another important issue for the meat poultry industry is the reduction of abdominal fat, which is regarded as the main source of waste in birds. Not only is abdominal fat a loss, but it also represents an added expense for the processing effluent treatment in further processing (Griffiths et al., 1978; Becker et al., 1981; Salma et al., 2007). Excessive fatness is a problem in modern strains of rapidly growing broiler chickens that are reared for meat production (Griffin et al., 1991); the same was found for meat type quails. Infact, Ogũz et al. (1996) showed that the selection for increased 4-week body weight in Japanese quail resulted in an

increased abdominal fat compared to the control line. Marks (1990) also shown that the percentage of abdominal fat was higher in high body weight lines than in low body weight lines, and correlation coefficients between body weight and abdominal fat were moderate to high. In a recent study Lotfi et al. (2011) found that the selection against abdominal fat and subcutaneous fat does not change intramuscular fat and the quality of breast meat.

In light of this, some of the studies on Japanese quails aimed to obtain information that might be utilized in production in terms of the improvement of the characteristics with economic significance, whereas some of them aimed to elucidate the basic issues that will also apply to other domestic poultry (Narinc et al., 2012).

Chapter 2

QUAIL FARMING SYSTEM

Domesticated Japanese quail, deriving from wild Japanese quail, have produced a flourishing industry as laying and meat animal. In the wild quails lay from 12 to 14 eggs per year, whereas domestic quail can lay about 280 eggs per year under normal feeding conditions (Chang et al., 2009; Mills et al., 1997). The domesticated Japanese quail has lost the instinct of nesting, so the only way of breeding quail is performed by artificial incubation of eggs.

2.1 Incubation and hatching

As for other poultry productions, one of the main prerequisites for efficient and profitable quail breeding is to produce fertile eggs and to obtain the highest hatchability of the eggs. Hatchability is a function of number of chicks hatched, and is affected by numerous factors such as fertility, egg quality, handling of eggs and management conditions during incubation and hatching (Alkan et al., 2008). In particular, quail eggs fail to hatch for three main reasons:

- infertile eggs that have never contained a living germ cell and for this reason at candling, infertile eggs will appear “clear”;
- the fertile germ has died between the time the eggs was laid and the time of setting in the incubator;
- the fertile germ has not developed properly, or has died, between the time of setting and the time of hatching (Shanaway, 1994).

In quails, the production of fertile eggs is affected by many factors related to both parents and the environment, such as:

- male : female ratio; too many or too few quail cocks in the unit could lead to a higher proportion of infertile eggs. Quail cocks are aggressive and, when there are too many of them, a phenomenon called “psychological castration” often arises as a result of forming a peak order or dominance pyramid. On the other hand, placing too few cocks in the unit means that not all the hens are mated. Male : female ratios from 1:2 to 1:5 appear to give comparable fertility rates, while extreme ratios of 1:1

or 1:6 usually result in lower fertility rates. However, the correct ratio depends on the type of housing system used; in cages a ratio of 1:2 it is recommended, rising to 1:3 if the size of the cage allows;

- preferential mating; this tendency for the male to mate more often with certain females in the flock than others can be solved changing periodically the males. However, preferential mating has not been observed with quail kept in cages, where mating ratios are lower;
- age of the breeding flock; in Japanese quail, the fertility rate increases gradually until a maximum is reached at between 12 and 15 weeks of age, after which it declines gradually;
- health of breeding flock; obviously a sick or poor bird has no hope of breeding;
- inheritance; there is a great variation in inherent fertility from one species or strain of quail to another. As in domestic fowls and turkeys, semen production in quail is inherited, and certain semen characteristics (motility and concentration of spermatozoa) and semen volume are more likely to be inherited by meat type males (e.g. Bobwhite) than egg type males;
- nutrition; the nutrition of growing and adult quail is important for achieving maximum reproductive performance. Gross or marginal deficiencies in either the quantity or quality of feed can adversely affect fertility. When the dietary deficiency is marginal, a quail hen can still produce almost twice her own weight of eggs but a cock cannot produce the quantity of semen needed to fertilize them. Calcium has a profound effect on the function of the reproductive organs, especially in the female (Shanaway, 1994). Also some supplementations, such as selenium, zinc and vitamin E, were recorded as crucial factors in maintaining the high reproductive characteristics of poultry (Gallo et al., 2003, 2005);
- managerial factors; in example, the duration and intensity of light exert a profound influence on the sexual development of quail, fertility of males and egg production. Generally, long days during the growing period accelerate gonadal growth and maturity, while short days restrain it and delay maturity (Shanaway, 1994). Some authors reported, also, that an increasing of light intensity determine an increase of stress in quails, and thereby result in decreased mating performance and fertility rates. Coban et al. (2008) reported that an increase in light intensity decrease the hatchability of eggs. Also, Renema et al. (2001) have reported that an increased

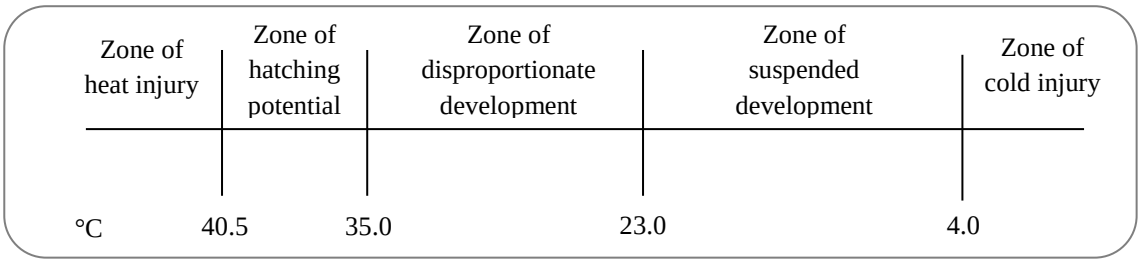
light intensity cause a decrease in egg shell thickness and quality. Extremes of ambient temperature also affect fertility, indirectly by influencing feed consumption, and directly by reducing the frequency of mating. In addition, insufficient feeding or drinking space overcrowds the birds and could deny them free access to feed and water, as well as reducing the space available for courting and successful mating (Shanaway, 1994).

As mentioned before, successful hatches depend also by the pre-incubation period. The laid eggs are often collected and stored for a period before being incubated. This is common practice particularly in commercial enterprises where eggs are held until there are sufficient numbers to fill the incubators in one go. Proper handling of eggs prior to incubation is as important as incubation itself if the highest hatchability is to be attained. Rough handling of hatching eggs can disrupt their delicate internal structure and usually leads to the death of embryos. The problem is even more pronounced in quail eggs because of the frail nature of their shells. From the moment of laying, the egg begins to deteriorate physically, and is subjected to bacterial attack (Shanaway, 1994). Infact, the loss of CO_2 rapidly increases albumen alkalinity at pH levels over the optimum of 8.2 for preserving embryo vitality. Depending on the stage of embryo development by the time of egg laying, its tolerance to increased pH values is different. Their negative impact is further enhanced when pH approaches 9 causing embryonic death. Another factor that has an effect upon hatchability is the loss of water during egg storage; it depends on eggs' storage conditions and duration, as well as on the changes in the thickness and porosity of eggshell related to the age of breeding flock. On the other hand, the loss of weight during incubation is a main parameter of biological control providing information for the development of the embryo. This trait is dependent on the physical conditions in the incubator, the thickness and porosity of shells, but also on the intensity of embryonic metabolism. The optimum water loss during incubation for the normal embryonic development ranges between 13-15 % of the initial egg weight (Genchev, 2009). For this reasons eggs cannot be stored indefinitely prior to incubation. An egg will remain hatchable up to a certain point of deterioration, beyond which hatchability falls off rapidly. The period for which an egg can be held without impairing its hatchability is short (Shanaway, 1994).

After the storage period, collected eggs are incubated. The incubation period for quail is 17–18 days, depending on the strain and the incubation procedures.

Maintenance of optimal temperature, humidity and ventilation is of prime importance for satisfactory hatching results. The temperature range in which development of the embryo will proceed correctly is very narrow (Figure 2.1).

Figure 2.1. Effect of temperature on the developing embryo (*source: Shanaway, 1994*)



In the first few days after laying, when the chick is being formed, great harm can be done by minor changes in temperature, whereas in the later stages the same changes will have little or no effect except to alter the time of hatching. Changes in temperature often appear to do no harm at the time, but the late mortality rate will be very high. If the temperature is too high throughout incubation embryos will start developing, but a large number will die after three to four days. On the other hand, whereas marginally low temperature will only delay hatching, significantly low temperature could prevent embryonic development (Shanaway, 1994). The optimal incubation temperature is not constant, but varies with the humidity of the air. Humidity is important for an embryo, helping it to develop properly and to transform into a chick of normal size. For this to happen, the egg contents must evaporate at an established rate (11-13 % of fresh weight). If the rate of evaporation is high, the egg contents dry out too rapidly and the chick will be smaller than normal; if evaporation is not fast enough the chick will be large. In either case the embryo is weakened, resulting in reduced hatchability, poor-quality chicks or both. To control the rate at which the egg contents are evaporated, the moisture content of the air surrounding the egg must be controlled, as it is this outside moisture which determines the rate of water loss from the egg (Shanaway, 1994). Quail eggs require a higher relative humidity (RH) than chicken eggs for optimum hatching, notably at the beginning of incubation. High hatching rates in quail are obtained with humidity in the range 65-72 % RH (at a temperature of 37.7°C).

Also, ventilation has a significant effect on hatchability; in fact, poor ventilation and air movement inside the incubator can result in an irregular distribution of heat and moisture, a lethal level of CO_2 and an insufficient oxygen supply, leading to poor hatchability. The developing embryo is normally capable of withstanding marginal

reductions in oxygen levels to 18 %. It is generally agreed, however, that a drop in hatchability of 4-5 % is expected for every 1 % drop in oxygen level below 18 %. A high concentration of carbon dioxide inside the incubator is extremely damaging (Shanaway, 1994).

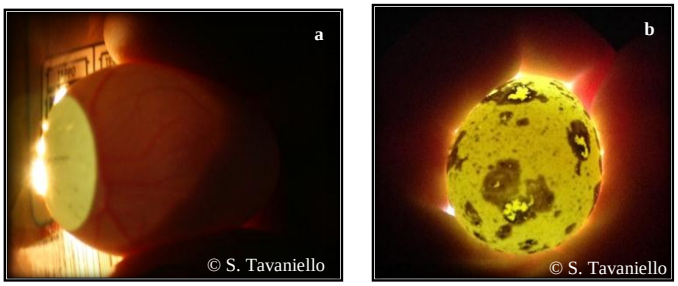
The two types of incubators generally available are fan-ventilated (forced-draft) and still-air machines. A forced-draft incubator is preferable, but a still-air machine works well if carefully operated. Some models are designed especially for quail. Japanese quail eggs can be incubated in any chicken egg type of incubator, although the egg trays in some machines may need modifying. Eggs should be placed large end up in the setting tray (Randall and Bolla, 2008). Forced-draft incubators should maintain an incubating temperature of 37.5 ± 0.3°C and a relative humidity of 60 % wet bulb reading of 30 ± 0.5°C until the 14th day of incubation. Eggs should be turned every 2–4 hours to prevent embryos from sticking to the shell. On the 14th day, candle and remove any cracked eggs, infertile and dead embryos. Transfer the eggs to hatching trays and stop turning. A separate hatcher should be operated at 37.2°C and a relative humidity of 70% wet bulb 32.2°C. If the incubator is a combined setter and hatcher, it should be operated at a temperature of 37.5°C, but the relative humidity should be increased to 70% wet bulb 32.2°C during hatching. If a still-air incubator is used, normal incubating temperature is 38.3°C for the first week, 38.8°C for the second week and not exceeding 39.5°C until hatching is completed. Temperature should be measured at the top of the eggs. Humidity should be less than 70 % wet bulb 29.4 – 30.5°C until the 14th day of incubation; it should then be increased to 70 % wet bulb 32.2°C until hatching is completed in 17 or 18 days. Maintaining proper humidity in small still-air incubators can be a problem; do not open the incubator more frequently than is needed to turn the eggs, and do not leave it open for long periods of time (Randall and Bolla, 2008).

The position and turning of eggs are further important factors during artificial incubation. The part of the yolk that is in contact with the germinal disc is lighter than the rest, so tends to float to the top of the egg. Each movement of the egg therefore brings the germinal disc into contact with fresh nutrients. This is of paramount importance before the embryo has developed the blood circulation that brings the nutrients to it; failure to turn the egg can deprive the embryo of nutrients and oxygen at a very critical stage in its development. The yolk as a whole is lighter than the albumen (because of its higher lipid content) so tends to float to the upper surface of the egg. It is

only the yolk ligaments (chalazae) that delay, but do not stop, its movement to the top of the egg. If not turned to a new position frequently, the developing embryo touches the shell membrane and sticks to it. This hampers its movement inside the egg and could be fatal. At each stage of incubation the embryo takes up a definite position. Turning the egg is a necessary aid to these embryo movements within it, and without turning malpositions arise which, in many cases, result in the unsuccessful emergence of the chick from the egg at hatching time. Under natural incubation conditions, the quail hen moves and turns the eggs, on average, once every 15 to 20 minutes, i.e. 72 to 96 times in 24 hours. Under artificial incubation eggs should be held with the large ends uppermost for turning. Turning should be carried out at least three times a day if it is done manually (more if possible but always an odd number of time, e.g. five, seven, nine), or once every hour if done mechanically (Shanaway, 1994). Most commercial incubators are provided with plastic egg trays that hold the egg vertically, with the small end down. The tray is then tilted through an angle of about 40º either side of horizontal (an overall angle of 80º) at predetermined intervals, perhaps every hour for example.

After 14 days of incubation, eggs are candled to determine whether they are fertile and to check the growth and development of the embryos. Eggs are passed over a bright light (Figure 2.2) which shows up internal defects, such as blood and meat spots, infertile eggs, died embryos, and previously undetected cracked or weak shells. If the embryo dies, the blood draws away from the embryo and forms what is called a blood ring. All clear eggs and eggs showing blood rings or streaks are removed from the incubator.

Figure 2.2. Fertile (a) and infertile (b) quail eggs (*personal photo*)



The fertile eggs are then transferred to hatching trays in the hatching compartment of a setting and hatching machine or to separate hatchers. This is normally done at day 14 or 15 for Japanese quail. The chick draws the yolk sac inside its body at hatching and uses it is a food store for the first few days of its life after hatching. It is the presence of

this yolk sac that enables the transportation of chicks for several days without the need to provide them with food or water (Shanaway, 1994). Chick weight and chick quality may be influenced by egg parameters, pre-incubation storage duration, and age of breeder (Tona et al., 2004).

2.2 Brooding and care of young birds

It is generally recognized that the first two weeks of any chick's life are the most critical to its survival and the small hatching size of quail chicks makes them even more vulnerable and susceptible to stress. In the quail chick neither is fully developed until about the third week of its life so stress stimuli, such as temperature extremes, overcrowding, poor nutrition, injury and disease agents often result in decreased growth of chicks, lower disease resistance and, in many cases, death (Shanaway, 1994).

After hatching, the chick are housed either in rearing cages or on litter (Figure 2.3). The system can be single or multistage. In a single-stage system, chicks are brooded in cages or on the floor, and kept in the same type of accommodation until they are marketed or start laying at six weeks of age. At this age chick raised for egg production or breeding are transferred to layer or breeder quarters. In a multistage system, once the chicks no longer require the artificial heat of the brooder, they are moved from the brooder first to grower houses and from there to layer or breeder houses (Shanaway, 1994).

Figure 2.3. Litter (a) and rearing cages (b)

Temperature is particularly important for day-old quail chicks because they are exceedingly susceptible to chills and draughts and a commercial brooder or any other heat sources that provides sufficient heat can be used successfully, and should be placed 30-46 cm above the floor of the pen. The zone of thermal neutrality for quail chicks is

between 35 and 37°C at one day old, narrowing to 33°C at one week and 31°C at two weeks of age, respectively. Temperature should be maintained at about 35°C during the first three days and gradually decreased by 0.5°C every day (or 1°C every other day) until a temperature of 21-23°C is reached at about four weeks of age at which point the chicks should be fully feathered. As the chick starts to eat, its body temperature increases. The best guide for adjusting the temperature is chick's behaviour. Chicks that crowd near the heat source and seem cold indicate the temperature is too low. When the chicks tend to settle just outside the hottest area, the temperature is about right. Failure to provide adequate heat during the early days of the brooding period invariably results in increased mortality. Chicks should be protected from draughts of cold air, especially at night (Shanaway, 1994; Randall and Bolla, 2008). Also humidity is important for the well-being of quail chicks, in fact, it affects the rate of feather development, as well as the incidence of respiratory diseases in the growing chick.

Quail chicks should be brooded under continuous (24 hour) light for their first two weeks, after which the light programme depends on the purpose of production. If the birds are raised for meat production, they can be given 23 hours of light and one hour of darkness or by intermittent lighting (interrupted lighting). A programme which alternates three hours of darkness and one hour of light repeated six times could help reduce feed intake and improve the feed efficiency rate, in addition to the obvious saving in electricity (74 %) (Shanaway, 1994).

2.3 Housing systems

Depending on the type and scale of production, there are 3 basic housing systems:

- aviary system usually used to raise and keep exotic quail;
- floor system used by small to medium scale enterprises;
- cage system widely used in commercial quail enterprises for egg and meat production, as well as for rearing. Cage system is used alone or in combination with floor systems.

2.3.1 Housing system and management of meat type quails

The main guiding principle of raising broiler quail is the "all-in/all-out" system, in which only birds of the same age are kept on the same site. The single stage system is

ideal for this purpose. The birds can be raised from hatching to slaughter either on the floor or in battery cages (Figure 2.4). In most commercial operations birds are reared "as hatched" which means that males and females are not reared separately. The growth rate of quail chicks is normally rapid from hatching to five weeks of age and slows down thereafter. Adult weight is achieved at about 50 days. Unlike practically all other domestic avian species, sexual dimorphism in quail favors the female.

Figure 2.4. Cage system (*personal photo*)

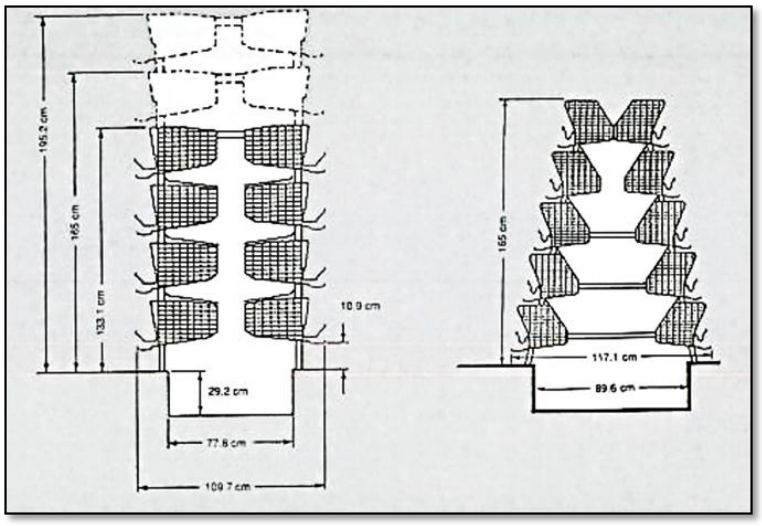
Growth in quail is affected by a number of factors including species, strain, sex, age, stocking density, nutrient intake, photoperiod and temperature. The optimal stocking density is 25 kg live weight/m^2 in naturally ventilated houses and 35 kg live weight/m^2 in environmentally controlled houses. Stocking density exerts a profound effect on growth rate of quails, in fact, it was observed that an increasing in feed intake is mainly associated with a reduction in the competition for feed (Shanaway, 1994). Optimal temperature for high body weight gain are related to the age of the birds. During the first week after hatching, highest gain is reached when the brooding temperature is 35°C; it drops in weeks two and three to 32°C and 28°C, respectively. From week four onwards, the optimal temperature range between 22 and 25°C. Also the light period affects the growth rate by directly influencing the duration of daily feeding. When birds are offered long light periods, their feed consumption increases in proportion to the duration of light. As a result, their body weight at marketing increase but, in most cases, the overall feed conversion ratio is not much altered (Shanaway, 1994; Randall and Bolla, 2008).

Nutrition plays an important role on the growth of quail, it represents about 70 % of the total production cost. Energy is considered to be the most important requirement from the standpoint of total cost and quantity of quail feed. In light of these, the diet for meat type quail should contain about 27 % protein and 12.1 MJ ME/Kg, for the first 2 weeks of life; from the second to the fourth week , protein content of the diet should be 23 % and 12.5 MJ ME/kg. Finally, from fourth to sixth week and follow the protein content can be further reduced to 20% by increasing the energy at 12.9 MJ ME/kg (Shanaway, 1994; Randall and Bolla, 2008).

2.3.2 Housing system and management of egg type quails

Egg type quails are usually reared using the cage system. The battery cage offer a high degree of automation for quail rearing and commercial egg production. The reverse cage pyramid system for layers (Figure 2.5) increase house capacity without increasing floor space. In the upright four or six stacked layer cage system, super high density can be achieved (Shanaway, 1994). To get a good fertility rates, the males are reared separately and are placed in the cage of females once every 2 days, after mating occurred the male is again separated (Shanaway, 1994).

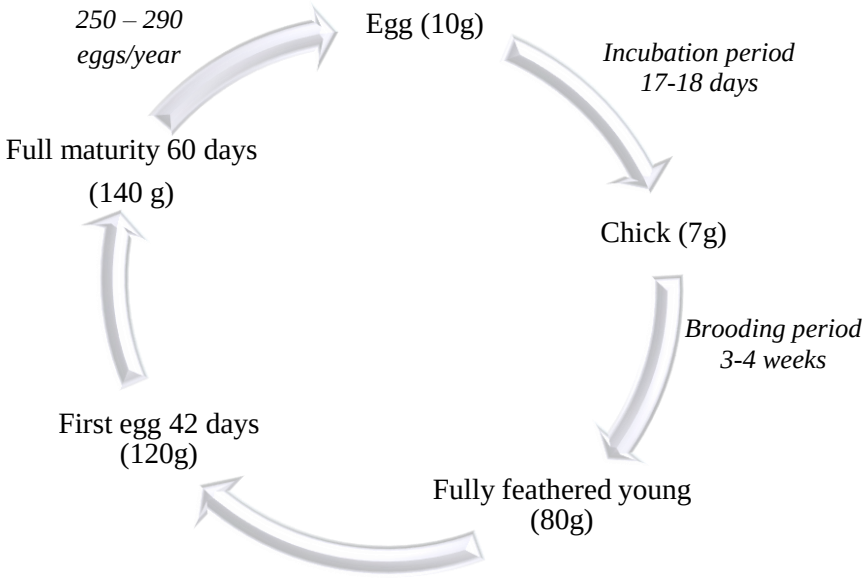
Figure 2.5. Cage systems used for commercial egg production (*source: Shanaway, 1994*)



Japanese quail reach puberty more quickly than any other domesticated birds at about 5 - 6 weeks of age; however, peak production is not reached until 3 to 5 weeks later (Figure 2.6). Quails exhibit relatively high fertility and egg production and remain reproductive if maintained on a long photoperiod. Fertility of quail breeders drops 30-50

by 70 weeks of age. In females, egg production becomes erratic and decline between 1.5 and 2 years of age (Shanaway, 1994; Ottinger, 2001).

Figure 2.6. Life cycle of Japanese quail female with approximate body weight (g)



Japanese quail eggs are a mottled brown colour and are often covered with a light blue, chalky material. Each hen appears to lay eggs with a characteristic shell pattern or colour. Some strains lay only white eggs. The average egg weighs about 10 g, about 8% of the bodyweight of the quail hen. Young chicks weigh 6–7 g when hatched and are brownish with yellow stripes (Randall and Bolla, 2008). Egg pigmentation are characterized by a variety of color patterns (Figure 2.7), ranging from dark brown, blue, and white to buff, each heavily mottled with black, brown, and blue. The eggshell pigments are ooporphyrin and biliverdin which start to be deposited on the shell approximately three and half hours before laying. The quail egg is one-fifth the size of chicken's egg, ranging in weight from 7 to 15g, with an average weight of 10g for Japanese quail. This weight represents between 8 - 9 % of the female's body weight. In chickens, egg weight represent only 3 %, while in turkey 1 % of body weight (Shanaway, 1994).

Figure 2.7. Japanese quail eggs

The photoperiod (day length) exerts a significant influence on the sexual development of quails. Long days, during the growing period, accelerate gonadal growth and maturity, while short days restrain it and delay maturity. The use of artificial lights to induce laying during any season of the year has been one of the most significant contributions to the improvement of egg production in quail (Shanaway, 1994). In general, Japanese quail require 14 – 18 hours of light per day to maintain maximum egg production and fertility. This means that supplementary lighting must be provided in the autumn, winter and spring months to maintain production (Randall and Bolla, 2008). Unlike all other domesticated birds, ovulation in quail occurs in the afternoon and egg laying (oviposition) takes place about 24 hours later (the following afternoon). The series of eggs laid on successive days is known as an egg clutch or sequence, while days on which no eggs are laid are known as pause days. Clutch length is long during the initial period of production, reaching a peak about four week after the first egg is laid. It declines gradually as the hen becomes older (Shanaway, 1994).

Other environmental factors such as temperature and stocking density could have profound effect on delay in sexual maturity.

Nutrition plays an important role on the growth of laying quails and on the egg production. Laying diets should contain about 24 % protein, 11.7 MJ ME/ kg, and 2.5–3.0 % calcium. In fact, the calcium in the form of calcium carbonate, is the main constituent of eggshells, so the level of calcium in the growing diet must be increased to allow the production of the shell of the eggs.

Chapter 3

QUAIL MEAT PRODUCTION

3.1 Quail meat as an alternative to conventional meat

Nowadays, consumers are increasingly becoming concerned about healthy and safe products, and the demand for these products is rapidly growing. Consumers expect the meat products on the market to have the required nutritional value, be wholesome, fresh, lean and have adequate juiciness, flavor and tenderness (Dransfield, 2001, 2003; Ngapo and Dransfield, 2006). However, the consumer's perception of meat not only depends on their inherent properties, but also on the way in which these properties interact with immediate external factors, such as animal welfare and the environment, and on the previous experiences of the consumer (Dransfield et al., 1998).

As a result of the changes in the demand for meat, interest in "new" meat products such as alternative meat, has increased in recent years. These trend are particularly visible in meat production; ostrich, pheasant or quail meat is more often available in big retail stores. The valuable taste and dietary properties of quail meat are pivotal in determining the growing interest of consumers in this product (Genchev et al., 2008a). In addition, when it comes to composition, quail meat has interesting technological properties because of the lower loss of moisture, which might aid in its marketing (Genchev et al., 2005, 2010). In terms of its basic composition, it is quite similar to broiler meat, and for this reason quail meat can easily satisfy the consumers' requirement about healthy food with low cholesterol content and high polyunsaturated fatty acids.

Together with the changing in consumers' choice there was also an increase in publications of scientific papers on meat quality of ostrich, quail, pheasant, even if the available information are still very scarce. In this perspective, there is still a big possibility for further improvement in about all aspects of production: selection of lines, nutrition, management of birds, protection from diseases, processing of eggs and meat, and research is needed in all these areas. The futures of quail research and of quail production are not independent, however, better links between both activities are needed

to promote relevant research, explore new avenues of quail production, and help maintain this bird as an essential animal model (Minvielle, 2004).

3.2 Quail meat production trend

Avian meat is one of the main products consumed by humans and allows the development of products with reduced cholesterol content, which is an urgent need in industrialized societies. In the poultry world, quail meat production is negligible when compared with that of broilers, but nevertheless occupies a relevant place in poultry breeding and contributes to the variety in poultry meat production (Maiorano et al., 2011). Actually, it is difficult to track precisely the production of Japanese quail meat around the world which has an unequal development (Minvielle, 2004). This is due to the fact that quail is quite far from being a regularly consumed product. Quail meat is mainly and still regarded as a food delicacy or a kind of food for special occasions; even though, in some countries quail meat represent an interesting niche business.

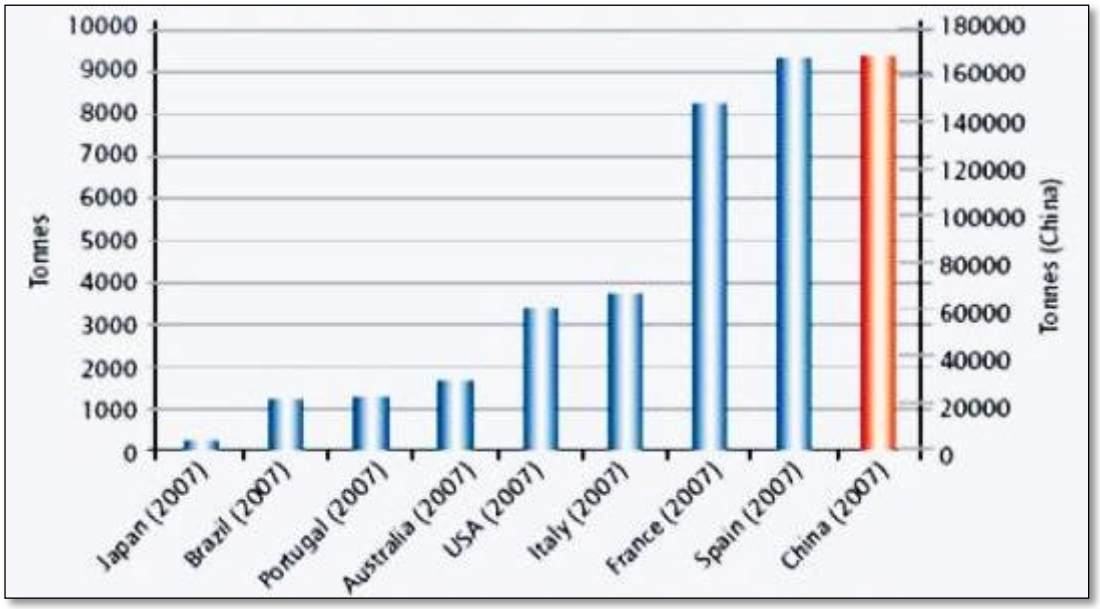
France, Spain, Italy, China and the USA are the largest producers, with other countries far behind. However, some initiatives to raise production were launched in India, Australia and Canada (http://www.worldpoultry.net).

China has the biggest quail meat production in the world. China's data is hard to interpret in the absence of official statistical time series. It is estimated that in China 1,040 to 1,360 million birds are slaughtered per year, and considering an average of 13 – 17 cycles per year and an average weight at slaughter of 200 g with a carcass yield of 70 %, it is estimated a production of 146,000 - 190,000 tons of quail meat per year (Figure 3.1). However, even these numbers might be a big underestimation of the real productive situation, due to the fact that the laying quails might be consumed after their productive period. Consequently, based on an estimation of 315 - 350 million of Japanese quail layers per year, the effective number of quail meat production in China would change drastically.

A significant quail meat production in Europe are recorded in Spain, France and Italy, which show a thriving export market, but little or no commercial meat production in Netherlands, Germany and U.K. (Shanaway, 1994; Minvielle, 2004). It is estimated that in Spain in 2004 the production of quail meat was about 9,300 tons with exports that reach in 2007 3,782 tons. France has similar values with an estimated production (2006) of 8,197 tons. In the recent years the production reached 9,000 tons. According

to the French Customs, the country also exports more than it imports, 1,644 and 1,504 tons, in 2007; but the largest portion of the trade is restricted to Europe. Belgium and Germany are the main importers of French quail, and Spain is the major exporter to the country. Next comes Italy with 3,300 - 3,600 tons of quail meat produced and an export of about 600-650 tons per year (http://www.worldpoultry.net). Recent data (ISTAT, 2012) reported that the number of slaughtered quails in Italy was of 16,8 million animals. Portugal has a modest production. During the past seven years were slaughtered about 8-13 million quails, and carcass weight surrounding between 960 and 1,600 tons.

Figure 3.1. Quail meat production in the world



Source: http://www.worldpoultry.net/Other-Poultry-Species/Other-Poultry-Species/2009/2/Quail-meat---an-undiscovered-alternative-WP006930W/

In 2002 the census of agriculture in USA reported 1,907 farms having sold just over 19.1 million quails. Assuming a weight range of 200-300 g, and a 70 % carcass yield, this would represent between 2,674 and 4,011 tons. Georgia is the largest producer, followed by North Carolina, Texas and Alabama. USA also imports quail meat, with the main supplier being Canada (http://www.worldpoultry.net). According to a report by the Rural Industries Research and Development Corporation (RIRDC, Australia), in 2001-2002 the country slaughtered 6,5 million quails (out of 17 million game birds). A single company located in New South Wales answered for 75 % of the quail production. Australia's supply capabilities to Asia are considered to be good by RIRDC, and France and China are the main competitors. Brazil can hold the promise to be a significant

competitor whenever it comes to poultry. Nonetheless, the meat sector in Brazil is still very young. Only one company (the giant Perdigão) has ventured into the commercial business. In 2007, the company processed 1,200 tons of quails, but it has been experiencing growth of about 10 % per year. The majority of the production supplies the domestic market. Exports go mostly to the Middle East. Egypt is another country with a similar productive situation, the data are restricted to a single company which claims to slaughter 6 million quails per year (http://www.worldpoultry.net).

A promising actor is Canada where in 2000 the University of British Columbia tracked the status and perspectives of British Columbia on game birds. In 2000, that state alone was producing 2 million quails per year through a single producer and slaughtered about 10,5 million game birds, and a "sizeable" proportion of it was said to be from quails. A substantial portion of such production goes to the USA, particularly California. In 2007 alone, Canada exported 628 tons of quail to the USA. The report foresaw a growth perspective between 50 and 60 % up to 2007 for British Columbia. Although there is no recent data to see how much production was reached, the forecasts were not confirmed, according to dr. Cheng, responsible for the report. She lists two unforeseen reasons for this: the avian influenza outbreak of 2004, which negatively impacted their production and export, and the more recent exchange rate between the USA and the Canadian dollar, which further hurt exports. India is another promising quail meat producer since the government is giving incentives for quail production. In the last three and a half decades there has been phenomenal growth in the production capacity of the Indian poultry industry. It attained an annual growth rate of 7 to 10 % in layer production and 15 to 20 % in broiler production. There are now 35 billion eggs and 4 million broilers at marketable age available annually. Japan has a low quail meat production while it is a big quail egg producer (http://www.worldpoultry.net).

In conclusion, it is not easy to track a precisely status of the quail meat production around the world; this is due to the fact that quail meat is quite far from being a regularly consumed product and also quail meat production is a young productive sector compared to that of other poultry species. It is hard to try and predict what quail production will become in the future. Of course, what will really takes place will depend on geography, sociology, economy and science.

The production and consumption of quail meat might take advantage from the image that this meat have in many countries; among all products of intensive poultry farming,

Japanese quail meat and eggs convey the image of a natural and festive food. This positive image could be preserved or even further developed. If this choice was made, quail production should incorporate well-publicized zootechnical practices (allocation of space, food source...) for the well-being of the birds, and might avoid fast growing birds (Minvielle, 2004). On the other hand, there may also be markets for standard 4 week–old quail, and for larger 300 g quail to be sold as broilers or processed. Finally, a segment of the production might consist of older, lower growing birds yielding high quality meat. Well established, parental or grand parental commercial lines covering a large range of body weights and corresponding management practices will then be needed to adapt quickly to the market (Minvielle, 2004). In addition, the development of new products from quail eggs and meat should help attract consumers which are increasingly interested on alternative and convenience foods. Moreover, quail meat, due to its high nutritive value, could be a relatively cheap providers of proteins representing a good strategy to reduce the protein deficiency in poor countries.

Chapter 4

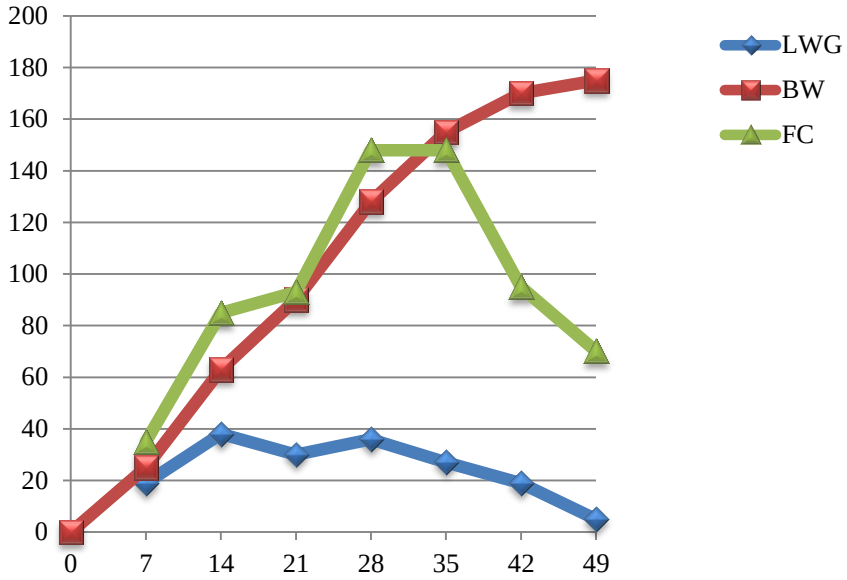
CARCASS COMPOSITION AND MEAT QUALITY IN JAPANESE QUAIL

4.1 Carcass composition

The main indicator of the quality of poultry meat, is the category of a carcass, which is determined by its nutritional status, taking into account the degree of fat and muscle tissue. The structure consists of a carcass muscle, fat, bone and connective tissue, as well as cartilage and ligaments. The less bone and cartilage and more muscle and adipose tissue in the carcass determine the higher categorical and nutritional value of meat (Maiorano and Bednarczyk, 2013). Generally, in quail boneless meat yields is about 77 % of carcass weight, the breast muscle represent 50 % of the total carcass meat yield while leg muscle contribute about 30 %. The ratio meat:bones increase from 3:3 at five weeks of age to 3:6 at 8 weeks (Shanaway, 1994). In the case of broiler chicken the content of muscle tissue of the carcass varies between 40 and 70%. Broiler crosses have the best proportion of muscle tissue in the pectoral muscle with an average of 94 - 98%, and in the leg about 92 – 97 %. The ratio meat:bones is about 4 - 4,5:1 (Maiorano and Bednarczyk, 2013).

Generally, broiler quails are slaughtered at about 5 - 6 weeks of age for the economic reasons (Genchev et al., 2008a). However, quails are also slaughtered as young/old broilers (8 - 13 weeks) and spent birds (8 months; Boni et al., 2010) and sold on the commercial market without any distinction being made on age (Shanaway, 1994; Minvielle, 2004). Slaughter age is essentially influenced by market forces and can be determined in a variety of ways; growth rate, feed consumption and feed conversion ratio are the economic considerations commonly used to decide the best slaughter age for Japanese quail. Under good condition of feeding, environmental and management, the body weight gain of quails increases till the 4th week, then starts to decline (Figure 4.1) (Shanaway, 1994; Seker et al., 2007).

Figure 4.1. Body weight (BW), Live weight gain (LWG) and feed consumption (FC) of Japanese male quails during 49 days feeding period (*source: Seker et al., 2007*)



Similarly, feed consumption shows a sudden decline after 35 days of age. This appears to be the reason for slower growth. Some authors reported a reduction in feed consumption after 35 days in separately reared male quails (reviewed in Seker et al., 2007). This can be, in part, explained by the start of sexual activity in male birds at 30-40 days. Hormonal changes during this period might have resulted in decrease in feed consumption. In addition, during this period there is the beginning of social hierarchy which can cause additional stress factors, resulting in reduced live body weight gain. Feed conversion ratio increase with age, but after 42 days it increases rapidly there was making uneconomical feed quails after that age (Seker et al., 2007).

The carcass yield (eviscerated carcass weight/live body weight) in Japanese quail ranges from 60 to 70 - 75 % depending on slaughter age, line and sex (Caron et al., 1990; Seker et al., 2007; Genchev et al., 2008a; Maiorano et al., 2009; Alkan et al., 2013). The effect of sex on slaughter and carcass characteristics is well known in quail and was reported as highly significant (Tserveni-Gousi and Yannakopoulos, 1986, Caron et al., 1990; Yalcin et al., 1995; Minvielle et al., 2000; Vali et al., 2005, 2008; Saatci et al., 2006; Khaldari et al., 2010; Narinc et al., 2010). For the sexual dimorphism female are heavier than males, but the latter are characterized by higher carcass yield. Large reproductive organs in females, such as ovary and oviducts, appear to be the main reasons behind higher body weight in females than males (Marks, 1993). Therefore, the weight of reproductive organs related to body weight in Japanese quail is higher than in

broiler chickens at 42 days of age and consequently, the carcass yield will be lower in Japanese quail (Lotfi et al., 2011). Despite Japanese quail is not a species with a high slaughter yield, the percentage of edible meat is high. It was reported that breast makes up a considerable part of the carcass in Japanese quail (Vali et al., 2005; Khaldari et al., 2010) and this is a clear advantage, because breast meat is favourable for consumers. The incidence on the carcass of breast muscle is ranging from 25 to 36 % and for legs is ranging from 16 to 22 % in Japanese quail of different ages (Caron et al., 1990; Panda and Singh; 1990; Seker et al., 2007; Genchev et al., 2008a; Alkan et al., 2013). In addition, quail breast meat is characterized by higher content of intramuscular fat compared to chicken breast muscle (reviewed in Lotfi et al., 2011), affecting the quality of breast meat in terms of flavour and juiciness (Chizzolini et al., 1999). This higher content of intramuscular fat in Japanese quail refers to the flying behavior of these birds (reviewed in Lotfi et al., 2011). Differently, the mean abdominal fat percentage in Japanese quail (0.8 %) is lower than in broilers at 42 d of age, whereas the skin percentage in Japanese quail (5.2 %) is higher than in broilers at the same age (Zerehdaran et al., 2004). Abdominal fat is the largest adipose tissue in broilers (Leenstra, 1986; Griffin, 1996). Although subcutaneous fat was not separately measured, it seems the size and importance of subcutaneous fat is more than abdominal fat in Japanese quail. The reason behind the importance of subcutaneous fat is that the Japanese quail is a flying bird. Boswell et al. (1993) reported a visible increase in the size of subcutaneous fat in European quail during migration and described that subcutaneous fat is the main source of energy in migrating birds. Piersma et al. (1999) also showed that subcutaneous fat contributes, on average, 71.0 % of total body fat in flying birds, whereas the contribution of fat in the abdominal cavity (the abdominal fat layer, plus the fat surrounding the intestines) is about 14.2 %. Abdominal and subcutaneous fats are being regarded as the main source of waste in birds (Griffiths et al., 1978; Becker et al., 1981). Abdominal fat is found to be highly correlated (0.6 to 0.9) with the total carcass fat, so it is used as the main criterion reflecting excessive fat deposition in birds (Chambers, 1990). During the last decades, intensive selection for rapid growth was found to result in higher fat deposition particularly in the abdominal region. Several studies have reported less abdominal fat accumulation in broilers fed diets containing high levels of polyunsaturated fatty acids than in those fed diets containing high levels of saturated or monounsaturated fatty acids (Sanz et al., 1999;

Crespo and Esteve-Garcia, 2001). This reduction could be accompanied by a reduction in total body fat. Sanz et al. (2000a) found lower total body fat in broilers fed diets rich in polyunsaturated fatty acids than in those fed diets rich in saturated fatty acids. This lowering effect could be due to changes in rates of lipid oxidation or lipogenesis, as suggested by some authors (Blake and Clarke, 1990; Cunnane and Anderson, 1997; Sanz et al., 2000b). So one of the efforts of the modern poultry industry is the selection against abdominal and subcutaneous fats without changing intramuscular fat and the quality of breast meat (Lotfi et al., 2011).

4.2 Meat quality traits of Japanese quail meat

Meat quality is a generic term used to describe properties and perceptions of meat. There is no standard definition of meat quality that meets all the quality components of the meat production; anyway one of the most accepted definition is that proposed by ISO (8402:1994): "Quality is the totality of characteristics of an entity that bear on its ability to satisfy stated and implied needs". The quality concept is an extremely complex notion that can be assessed from different points of view. It includes attributes such as carcass composition and conformation, the eating quality of the meat, health issues associated with meat such as *Escherichia coli* 0157 contamination, and production-related issues including animal welfare and environmental impact (Maltin et al., 2003). In particular, modern consumers are more and more sensitive to the ethical and cultural aspects of food consumption and there is an increasing interest towards alternative rearing and animal-friendly production systems, which can improve animal welfare as well as guarantee higher qualitative standards concerning food safety, nutritional and sensory properties (Magdelaine et al., 2008). From both the standpoint of consumers and slaughter industry, poultry should have not only high slaughter yields and desirable carcass conformation scores, but also a good aesthetic, sensory and nutritional characteristics (Maiorano and Bednarczyk, 2013). Even if there are a number of characteristics that determine the overall quality of meat, poultry meat quality is determined also by three extremely important traits: appearance, meat tenderness and flavour (Fletcher, 2002). Infact, one of the critical point of appraisal of meat quality occurs when the consumer eats the product, and it is this outcome, together with views of color, healthiness and price, that determines the decision to repurchase. Hence, consumer evaluation of eating quality is the major determinant of meat quality, with

tenderness, juiciness and flavour of meat being the most important elements. However, the main source of consumer complaint and the primary cause of failure to repurchase is the variability in eating quality, especially tenderness (reviewed in Maltin et al., 2003). The determinants of meat eating quality are multifactorial and complex. This situation is not surprising since muscle is intrinsically a highly organized and complex structure, so that the properties of meat are likely to be determined at different levels ranging from the molecular to the mechanical, including genotype, feeding, housing systems, pre-slaughter handling, slaughtering and processing (Maltin et al., 2003; Cavani et al., 2009).

4.2.1 *Chemical composition and nutritional aspects of quail meat*

The quality and composition of quail meat are influenced by numerous factors such as genotype of birds (Genchev et al., 2005; Alkan et al., 2010), divergent selection (Maiorano et al., 2009), feeding (Gardzielewska et al., 2005), sex (Genchev et al., 2008a), age (Tserveni-Gousi and Yannakopoulos, 1986), and stress (Gonzalez et al., 2007).

Meat tissues are composed of five primary chemical constituents: water, proteins, lipids, carbohydrates and inorganic matter (ash or minerals). Other components include non-protein nitrogen compounds (e.g. nucleotides, peptides, creatine, creatine phosphate, urea, inosine monophosphate, nicotinamide–adenine dinucleotide) and non-nitrogenous substances (e.g. vitamins, glycolytic intermediates, organic acids). Skeletal muscle tissue is composed of approximately 75 % water, 19 % protein, 2.5 % lipid, 1.5% non-protein nitrogenous compounds, 1 % carbohydrate and non-nitrogenous components and 1 % inorganic matter. The meat tissue composition varies according to differences in species, maturity, stage of growth, plan of nutrition, anatomical location of cuts within carcass and the inclusion of skin and bone. Carcasses are chemically more diverse, while individual muscles from each species are more similar in gross composition (moisture, protein, fat). However, muscles vary in the proportions of specific chemical components (e.g. collagen content, myoglobin concentration, sarcoplasmic proteins) or nutrients (e.g. saturated, monounsaturated and polyunsaturated fatty acids; iron). Generally, the percentages of water, protein and ash are inversely related to the percentage of fat; in other words, the percentages of moisture, protein and ash decrease with increasing amounts of fat in the tissues. The percentage of

carbohydrate, however, remains rather constant as the fat content of meat increases (Keeton and Eddy, 2004).

When it comes to composition, quail meat has some interesting properties, which might aid in its marketing. In terms of its basic composition, it is quite similar to broiler chicken meat (Tables 4.1 and 4.2). Accordingly, it has a high protein content and a relatively low fat content (especially when skin is taken out). Poultry meat is recognized by consumers as a healthy type of meat for the low fat content with a high unsaturation degree of fatty acids (FA) and low sodium and cholesterol levels (Cavani et al., 2009). Poultry meat may be also considered as "functional food", which provide bioactive substances with favorable effects on human health, like conjugated linoleic acid (CLA), vitamins and minerals, and a balanced n-6 to n-3 polyunsaturated fatty acids (PUFA) ratio (Barroeta, 2006; Grashorn, 2007). From this point of view, quail meat could be an interesting alternative meat for those consumers interested in good tasting and healthy foods.

Table 4.1. Chemical composition of whole carcass of quail and broiler chicken (value per 100g)

Item	Quail[1]	Chicken[2]
Water (g)	69.65	65.99
Protein (g)	19.63	18.60
Total lipid (g)	12.05	15.06
Carbohydrate, by difference (g)	0.00	0.00
Fiber, total dietary (g)	0.00	0.00
Energy (Kcal)	192	215

[1]Quail, meat and skin, raw;
[2]Chicken, broilers or fryers, meat and skin, raw.
Source: http://ndb.nal.usda.gov/ndb/search/list

Table 4.2. Chemical composition of breast muscle meat of quail and broiler chicken (value per 100g)

Item	Quail	Chicken
Water (g)	71.67	73.90
Protein (g)	22.59	22.50
Total lipid (g)	2.99	2.62
Carbohydrate, by difference (g)	0.00	0.00
Fiber, total dietary (g)	0.00	0.00
Energy (Kcal)	123	120

Source: http://ndb.nal.usda.gov/ndb/search/list

4.2.1.1 Proteins

Proteins constitute 16–22 % of skeletal muscle tissue and are generally categorized according to function: myofibrillar 11.5 % (contractile), sarcoplasmic 5.5 % (metabolic) and stromal 2 % (connective or support) proteins. The metabolic turnover or replacement rates for each of these tissues are intermediate, rapid and very slow, respectively (Keeton and Eddy, 2004; Lawrie and Ledward, 2006).

As mentioned before, quail meat like other poultry meat, is a concentrated source of protein with high biological value with a good content of essential amino acids and high digestibility. The variability of protein quality of meat depends essentially by the relative amount of connective tissue in muscle. In fact, connective tissue is characterized by a poor protein quality being devoid of an essential amino acid, the tryptophan, and characterized by high amounts of glycine (about 33 %), alanine (11 %), proline (9 - 10 %) and hydroxyproline (13 – 14 %) which are specific amino acids of collagen (Keeton and Eddy, 2004; Carnovale and Sambuy, 2006).

Genchev et al. (2008a) reported that the ratio between essential and non-essential amino acids in quail meat was 1.25, indicating the high biological value of quail meat. The essential amino acids accounted for about 40 % of the meat protein (Table 4.3). Genchev et al. (2008a) reported that the daily consumption of 2 quails is equal to an average intake of 125 - 130 g of pure meat which provide a total of 27 - 28 g of protein, including 11 g of essential amino acids, corresponding to 40 % of human protein needs. The sum of consumed essential amino acids plus cysteine and tyrosine, presented as percentage of meat protein, is equivalent to 43.6 %, that is more than the requirements for “ideal protein” of 35 %. The consumption of meat from two quails satisfies human minimal daily needs of lysine, leucine, phenylalanine + tyrosine and valine, which depending on the age, physiological status and physical workload of men (reviewed in Genchev et al., 2008a). In light of these results quail meat could be considered a valuable source of protein and in particular of essential amino acids.

Table 4.3 Amino acids content of meat from 35 days old Japanese quail

Amino acids (%)	Breast	Legs
Essential amino acids		
Lysine	2.19±0.06	2.12±006
Methionine	0.56±0.04	0.52±0.04
Isoleucine	1.22±0.03	1.11±0.03
Leucine	2.09±0.05	1.96±0.05
Phenylalanine	0.97±0.01	0.97±0.02
Threonine	0.74±0.04	0.69±0.02
Valine	1.29±0.04	1.15±0.04
Cysteine	0.20±0.02	0.16±0.01
Tyrosine	0.61±0.02	0.54±0.02
Non – essential amino acids		
Histidine	1.13±0.03	0.70±0.02
Arginine	1.40±0.04	1.31±0.05
Glutamic acid	3.96±0.09	3.81±0.23
Glycine	1.02±0.03	1.11±0.04
Serine	0.43±0.05	0.38±0.01
Alanine	1.34±0.04	1.30±0.04
Proline	0.99±0.03	0.99±0.03
Asparagine acid	2.05±0.04	1.93±0.05
Protein content	22.21±0.52	20.74±0.49
Total essential amino acids[1]	9.07±0.21	8.52±0.22
Essential + Cysteine and Tyrosine[2]	9.88±0.21	9.22±0.23
Ratio non-essential : essential	1.25	1.25

[1]amount of essential amino acids is without tryptophan;
[2]Cysteine and Tyrosine can be essential in determined conditions.
S*ource: Genchev et al., 2008a*

4.2.1.2 Collagen: composition, organization and crosslink formation

Collagen, the most abundant mammalian and avian protein, is a connective tissue constituent that is present in all tissues. Four decades ago, collagen was thought to be a single molecule but in the last years at least 29 distinct collagen type, the products of more than 30 genes, have been identified (reviewed in McCormick, 2009). All collagen types are identified by a Roman number and collagen genes are named after the collagen type and the α-chain that is encoded (e.g., COL1A2 for the α-chain for Type I collagen). Collagens are divided into four main groups:

- The fibrillar collagen (types I, III and V);
- The network collagen (type IV),
- The filamentous collagen (type VI);
- The fibril-associated collagen or FACITs (type XII and XIV)

and several subgroups including membrane collagen (McCormick, 2009).

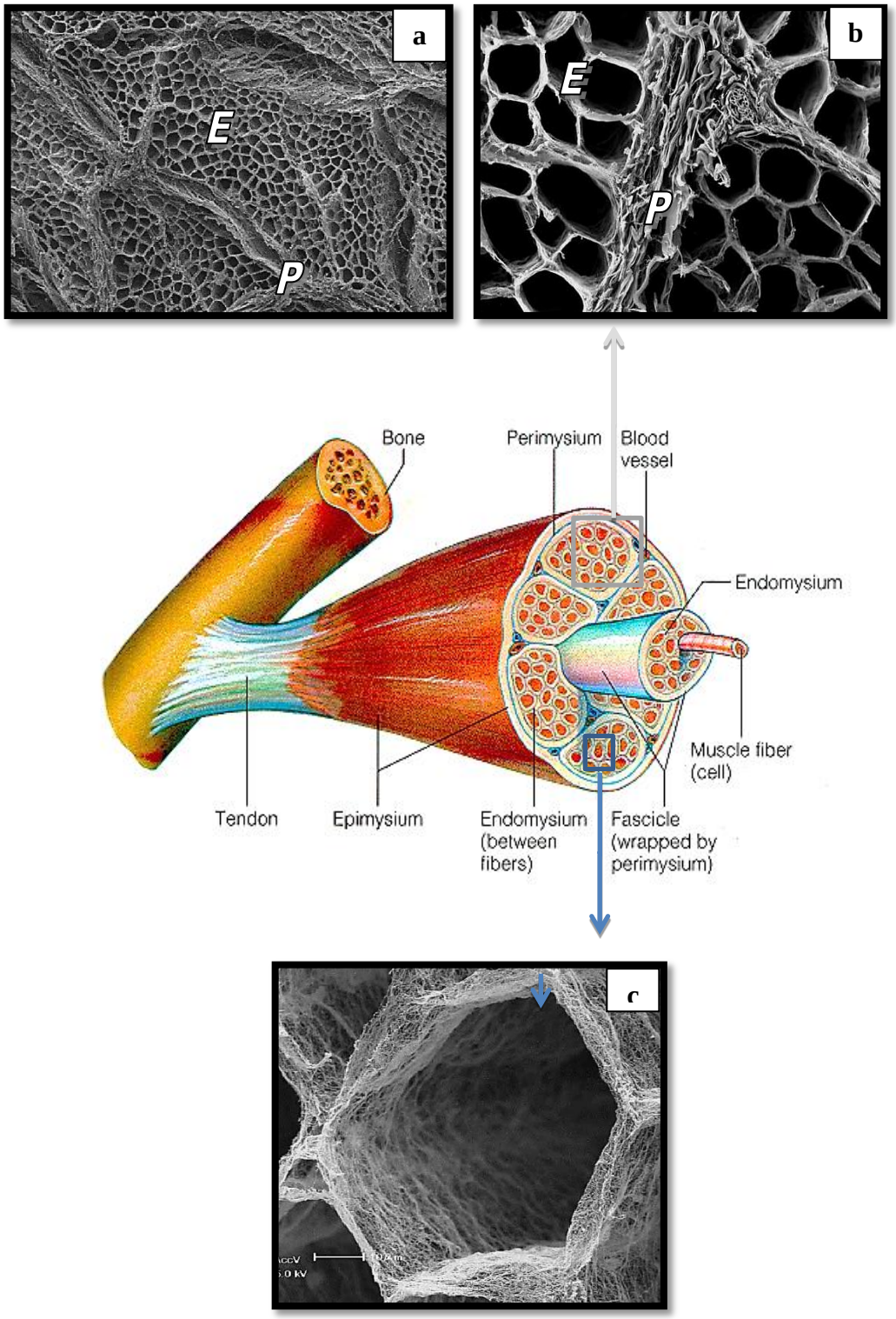
Types I and III collagen are the primary proteins of the muscle extracellular matrix (ECM), with lesser amounts of Type V and IV collagens associated with the perimysium and basement membranes, respectively (Bailey and Light, 1989).

The structure of muscles is mainly defined by connective tissue sheaths which can be divided into three subgroups (Figure 4.2):

- the endomysium is the thin connective tissue layer separating individual muscle fibers. The vast majority of its thickness is made up of a near-random feltwork of fine, wavy collagen fibers (Figure 4.2 c). This collagen feltwork can easily reorientate with changing muscle length;
- the perimysium is the connective tissue layer that separates each muscle into muscle fiber bundles, or fascicles. There are large (primary) fascicles and smaller (secondary) fascicles, and therefore primary and secondary perimysial layers separating them. Collagen fibers in the perimysium are arranged in a crossed-ply arrangement of two sets of wavy collagen fibers, with the fibers in each ply parallel to each other but at an angle to the muscle fiber axis. Again, reorientation of this collagen network allows the perimysium to easily follow elongation or shortening of the muscle fascicles;
- the epimysium is the connective tissue sheath delineating and separating individual muscles. In many muscles collagen fibers in the epimysium take on the same crossed-2-ply arrangement in the perimysium. Instead, in muscles where the epimysium clearly participates in transferring load to adjacent structures, the collagen fibers are more close-packed and longitudinally arranged, like a tendon (reviewed in Purslow, 2005).

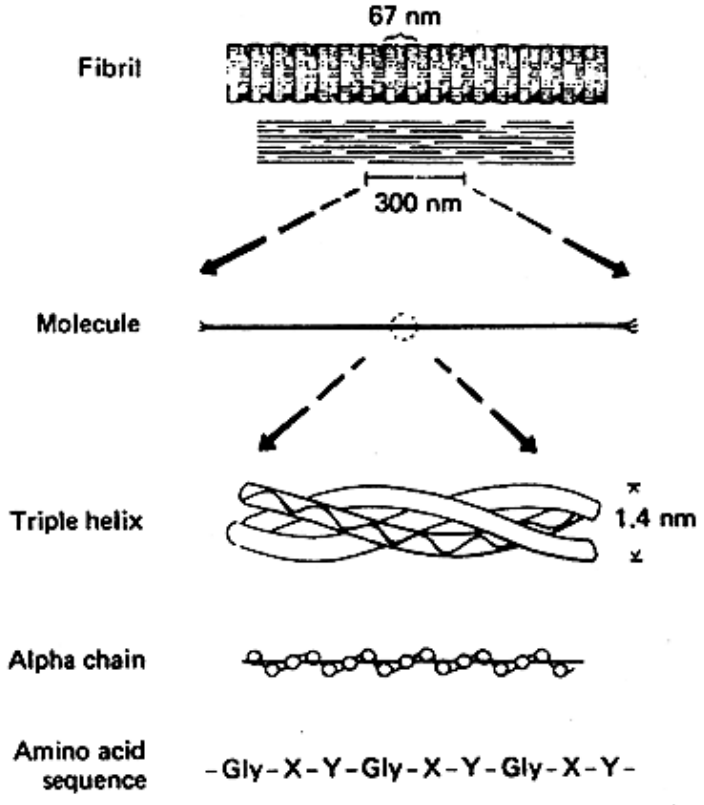
The epimysium is often thick and tough and resistant to both shear and solubilization. However, it is easily (and usually) separated from cuts of meat and is generally not considered to be a factor in meat quality. The intramuscular connective tissue (IMCT) is, thus, the combined perimysium and endomysium depots. The IMCT is composed mainly (90 %) of perimysium which play the major role in determining meat texture differences that are related to connective tissue (Light et al., 1985).

Figure 4.2. Gross composition of muscle indicating epimysium, perimysium and endomysium (*source: http://www.tarleton.edu/Departments/anatomy/musclepix2.html)* with SEM photographs of chicken Pectoralis muscle: (a) low magnification, (b) middle magnification, (c) middle magnification of endomysium; E = epimysium; P = perimysium (*source: Roy et al., 2006*).



The collagen molecule consists of three α-chains with a [Gly-X-Y] repeating motif, where X is commonly proline and Y can represent any amino acids but is often the modified amino acid hydroxyproline (Figure 4.3). The α-chains may be identical (homotrimer) or different (heterotrimer). Type III collagen is a homotrimer; it consists of three identical α-chains. Type I collagen is a heterotrimer with two α-chains alike and one different. These individual chains are unstable but when three chains are wrapped round each other they form the very stable right-handed triple helix characteristic of collagen. Short, non-helical regions called telopeptides are found at the carboxyl- and amino-terminal ends. In skeletal muscle tissue, collagen is produced primarly by fibroblasts and myositis. Collagen is synthetized intracellularly in the lumen of the endoplasmatic reticulum and extensively modified after translation, both intracellularly and extracellularly. In the extracellular space, the collagen molecules align themselves into microfibrils. The lateral displacement of each molecule relative to the other is about a quarter of its length. Microfibrils undergo lateral and end-to-end aggregation to form fibrils. This pattern of displacement and aggregation gives rise to the striated appearance of collagen fibrils (Bailey and Light, 1989; McCormick, 1999, 2009).

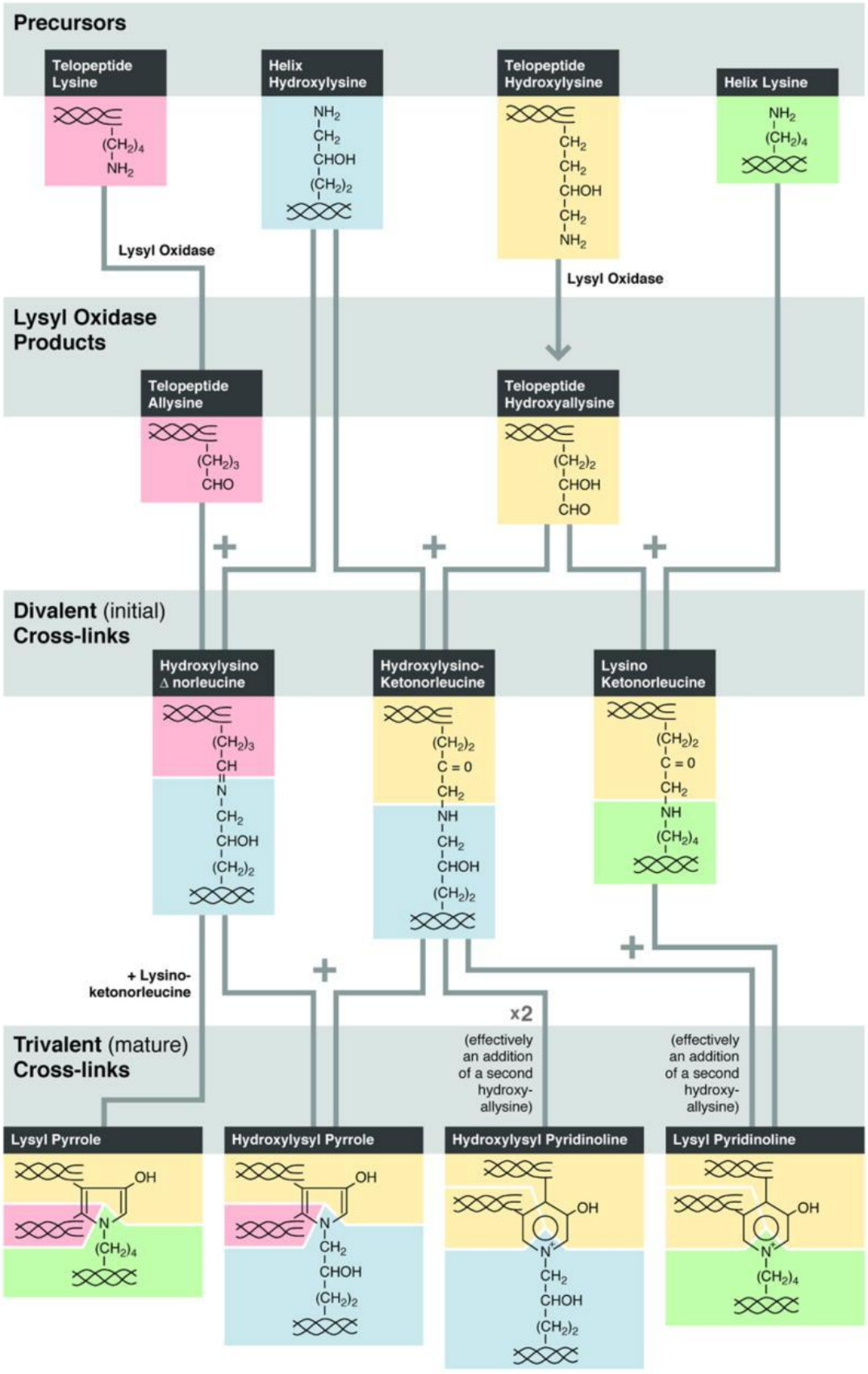
Figure 4.3. Collagen molecule and its organization



Source: http://www.nbs.csudh.edu/chemistry/faculty/nsturm/CHE450/06_Proteins-Structural.htm

The collagen fibers have a high tensile strength due to intra- and intermolecular cross-linkages. These chemical bridges are covalent in nature (Bailey and Light, 1989). Crosslinking is initiated by the oxidative deamination via the enzyme lysyl oxidase of specific lysine or hydroxylysine which produces peptidyl aldehydes, termed allysine or hydroxyallysine, respectively. The head-to-tail lateral alignment of collagen molecules in a quarter-stagger array allows the aldehyde functions to react with other peptidyl aldehydes or unmodified lysine or hydroxylysine residues on adjacent alpha chains. The initial condensation products form reducible crosslinks, so named because they contain Schiff base double bonds which can be reductively labelled. There are two major pathways by which crosslinks form: the first, the allysine pathway which is based in lysine aldehydes and produces aldimine crosslinks; the second, the hydroxyallysine pathway, produces crosslinks arising from hydroxylysine aldehydes. Amadori rearrangement of the initial aldimine crosslinks formed between lysine and hydroxylysine aldehydes can produce ketoamine derivatives. In muscle tissue crosslinking follows the hydroxyallysine pathway. The reducible crosslinks vary in their stability, with ketoamine crosslinks being heat stable and aldimine crosslinks heat labile. Crosslinking of collagen is a progressive process, and the reducible crosslinks undergo further condensation reactions and are replaced with mature non reducible crosslinks. The known mature crosslinks on the hydroxyallysine pathway are trivalent, hydroxylysylpyridinium (HP), and lysyl pyridinium residues, with the latter present in negligible amounts in most tissues except bone (Figure 4.4). Both HP and LP are heat stable and their occurrence in muscle is also highly correlated with the thermal stability of collagen (McCormick, 1999; 2009). The steady increase in mature collagen crosslinking is due to progressive and ongoing crosslinking reactions that occur within fibrillar collagen with the slowing of collagen synthesis rates as animals reach maturity. Less collagen synthesis and turnover provide existing fibrillar collagen time to progressively crosslink or mature (McCormick, 1994).

Figure 4.4. Precursors, divalent crosslinks, and mature trivalent crosslinks on the hydroxyallysine pathway (*source: Eyre and Wu, 2005*)



It has been established that collagen plays a key role in determining the background toughness of meat from different domestic animals including birds (reviewed in Maiorano et al., 2012). Lepetit (2007) analyzed various studies in which collagen crosslinks in muscle tissue were measured. He suggested that measurement of crosslinks (pyridinoline) is a reasonable predictor of tenderness. In addition, McCormick (1999) suggested that mature crosslinks and collagen concentration have an additive effect on the toughening of meat.

Very few study are available in literature about intramuscular collagen properties of Japanese quail meat. In different studies conducted by Maiorano et al. (2009, 2011) on different breeds and strains of quails slaughtered at 35 days of age, it was reported that the collagen content in quail pectoral muscle ranging from 17.25 to 18.90 μg/mg, and an average of collagen maturation ranging from 0.141 to 0.175 moles of HLP/moles of collagen (Maiorano et al., 2009). These values found by Maiorano et al. (2009) for collagen content and HLP crosslinks are lower and higher, respectively, to the values reported in pectoral muscle of 6 week old control broiler chicken (Ross 308) (Maiorano et al., 2012). It is known that collagen synthesis and maturation differ between species, muscles, animal age, growth rate and management practices (McCormick, 1994; Purslow, 2005).

4.2.1.3 Lipids (fat and fatty acid content of meat)

Animal adipose tissue is composed primarily of neutral lipids (triglycerides or triacylglycerols) and phospholipids that collectively range from 1.5 to 13 % in muscle tissue. Lipids also exist as sterols and sterol esters (cholesterol and cholesterol components) and cerebrosides. Various lipid forms serve as an energy source for the cell, as a structural and functional component of the cell wall, as insulation or protection for vital organs, and as solubilizing agents for certain hormones and vitamins (A, D, E, K) (Keeton and Eddy, 2004). Lipids contribute substantially to the caloric content of meat and also have marked effects on mouthfeel and flavour of meat, which are primary components of palatability. Additionally, the kinds of fatty acids present in meat and its cholesterol content influence the perceived healthfulness of meat. Several factors influence both the quantity and the quality of lipids in animal products. Endogenous factors such as age, or weight, gender, genotype and castration have mainly an influence on the quantity of lipids in these products. Among these factors, genetics is very

important. Selective breeding results not only in changes of total fat content, but also in changes in fat distribution between different depots, which allows to produce animals with lower subcutaneous fat without decreasing intramuscular fat which is very important for organoleptic meat quality (reviewed in Kouba and Mourot, 2011; Lotfi et al., 2011). The fat content and fat composition is also affected by animal feeding, a fact that is exploited for modification of the meat fatty acid composition, with the relatively best results in single-stomached pigs and poultry (Valsta et al., 2005). In relation to different tissues, the fatty acids composition of intramuscular fat can vary less than that of separable fat depots such as abdominal and subcutaneous fat. The minimum feeding time required to achieve substantial FA modification in thigh and breast meat is one or two weeks before slaughtering, respectively (reviewed in Cavani et al., 2009). All fats do not have the same metabolism, and therefore the extent to which the composition of meat and meat derivatives should be modified is closely linked to cholesterol levels, fat intake and the fatty acid profile (Jiménez-Colmenero et al., 2001).

The lipid content in edible lean meat today is less than 5 %, in particular poultry meat including quail meat is regarded as a low-fat meat (Table 4.4 and 4.5), fulfilling the consensus of many consumers becoming more health conscious.

Table 4.4. Lipid composition of whole carcass of quail and broiler chicken (value per 100g)

Item	Quail[1]	Chicken[2]
Total lipid (g)	12.05	15.06
Fatty acids, total saturated (g)	3.380	4.310
Fatty acids, total monounsaturated (g)	4.180	6.240
Fatty acids, total polyunsaturated (g)	2.980	3.230
Cholesterol (mg)	76	75

[1]Quail, meat and skin, raw;
[2]Chicken, broilers or fryers, meat and skin, raw.
Source: http://ndb.nal.usda.gov/ndb/search/list

Table 4.5. Lipid composition of breast muscle meat of quail and broiler chicken (value per 100g)

Item	Quail	Chicken
Total lipid (g)	2.99	2.62
Fatty acids, total saturated (g)	0.870	0.563
Fatty acids, total monounsaturated (g)	0.840	0.689
Fatty acids, total polyunsaturated (g)	0.770	0.424
Cholesterol (mg)	58	73

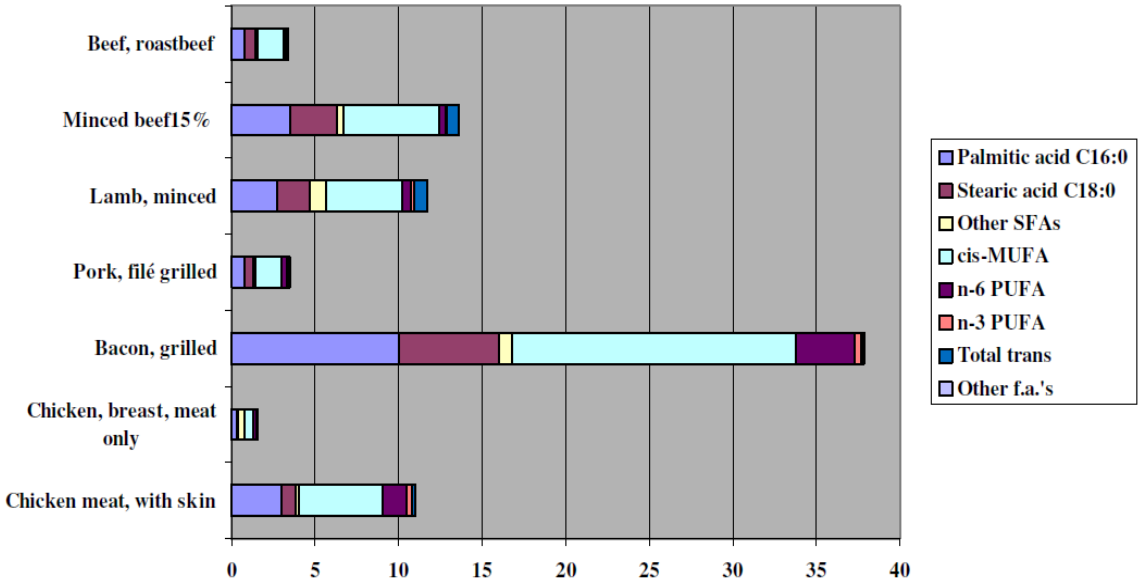
Source: http://ndb.nal.usda.gov/ndb/search/list

The difference in fat content between whole carcass and breast meat in both chicken and quail provide evidence that the skin is an important fat depot influencing the fat content and composition of the carcass. Poultry skin is rich of polyunsaturated fatty acids (PUFA) and it could be considered as subject of potential risk during long storage, even frozen. PUFA of the phospholipid fraction of cell membranes are highly susceptible to oxidation. Thus, the lower phospholipid content in carcasses without skin allows to assume that this type of carcass processing, although not traditional in poultry industry, has advantages in regard to grill quality and its shelf life. Poultry carcasses without skin could be more appealing for consumers from the point of view of dietetics and for a longer shelf life (Genchev et al., 2008a).

Apart from the fat content in meat, the composition of fat is of great interest for poultry industry and for consumers. In fact, interest in meat fatty acid composition stems mainly from the need to find ways to produce healthier meat with higher ratio of polyunsaturated to saturated fatty acids and a more favorable balance between n-6 and n-3 PUFA. In general, meat fat comprises mostly monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). The most ubiquitous fatty acids are oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids. The main fatty acids behind the cholesterol elevating effect are myristic (C14:0) and palmitic acids; in particular myristic acid is the most atherogenic with about four time the cholesterol-raising potential of palmitic acid (Ulbricht and Southgate, 1991; Valsta et al., 2005). Stearic acid is partially converted to oleic acid in vivo and has not been shown to elevate blood cholesterol. Poultry and pork contain somewhat more unsaturated fatty acids (10 – 15 of total fatty acids) than beef and lamb, and also a notable amount of polyunsaturated fatty acids (PUFAs). Linoleic acid (C18:2) is the predominant PUFA (0.5 – 7 %), followed by α-linolenic acid (C18:3) (up to 0.5 %). Trans-fatty acids comprise about 1–2 % of total fatty acids across all types of meat; in ruminant meats they represent 2–4 %. Conjugated linoleic acid (CLA), a group of polyunsaturated fatty acids that appear in dairy products and are thought to have beneficial effects on health, are also found at low levels in meats, especially in beef and lamb (reviewed in Valsta et al., 2005; Figure 4.5). Muscle contains significant proportions of long chain (C:20-22) PUFAs which are formed from 18:2 n-6 and 18:3 n-3 by the action of Δ_5 and Δ_6 desaturase and elongase enzymes. Important products are arachidonic acid (20:4 n-6) and eicosapentaenoic acid (EPA,

20:5 n-3) which have various metabolic roles including eicosanoid production (Wood et al., 2008).

Figure 4.5. Common fatty acid composition of selected meats (g fatty acids/100 g foods) (*source: Valsta et al., 2005*)



The fatty acid profile of quail meat it is quite similar to that of broiler chicken (Table 4.6), even if it is characterized by higher amount of oleic acid (C18:1) and linoleic acid (C18:2 n-6) and lower amount of arachidonic acid (C20:4 n-6). Similar amount of α-linolenic acid (C18:3 n-3) are reported for chicken and quail meat.

During the last decades, there was an increasing recognition of the health benefits of polyunsaturated fatty acids (PUFA) in general, and of n-3 fatty acids in particular, because these fatty acids are essential for humans The importance of n-3/n-6 ratio is recognized since both n-3 and n-6 fatty acids are precursors of eicosanoids, biological effectors such as prostaglandins, leucotriens, and thromboxanes regulating mainly the cardiovascular system and immunological processes. Eicosanoids built from n-6 fatty acids contribute to cardiovascular diseases and inflammation processes, whereas eicosanoids built from n-3 fatty acids act as opponents (Grashorn, 2007). Furthermore, there is also competition between PUFA n-3 and PUFA n-6 for the desaturation and elongation enzymes (Högberg et al., 2003).

Table 4.6. Fatty acid composition (% of total FA) of chicken and quail meat

Fatty acids	Chicken[1] *(Rule et al., 2002)*	Quail[2] *(Botsoglou et al., 2004)*	Quail[3] *(Genchev et al., 2008)*	Young quail[4] *(Boni et al., 2010)*	Spent quail[4] *(Boni et al., 2010)*
C 10:0	--	2.32	--	--	--
C 12:0	--	2.40	--		--
C 14:0	0.48	1.44	0.95	0.71	0.76
C 14:1	--	--	--	--	--
C 15:0	2.48	--	--	--	--
C 16:0	21.8	22.08	24.39	17.66	21.07
C 16:1	5.30	3.32	5.32	4.88	5.06
C 17:0	0.04	--	--	--	--
C 17:1	0.74	--	--	--	--
C 18:0	8.83	12.28	8.79	7.46	7.23
C 18:1	28.1	24.76	35.38	34.62	33.42
C 18:2	17.0	19.52	19.70	27.98	24.23
C 18:3	1,75	1.21	1,75	0.30	0.27
C20:0	--	1.06	--	--	--
C 20:1	--	0.54	--	0.30	0.09
C 20:2	0.36	--	--	--	--
C 20:3	0.16	0.24	--	--	--
C 20:4	4.69	3.08	2.69	2.78	1.61
C 20:5	0.18	0.44	--	0	0.86
C 22:0	0.56	--	--	--	--
C 22:1	1.19	--	--	--	--
C 22:2	--	--	--	--	--
C 22:4	1.05	0.50	0.84	--	--
C 22:5	0.31	0.03	--	--	--
C 22:6	0.26	1.24	--	--	--
C 24:0	0.15	--	--	--	--
SFA	34.7	41.58	34.13	25.84	29.07
PUFA	24.6	26.26	24.98	41.90	42.76
P/S	0.71	0.63	0.73	--	--
n-6/n-3	18.5	--	15.30	--	--

[1]Chicken breast (skin-off) of commercially produced animals purchased from a grocery store; [2]breast meat from 21 d old Japanese quail; [3]breast meat from 35 d old Japanese quail; [4]bulk samples of mechanically deboned meat from 8 week and 8 months old Japanese quail.

Both chicken and quail meats are characterized by higher n-6/n-3 ratio this is due to the higher n-6 fatty acids amount compared to other species (Rule et al, 2002; Valsta et al., 2005) (Figure 4.5). However, it is reported that birds are able to deposit increasing amounts of α-linolenic acid when their ration is rich in this n-3 PUFA. Alga extracts, linseed oil, hemp oil or rapeseed oil are suitable sources for n-3 PUFA enrichment (Grashorn, 2007). It was reported that supplementation of flaxseed to broilers diet for 14 days prior to slaughter increases the α-linoleic acid by 68.4 %, whereas the supplementation for 27 days increase the α-linoleic content more than twice, reducing the n-6/n-3 ratio to 7.63 (reviewed in Genchev et al., 2008a). Also the ratio between PUFA and SFA (P/S) have great nutritional implications and it is taken as a measure of the propensity of the diet to influence the incidence of coronary heart disease; the recommended ratio should be in the range of 0.4 - 0.7 (Wood et al., 2003). From this point of view poultry meat including quail meat is characterized by a good P/S ratio (> 0.60) due to the higher incidence of PUFA fractions. Compared with muscle of the other species, poultry breast muscle have higher C18:2 and C20:4 and lower C18:0, which are largely responsible for the higher P/S ratios observed for other species (Rule et al., 2002).

In addition to fatty acids, cholesterol is a nutritionally important component of meats and it is an essential constituent of the animal cells. Cholesterol in meat exists in two forms: as free cholesterol and as cholesterol ester. Free cholesterol is associated primarily with cellular and subcellular membranes of muscle and intramuscular adipocytes. Because intramuscular adipocytes are essentially lipid-filled spheres with very little membrane content, the amount of cholesterol associated with membranes is small (approximately 25 %). Cholesterol ester, located within the triacylglycerol-rich central lipid vacuole, comprises about 75 % of the total cholesterol in adipose tissue. Muscle fibers, which are rich in membranes but contain comparatively little lipid, have approximately 75 % of their total cholesterol associated with membranes and the other 25 % associated with their neutral lipids (Smith et al., 2004).

The amount of cholesterol in meat and meat products depends on numerous factors and varies between about 38 and 123 mg/100 g of edible portion, except in the case of some edible offal (heart, kidney, brains, etc.) where the concentrations are much higher (Chizzolini et al., 1999). Poultry meat is characterized by a low cholesterol content (broiler *Pectoralis* muscle, 47.41 mg/100 g muscle; Chizzolini et al., 1999) and thus is

considered to be healthier than other meat products, especially the red meat of mammalian origin, i.e. beef 66 mg/100 g, pork 65 mg/100 and lamb 50 mg/100 (Chizzolini et al., 1999). However, other studies on broiler chicken reported higher cholesterol value; for example, Salma et al. (2007) reported an average cholesterol content of 93.6 mg/100g of meat in *Pectoralis major* of 56 days old male Chunky broilers; while, Maiorano et al. (2012) reported values of cholesterol content ranging from 70.45 to 78.12 mg/100g in 42 days old broiler chickens. The observed differences in cholesterol content could be due to the use of different methodologies for cholesterol quantification or for sampling (Bragagnolo and Rodriguez-Amaya, 2002), but also cholesterol content in chicken meat can be altered by varying the composition of diet, age, and gender (Wang et al., 2005).

Japanese quail meat is characterized by a lower cholesterol content compared to broiler chicken. Maiorano et al. (2011) reported a cholesterol level of Pectoral muscle in quail ranging from 23.57 to 37.20 mg/100 g, lower than the cholesterol content found by Maiorano et al. (2009) in breast muscle of 35 days old Japanese quail (ranging from 27.83 to 43.38 mg/100 g). The cholesterol content obtained in the above mentioned study is similar to results from breast pigeon muscle (ranging from 23.6 to 44.4 mg/100 g; Pomianowski et al., 2009) or ostrich meat (ranging from 63.0 to 68.4 mg/100 g; Horbanczuk et al., 2004). Thus, from this point of view it seems that quail meat well fit the current consumers demand for a low-fat meat with high unsaturation degree of fatty acids and low cholesterol levels, taking into account also the nutritional recommendations of various international institutions (e.g., World Health Organization) which include limitations that refer not only to the amount of fat and the fatty acid composition but also the cholesterol levels in foods, of which meat and meat products constitute a major part (WHO, 2003).

4.2.1.4 Vitamin and mineral content of meat

Apart from proteins, meat products are an excellent source of several vitamins and minerals. Red meat provides around 25 % of the recommended dietary intakes for riboflavin, niacin, vitamin B6 and pantothenic acid per 100 g and practically two thirds of the daily requirement (DR) of vitamin B12 in the same serving. In poultry, chicken breast is a particularly good source of niacin (100 g supplies 56 % of DR) and vitamin B6 (27 % of DR) while 100 g of turkey breast supply 31 % of niacin DR and 29 % of

vitamin B6 DR. Both supply between 6 to 8 % of DR (reviewed in Pereira and Vincente, 2013). Vitamins are essential to maintaining optimal physiological conditions of the body because they act as biocatalysts (coenzymes) in the metabolic processes of nutrients and energy processes. They are also called "protective foods" because without them the cells fail to build the protoplasm and release energy. Vitamins can be divided into water-soluble and fat-soluble (Mariani et al., 1999). Meat is an excellent source of complex B vitamins, especially B12, the most complex and largest vitamin. Animal food are considered the major dietary source of vitamin B12 (Watanabe, 2007)

Japanese quail meat, as well as chicken meat, is an excellent source of pyridoxamine (vitamin B6), niacin (vitamin B3), but also of pantothenic acid, riboflavin and thiamine and have higher vitamin C and vitamin A content compared to chicken meat, with positive feedback from the nutritional point of view (Table 4.7).

Table 4.7. Vitamin content for quail and broiler chicken meat

Item	**Quail**[1]	**Chicken**[2]
Vitamin C, total ascorbic acid (mg)	6.1	1.6
Thiamin (mg)	0.244	0.060
Riboflavin (mg)	0.260	0.120
Niacin (mg)	7.538	6.801
Vitamin B-6 (mg)	0.600	0.350
Folate, DFE (µg)	8	6
Vitamin B-12 (µg)	0.43	0.31
Vitamin A, RAE (µg)	73	41
Vitamin A, IU (IU)	243	140
Vitamin E (alpha – tocopherol) (mg)	-	0.30
Vitamin D (D2 + D3) (µg)	-	0.2
Vitamin D (IU)	-	10
Vitamin K (phylloquinone)	-	1.5

[1]Quail, meat and skin, raw;
[2]Chicken, broilers or fryers, meat and skin, raw.
Source: http://ndb.nal.usda.gov/ndb/search/list

Meat is also one of the best sources for zinc, selenium, phosphorus and iron (Pereira and Vincente, 2013). These elements are important for health since they are essential for the activity of certain enzymes that are necessary for the normal function of the human body. For example, antioxidant enzymes such as superoxide dismutase (SOD) and glutatione peroxidase (GSH-Px) need mineral cofactors to be functional and protect cells against reactive oxygen species. The activity of these antioxidant enzymes is also important in meat quality since they impact the shelf-life of foods such as meat products

(reviewed in Bou et al., 2009). Meat is a good carrier of several trace elements, providing these elements mostly in organic and well-absorbable form; for example iron has a crucial role in human health, it can be found in a broad variety of foods however it is present in two different forms: heme-iron and non-heme iron. Heme-iron comes from hemoglobin and myoglobin thus it is only present in animal foods. It is highly bioavailable and easily absorbed in the intestinal lumen because it is absorbed as an intact molecule by enterocytes (reviewed in Pereira and Vincente, 2013). Beef has the highest content of heme-iron, loin can have 45 % to 77.58 %, while the average reported value was 58.10 %. Iron and heme-iron contents are lower in lighter meats such as chicken. Pork has intermediate values between beef and chicken. In loin cuts heme-iron can range from 38 % to 60 % while tenderloin has only 23 %. Meat and meat products can contribute up to 18 % of iron daily requirements which makes it important in a healthy balanced diet and crucial in preventing one of the most common nutritional deficiencies (reviewed in Pereira and Vincente, 2013).

Quail meat reveal a good mineral profile being a significant source of of phosphorus, potassium and iron that are higher compared with chicken meat (Table 4.8). Thus, in relation to chemical and nutritional aspects, quail meat reveal interesting properties, which might aid in its marketing.

Table 4.8. Mineral composition (mg) for quail and broiler chicken meat

Item	**Quail[1]**	**Chicken[2]**
Calcium, Ca	13	11
Iron, Fe	3.97	0.90
Magnesium, Mg	23	20
Phosphorus, P	275	147
Potassium, K	216	189
Sodium, Na	53	70
Zinc, Zn	2.42	1.31

[1]Quail, meat and skin, raw;
[2]Chicken, broilers or fryers, meat and skin, raw.
Source: http://ndb.nal.usda.gov/ndb/search/list

4.2.2 *Technological properties of quail meat*

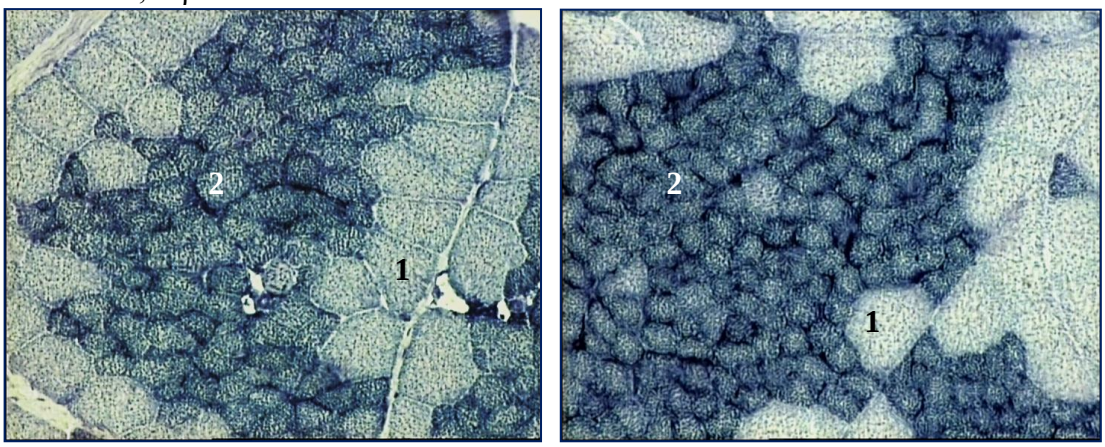
4.2.2.1 pH

It is well known that the ultimate pH of the muscle is an important contributing factor to meat quality expressed as tenderness, colour, and storage life (Van Laack et al., 2000). Measurement of muscle pH at different times *post mortem* provides information about forthcoming quality characteristics. Differences in pH could be due to the variation in muscle glycogen content (Berri et al., 2005). However, ultimate pH value is also dependent on the *ante mortem* stress, type of breed and the genetic variation within breeds (Terlouw, 2005).

Meat pH changes dramatically during the first hours after slaughter. This is the period of most enhanced glycolysis and lactate accumulation into muscle tissue. The rate of these processes and the lowering of muscle pH in the early post slaughter period are essential for meat quality. Glycolysis occurs very fast in poultry muscles, some authors reported that in broiler chickens, breast muscle glycolysis was complete until 45 min *post mortem*, and afterwards average pH remained almost unchanged. One investigation on physicochemical properties of goose meat reported that during the first 45 min after slaughter, pH decreased by 1.1 and 7.1 %, whereas over 24 hours, the decrease was by 9.7 and 19.3 %. A similar dynamics of pH reduction was exhibited by Japanese quail meat with a pH reduction during the first 24 hours of 7.5-11 % (reviewed in Ribarski and Genchev, 2013). Singh and Verma (1995) found that the pH in quail breast muscle declined rapidly within first 2 h after slaughter and leveled off after 4 h of aging as 5.8 to 5.9. However, contradictory data regarding quail meat pH exist. Narinc et al. (2013) reported an average pH value of 5.94 for 35 days old Japanese quail, whereas other authors reported higher values of pHu in quail breast meat (6.00, 6.17 and 6.38, respectively Genchev et al., 2010, Genchev et al., 2008a and Karakaya et al., 2005). In general, broiler chicken meats with pHu between 5.7 and 6.1 are called normal and do not reveal any quality problems (Barbut, 1997; Zhang et al., 2005). Genchev et al. (2008a) suggest that the main reason behind the high pH values found in quail breast muscle were due to the morphology of muscle. In fact, unlike other avian species (chicken and turkeys), where the pectoral muscles is entirely formed of glycolytic type fibers (white), in Japanese quails, the oxidative type fibers (red) prevail (Rosser et al., 1986; Riegel et al., 2003; Walasik et al.,2006) (Figure 4.6). The muscles

of the glycolytic type are characterized by high glycogen and low creatine phosphate content. This supposes a more rapid depletion of glycogen stores of the muscle without possibility for re-synthesis of ATP. On the contrary, the muscles of oxidative type possess a big reserve of creatine phosphate that supplies the energy for ATP re-synthesis, and the higher content of mitochondria provides oxygen for the slower aerobic glycolysis. As a result, these muscles are characterized by a slower development of *rigor mortis*, and therefore, higher pH values.

Figure 4.6. Cross section of the pectoral muscle in (a) meat type quails and (b) in egg type quails. Mag 12,5x10 (*source: Walasik et al.*,2006)
1-αW fibers; 2-βR fibers



4.2.2.2 Water holding capacity (WHC)

Water holding capacity (WHC) is known to be one of the major quality characteristics of fresh meat, as it affects some major characteristics of the cooked meat such as potential drip loss, technological quality, appearance and sensory properties. It can be said that appearance and technological characteristics are connected. WHC is obvious to the consumer when examining the packaging in the retail stores. Consumers discriminate against packages of fresh meat showing free fluid surrounding the products (poor WHC) and also against further-processed products showing exudates in the package. The juiciness of the meat after cooking is also affected by the WHC. Poor WHC meat may be dry or taste may be negatively affected. (Warris, 2000; Karakaya et al., 2005).

The WHC is the ability of meat to hold onto its own or added water when force (heat, pressure) is applied. The majority of water holding capacity resides in the water located in the intermolecular spaces between the salt-soluble proteins (actin, myosin) of

muscle tissue where it is held in place by capillary force. The interfilamental space is maintained by electrostatic forces that operate over relatively long distances. Factors that alter the spatial arrangement of the myofilaments can alter the amount of water immobilized in this compartment. Such factors, both intrinsic and extrinsic, include alterations in net charge induced by pH changes, screening of charges by anions or cations, the presence of divalent cations (Mg^{2+}, Ca^{2+}), denaturing conditions that alter protein conformation (rapid pH decline while temperature is still high), and the presence and condition of plasticizing agents such as adenosine triphosphate (ATP) as well as enzymes (ATPase) and cofactors necessary for plasticizer function in prevention of myofibrillar protein crosslinking (Brewer, 2004). In particular, the influence of pH on WHC has been demonstrated by Warriss et al. (2000), reduction of pH alters the spatial structure of myofibrillar proteins, which results in lower WHC of meat. The extend of myosin shrinkage depends on the rate of glycolysis and ultimate pH values, as well as on the rate of *post mortem* temperature decrease (Ribarski and Genchev, 2013).

As mentioned before, quail breast meat is characterized by high pH values which is accompanied by high WHC values. Genchev et al. (2010) reported that WHC of meat from Manchurian Golden Japanese quail slaughtered at different age varied within 18.05 and 21.7 %. Similar WHC values (an average of 15.5 %) were reported by Genchev et al. (2008a) in 35 days old Japanese quails. The good water holding capacity of breast and leg meat guarantees excellent technological properties. Furthermore, these WHC values combined with the higher carcass yield and the easier way of deboning of the muscle, make breast meat very attractive as a source for the production of gourmet products (Genchev et al., 2008a, 2010).

4.2.3 *Sensorial properties of quail meat*

Meat palatability is important because it relates to the eating quality and consumer acceptance of meat. Meat palatability relates to how meat tastes and is defined in terms of juiciness, tenderness and flavour. These three attributes have been related to consumers' perception of overall acceptability and preference. Juiciness is the amount of perceived juices in the meat during chewing or mastication. Tenderness is how easily meat breaks down during chewing. Flavour is a combination of smell, the aromatics perceived during consumption of the meat from the olfactory senses. Flavour also consists of aftertastes perceived after consuming the product. While these three

palatability components have been shown to individually impact consumer preferences, they also are interrelated; changing one component may affect another component. There are many factors, both *ante-mortem* and *post-mortem*, that affect meat palatability. These factors influence meat palatability by affecting the underlying chemical and physical components within the meat. The three major components of meat (fat, lean and connective tissue) exert a profound effect on meat palatability with different ways of action (Miller, 2004).

4.2.3.1 Color

It is generally recognized that the two most important attributes for poultry meat are appearance and texture. Poultry is unique because it is sold with and without its skin and the appearance (color) of both skin and meat is a critical food quality attribute; it is important for both the consumer's initial selection of a raw meat product in the marketplace and for the consumer's final evaluation and ultimate acceptance of the cooked product upon consumption (Fletcher, 1999; Fletcher et al., 2000). Because of the great importance of meat color to final product quality, factors affecting poultry meat color such as bird sex, age, strain (exercise), diet, intramuscular fat, meat moisture content, pre-slaughter conditions, method of processing, exposure to chemicals, have been extensively examined (Fletcher, 1999; Fletcher et al., 2000; Petracci et al., 2004). In the last decade, a number of researchers have suggested the possibility of using color measurements to predict functional properties of poultry meat. Specifically, pale, soft, and exudative-like conditions and water-holding capacity are the most common functional properties mentioned Relationships were also found between color and shelf life and between color and composition of broiler breast meat. Some researchers have also indicated lightness values to be useful as an indicator of poultry breast meat quality for further processing (reviewed in Bianchi and Fletcher, 2002).

Color of meat depends primarily on:

- ✓ the presence and the quantity of pigments in muscle and their chemical status;
- ✓ the type of muscle fibers and their spatial relationships, which determine the scattering grade of light and thus its deepness of penetration;
- ✓ the intramuscular fat and surface dehydratation which confer different degrees of glossiness and thus affect light scattering and reflection.

The pigment most responsible for the color of meat is myoglobin, although there are also hemoglobin, present only in small amount in well-slaughtered animals, and cytochrome C which plays a minor role because of its low concentration. The chemical forms of myglobin are primarly responsible for meat color: purple-red deoxymyoglobin in fresh meat in the absence of air; bright red oxymyoglobin formed in the presence of oxygen; brown metmyoglobin, the result of myoglobin oxidation. The pigments myoglobin, oxymyoglobin and metmyoglobin can be changed from one to the other, depending on the store conditions of meat. This reaction is reversible and dependent on the availability of oxygen, active enzymes and reducing compounds in the muscle (Mancini and Hunt, 2005).

According to the studies carried out on broiler chicken meat quality, ideal values of lightness (L*) should be between 46 and 53 (Barbut, 1997; Zhang and Barbut, 2005), and meats with an L* value below 46 are called to be dark, firm, dry, which means they have a dark color, high water holding capacity, and short shelf life. Previous literature found redness (a*) values for broiler chicken breast meat ranging between − 0.96 and 4.50, and yellowness (b*) values were in the range of 6.7 to 13.5 (reviewed in Narinc et al., 2013).

In a recent study conducted on 35 d old Japanese quails, Narinc et al. (2013) reported that the average L*, a*, and b* values of quail breast meat were 43.09, 19.24, and 7.74, respectively. Oğuz et al. (2004) and Gevrekci et al. (2009) reported the average L*, a*, and b* values of breast meat were 54.92, 9.70, and 5.59, and 54.87, 9.68, and 3.23, respectively. Lower values of L* were found by Riegel et al. (2003) in 24 weeks old Japanese quails who reported a continuous reduction of L* values until 38.9 – 40 and values of a* and b* of 10.9 – 12.4 and 2.0 – 2.5, respectively. Similar values were reported by Genchev et al. (2010) in 35 days old Manchurian Golden Japanese quails; they found that the color of breast meat became darker (lower L* values) with advancing slaughter age. Genchev et al. (2010) suggested that the main reasons behind the lower L* values of quail breast meat compared to those reported in literature for broiler chicken (Qiao et al., 2001; Fletcher, 1999) could be related to the morphological profile of the pectoral muscle in quails which show a higher incidence of red fibers. In addition, the red fibers, rich in myosin, give a more saturated color in both the red and yellow zone of the spectrum (Genchev et al., 2010).

4.2.3.2 Meat tenderness

As mentioned before, the appearance and tenderness are two extremely important traits in poultry meat quality (Fletcher, 2002). In particular, meat tenderness is the single most important sensory property affecting final quality assessment (Fletcher, 2002). Tenderness is affected by several factors, such as breed, sex, age, fiber resistance, sarcomere length, pH, and collagen morphology (Lepetit, 2007; Maiorano et al., 2009, 2012). It is not surprising that coming to definitive conclusions about the causes of toughening in meat is very difficult. Differences in meat tenderness have been mainly attributed to myofibrillar and connective tissue proteins, and intramuscular fat.

Post-mortem myofibrillar protein degradation thought to play a crucial role in meat tenderness. Ageing can be used to decrease shear force values during post-mortem storage as a result of the proteolysis of myofibrillar proteins, which is mediated in part by calpain system which is activated by increasing levels of calcium. This tenderization through ageing involves several aspects, among them pH; meat with high ultimate pH is associated with a higher rate of tenderization or with a better ultimate tenderness. Although the tenderness is associated with the rate of glycolysis as well as the decrease of temperature post-mortem and the ultimate pH of the muscle, a careful review of the literature reveals numerous, often conflicting, reports regarding the relationships between pH and tenderness. Therefore, it appears that the relationship between pH and meat tenderness is very complex. Some authors indicate a linear dependence between tenderness and ultimate pH, whereas others found a curvilinear dependence with minimum tenderness between 5.8 and 6.2 pH values due to a differential proteolytic activity that was responsible for the toughness of meat (reviewed in Silva et al., 1999).

Following several decades of works in meat tenderness, a lot of information is now available on the different characteristics of connective tissues, which concern quantitative, chemical and structural aspects (Lepetit, 2008). In particular, the role that intramuscular connective tissue (IMCT) plays in meat tenderness was recently reviewed by Purslow (2005), with emphasis placed on structural and morphological aspects of connective tissue and their relationship to meat tenderness. The morphology, composition and amount of the intramuscular connective tissue vary greatly between muscles, species, breeds and with animal age. Endomysium, perimysium, and epimysium make up a network of collagen and elastin fibers embedded in a matrix of proteoglycan. Perimysium represents about 90 % of total connective tissues in muscles

(McCormick, 1999) and its amount varies much more from one muscle to another than the amount of endomysium. Collagen is an abundant connective tissue protein and is a contributing factor to variation in meat tenderness and texture. Perimysium contains the main proportion of collagen, whereas the endomysium contains lesser of it. The thickness of perimysium was shown as an indicator of beef tenderness (reviewed in An et al., 2010). Liu et al. (1996) found that the raw meat shear force value (in kg/cm^2) increased linearly with increasing thickness of perimysium in chicken muscles and was highly correlated with the collagen content in muscle. These were supported by the results of Lachowicz et al. (2004) who found a positive correlation between width of perimysial and endomysial space and instrumental hardness of muscles from wild boars and domestic pigs. They also reported positive correlations between instrumental hardness of muscles and muscle fiber cross sectional area (reviewed in Voutila, 2009). Most studies have shown that the smaller the fiber size, more tender is the meat (Lepetit, 2008). These suggested that that besides myofiber size, muscle membrane was also an important factor for meat tenderness formation in birds. In conclusion, the perimysium and endomysium thickness, and fiber diameter are involved in determining meat tenderness of the chicken (An et al., 2010).

In addition to the structural aspects of intramuscular connective tissue, also the quality of intramuscular collagen (IMC) decisively influence the tenderness of meat of different domestic animals including birds (Maiorano et al., 2011, 2012). Collagen and its hydroxypyridinoline crosslinks (the main intramuscular collagen mature crosslink) contribution to meat toughness was recently reviewed by McCormick (2009), reporting that collagen (content and cross-linking) affect the background toughness of meat. As previously reported, McCormick (1999) suggested that mature crosslinks and collagen concentration have an additive effect on the toughening of meat. In other words, the role of collagen on meat tenderness depends not only on the crosslinks but also on the amount of collagen. Maiorano et al. (2011) give a tenderness index, which is the amount in HLP crosslinks per gram of lyophilized muscular tissue in different muscles in goat meat. It is well known that the proportion of mature to reducible crosslinks increases with age, resulting in older animals that often have less tender meat than younger animals. However, the expression of connective tissue within muscles is greatly variable, depending on developmental stage, muscle position/function, animal breed, nutrition, exercise and injury (Purslow, 2005).

In recent years was reported a new emerging quality issue in poultry that is the poor cohesiveness of meat due to immaturity of intramuscular collagen tissue, in relation to the very early slaughter age of modern chicken and turkey strains (Petracci and Cavani, 2012). Today, in the post-genomic era there are more possibilities to further investigate these issues, the manipulation of the state of IMCT maturity may be a possible means of reducing unwanted variability in meat tenderness (Purslow, 2005).

4.2.3.3 Juiciness and flavor

Juiciness of the meat is mainly related to the WHC and fat content of meat. In general, as fat content increases, palatability increases, but the rate of improvement in palatability with each incremental increase in fat is not constant. Meat with higher fat content will have longer sustained perception of juiciness. Fat also affects juiciness by lubrificating the muscle fibers during cooking and by increasing the tenderness of meat.

Flavour and odour are closely related. Generally, flavour is linked to water-soluble materials, and odour is related to fat-soluble volatile elements. If the meat smells unpleasant, it is mostly related to the quality of the meat. It can be an indicator of the spoilage. But it is not always the case (Warris, 2000). Flavor is an important quality attribute which relates to the organoleptic characteristics of meat; it is another quality attribute that consumers use to determine the acceptability of poultry meat. Although perception of flavor is a complex phenomenon, odor is the most important single factor contributing to the overall characteristics of flavor. A large number of compounds have been identified in the volatile fraction of red meats and poultry. An overview of the chemical constituents present in the volatiles of beef, pork, mutton and chicken is presented according to species and arranged by chemical class (hydrocarbons, alcohols, acids, aldehydes, ketones, sulfides, hetorocyclic compounds) (reviewed in Maiorano and Bednarczyk, 2013).

PART 2.

AIM OF DISSERTATION

In the recent years, Japanese quail (*Coturnix japonica*) meat has attained economic importance as an agricultural species producing eggs and meat that are enjoyed for their unique flavour. However, far too little attention has been paid to the determination of meat quality traits and the genetic parameters of Japanese quail from different breeds, lines and generations, and also very scarce are the study conducted on the evaluation of the carcass and meat quality traits of adult or spent quails at the end of their productive life.

Therefore, this research was conducted 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 gender (males and females) on growth performance, carcass traits and meat quality characteristics (pH, IMC properties, muscle cholesterol content and fatty acid composition) of Japanese quail. This results giving also the basis for the study of QTLs to identify the linkage between the genotypic and phenotypic data.

PART 3

Chapter 5

MATERIAL AND METHODS

This research work was a part of a larger research project conducted by the University of "Life Sciences" in Lublin (Poland) and the University of "Technology and Life Science" in Bydgoszcz (Poland), in collaboration with our Department of Agricultural, Environmental and Food Science in Campobasso (University of Molise, Italy). This research was partially financially supported by the Polish Ministry of Scientific Research and Information Technology (Warsaw), grant number N N311633638.

5.1 Experimental population

The experiment was performed with two Japanese quail (*Coturnix japonica*) population (meat type and egg type) reared at the Didactic Experimental Station of the University of Life Sciences in Lublin (Poland). 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 has been created by mating one F_1 male with one F_1 female, full siblings. The constitution of the three generations population of Japanese quail is subordinated to the main aim of the research project which consisted in the genetic mapping of QTLs in Japanese quails. Successful QTL identification using molecular markers depends on the availability of suitable markers and the use of a resource population with sufficient genetic variation to detect linkage between a segregating QTL and a genetic marker. To ensure sufficient genetic variation, two genetically distant parental lines for the trait(s) of interest must be crossed and F2 or backcross populations produced (Deeb and Lamont, 2002). 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 two flanking

molecular markers. In this method, it is assumed the existence of the reference population derived by crossing two lines 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.

For the experiment, the birds randomly chosen from:

- F_0 (22 males and 22 females);
- F_1 (22 males and 22 females);
- F_2 (84 males and 152 females), obtained in two consecutive hatches;

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) (Figure 5.1).

Figure 5.1. One day old quail chicks (a), adult quails in collective cages (b)

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. Afterward it was maintained at 18 to 20°C. Quail were fed ad libitum commercial diets according to age (Table 5.1). Birds had free access to water during the experiment.

Table 5.1. Composition and nutritional value of diets

	Period		
Item (% unless noted)	1 to 7 d	8 to 28 d	29 d to 20wk
Component			
Maize	37.0	54.2	61.6
Soybean meal	41.5	31.9	16.8
Rapeseed meal	—[1]	—	8.0
Fish meal	10.0	8.0	5.0
Milk powder, skimmed	3.0	2.0	2.0
Alfalfa dehydrated	—	—	4.0
Rapeseed oil	6.2	1.8	0.3
Dicalcium phosphate	0.5	0.4	0.8
L-lysine	—	—	0.2
DL-methionine	0.5	0.4	—
NaCl	0.3	0.3	0.3
Premix IB-1[2]	0.5	0.5	—
Premix IB-2[3]	0.5	0.5	—
Premix IB-3[4]	—	—	1.0
Calculated nutritional value			
ME[5] (kcal/kg)	3,001.7	2,898.3	2,797.9
CP	2.0	24.0	20.0
Crude fiber	3.84	3.70	4.76

[1]Dash indicates not detectable.

[2]Provided the following per kilogram: vitamin A, 650,000 IU; vitamin D3, 200 mg; vitamin E, 3,000 mg; vitamin B1, 250 mg; vitamin B2, 600 mg; biotin, 20,000 μg; vitamin B6, 350 mg; vitamin B12, 2,500 μg; vitamin K, 175 mg; Ca pantothenate, 1,200 mg; niacin, 5,000 mg; folic acid, 150 mg; choline, 35,000 mg; lysine, 8,5%; methionine, 12%; threonine, 1%; Ca, 19.0%; P available, 4.0%; Na, 7.2%; Mn, 7,000 mg; Zn, 6,000 mg; Co, 35 mg; Se, 25 mg; Cu, 1,200 mg; Fe, 3,500 mg; I, 75 mg; Mg, 3,500 mg.

[3]Provided the following per kilogram: vitamin A, 620,000 IU; vitamin D3, 170 mg; vitamin E, 2,200 mg; vitamin B1, 150 mg; vitamin B2, 550 mg; biotin, 17,000 μg; vitamin B6, 300 mg; vitamin B12, 1,800 μg; vitamin K, 150 mg; Ca pantothenate, 950 mg; niacin, 4,500 mg; folic acid, 120 mg; choline, 32,000 mg; lysine, 11,5%; methionine, 11%; threonine, 1%; Ca, 17.8%; P available, 4.0%; Na, 7.2%; Mn, 6,000 mg; Zn, 5,500 mg; Co, 25 mg; Se, 25 mg; Cu, 1,200 mg; Fe, 3,000 mg; I, 60 mg; Mg, 3,500 mg.

[4]Provided the following per kilogram: vitamin A, 600,000 IU; vitamin D3, 150 mg; vitamin E, 2,000 mg; vitamin B1, 120 mg; vitamin B2, 500 mg; biotin, 15,000 μg; vitamin B6, 200 mg; vitamin B12, 1,500 μg; vitamin K, 120 mg; Ca pantothenate, 850 mg; niacin, 3,500 mg; folic acid, 100 mg; choline, 30,000 mg; lysine, 11.0%; methionine, 11%; threonine, 1%; Ca, 25.5%; P available, 4.0%; Na, 7.2%; Mn, 5,500 mg; Zn, 4,500 mg; Co, 20 mg; Se, 25 mg; Cu, 1,200 mg; Fe, 3,000 mg; I, 60 mg; Mg, 4,500 mg.

[5]ME (MJ/kg) = 1 to 7 d: 12.57; 8 to 28 d: 12.14; 29 to 35 d: 11.72.

5.2 Slaughter surveys

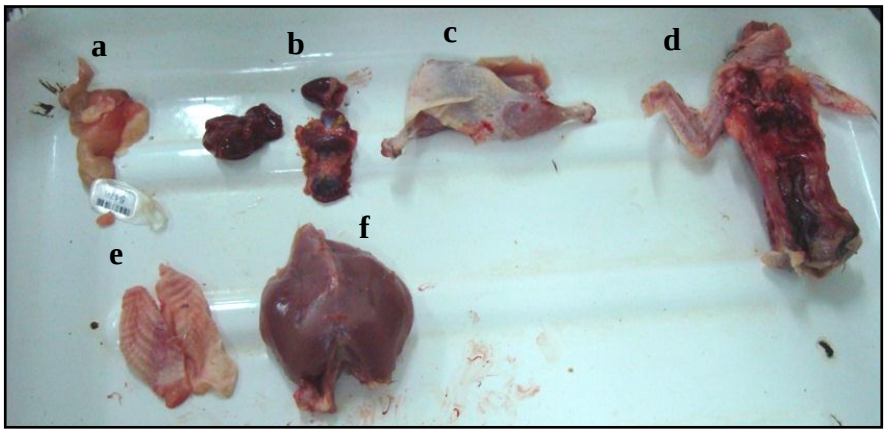
At slaughter (20 weeks of age), all birds were individually weighed (after a fasting period of 12 h), stunned, decapitated. Stunning was performed by a percussive blow to the back part of the head (occiput) and decapitation was performed with scissors between the cervical vertebrae and the base of the skull according to the EU regulations on the protection of animals at the time of killing (European Communities, 2009).

Carcasses were defeathered and eviscerated manually and hot carcass weight was recorded. The carcass without giblets makes the so-called "grill cut", that is fundamental in the slaughter analysis in poultry breeding and in industrial poultry meat production (Genchev et al., 2008b). Carcass yield was calculated based on live body weight. Then carcasses were dissected into the follow parts (Figure 5.2):

- edible giblets (heart, liver and gizzard);
- abdominal fat (from the proventriculus surrounding the gizzard down to the cloaca);
- breast (including the pectoral muscles, the sternum, the sternal rib part, the clavicle and coracoid bones);
- leg (thigh and drumstick).

The obtained parts were weighted and theirs percentages were calculated based on hot carcass weight.

Figure 5.2. Carcass parts: a) abdominal fat; b) edible giblets; c) legs; d) wings + back; e) skin; f) breast.



After the refrigeration period (24 h at 4°C), the right Pectoral muscle (PM) pH (pH_{24} or pH_u) was recorded using a portable pH-meter equipped with a glass electrode (R. Matthaüs, Pöttmes, Germany), electrode calibrated at pH 4.0 and 7.0 before measuring.

The left PM was removed, vacuum packaged, and stored frozen (− 40°C) for analyses of cholesterol, fatty acid composition and intramuscular collagen (IMC) properties.

5.3 Collagen analysis

Breast muscle samples from all birds of each generation were thawed, at room temperature, trimmed of fat and epimysium, lyophilized for 24 h (Genesis Pilot Lyophilizer, SP Scientific), and stored frozen (− 20°C) until collagen analyses.

The lyophilized muscle tissue (100 mg) was hydrolyzed in Duran tubes in 5ml 6N HCl at 110°C for 18 to 20 h (Etherington and Sims, 1981). The hydrolyzate was filtered (Whatman #1) and diluted with water plus. An aliquot of the hydrolyzate was removed for hydroxyproline determination and the remaining part was subjected to HLP (Hydroxylysilpyridinoline) crosslink analysis.

5.3.1 Collagen concentration analysis

The quantitative determination of hydroxyproline was performed using the colorimetric procedure of Woessner (1961). It was based on oxidation of hydroxyproline by adding chloramine T (sodium p-toluenesulfonchloramide). The chloramines T was then destroyed by adding perchloric acid. Finally, p-dimethylaminobenzaldehyde solution (Ehrlich solution) was added and placed in a 60°C water bath for 20 minutes. The absorbance of the solution was then determined spectrophotometrically (UV 8500, Techomp, Japan) at 557 nm (Figure 5.3). The hydroxyproline concentration was determined directly from the standard curve of L-hydroxyproline. Intramuscular collagen concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as µg hydroxyproline/mg of lyophilized tissue.

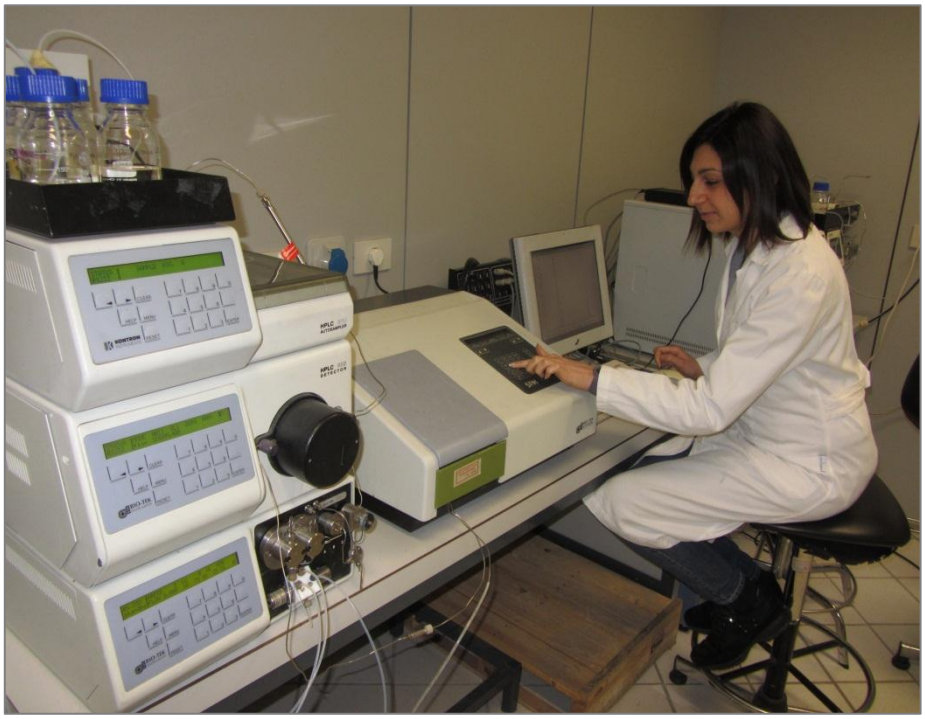
Figure 5.3. Spectrophotometer, UV-8500,Techomp

5.3.2 Crosslink concentration analysis

Hydroxylysylpyridinoline (HLP) concentration, the principal non-reducible crosslink of muscle collagen and highly correlated with the thermal stability of collagen (McCormick, 1999), was determined using the procedure described by Eyre et al. (1984). HLP was measured on breast muscle samples from 22 males and 22 females for each generation. Hydrolyzate HLP was concentrated and separated from the bulk of the other amino acids by elution from a CF1 cellulose column using the procedure described by Skinner (1982). The obtained eluate, added of pyridoxamine as an internal standard, was concentrated (Speed Vac® Plus SC110A, Savant Instruments, Farmingdale, NY), resuspended in 1% (v/v) n-heptafluorobutyric acid (HFBA) and filtrated (Nylon syringe filter 0.45µm, Whatman).

Quantitation of the HLP crosslink was performed by reversed phase high performance liquid chromatography (RP-HPLC) using the procedure described by Eyre et al. (1984). A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535 (Figure 5.4), equipped with a Luna C18 column (250 x 4.6 mm x 5 µm; Phenomenex, Torrance, CA), was used.

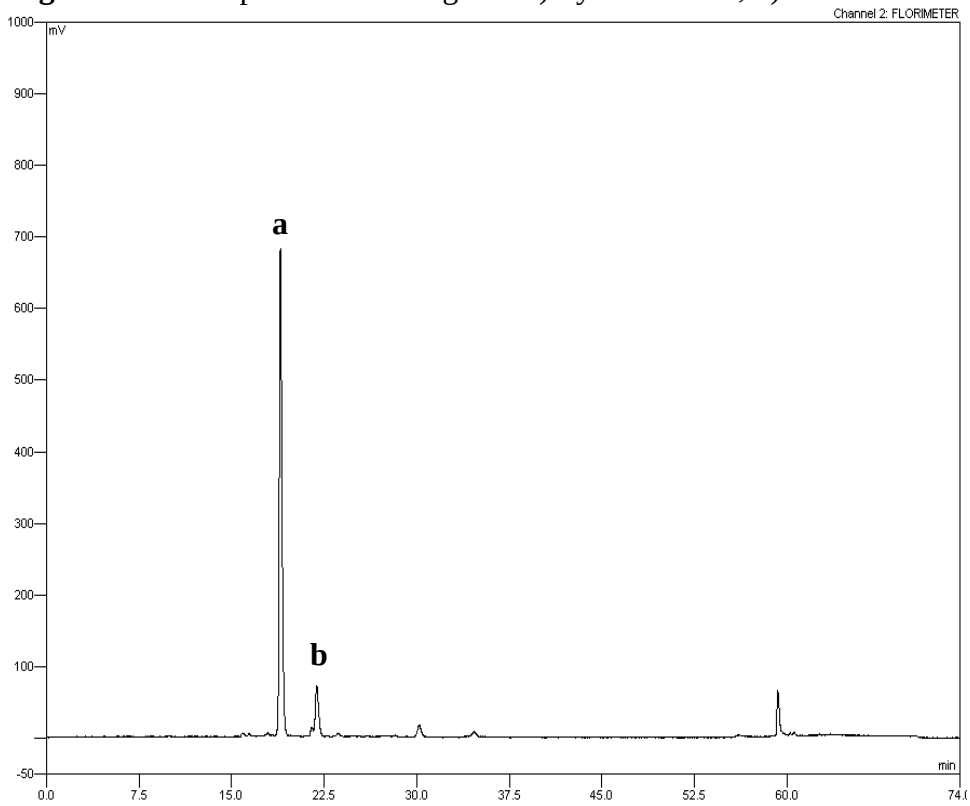
Figure 5.4. HPLC equipment



The HLP was resolved using a binary gradient of two solvents using HFBA as ion-pairing agent: solvent A was 0.01 M HFBA in 5 % acetonitrile (v/v) and solvent B was 0.01 M HFBA in acetonitrile. A flow rate of 0.700 ml/min was used. Samples (20 μl) were injected dissolved in 1 % HFBA (v/v). The column effluent was monitored for fluorescence by excitation at 295 nm and emission at 395 nm.

Quantitation of the HLP peak was performed using Piridoxamine (Sigma, St. Louis, MO) as internal standard (Figure 5.5). This form of vitamin B6 fluoresces with similar excitation and emission maxima to HLP and elutes about 1 min earlier in the chromatogram. Collagen HLP crosslink concentration was expressed as mole of HLP/mole of collagen, assuming that the molecular weight of collagen was 300,000 and the molar fluorescence yield of pyridoxamine was 3.1 times that of HLP (Eyre et al., 1984).

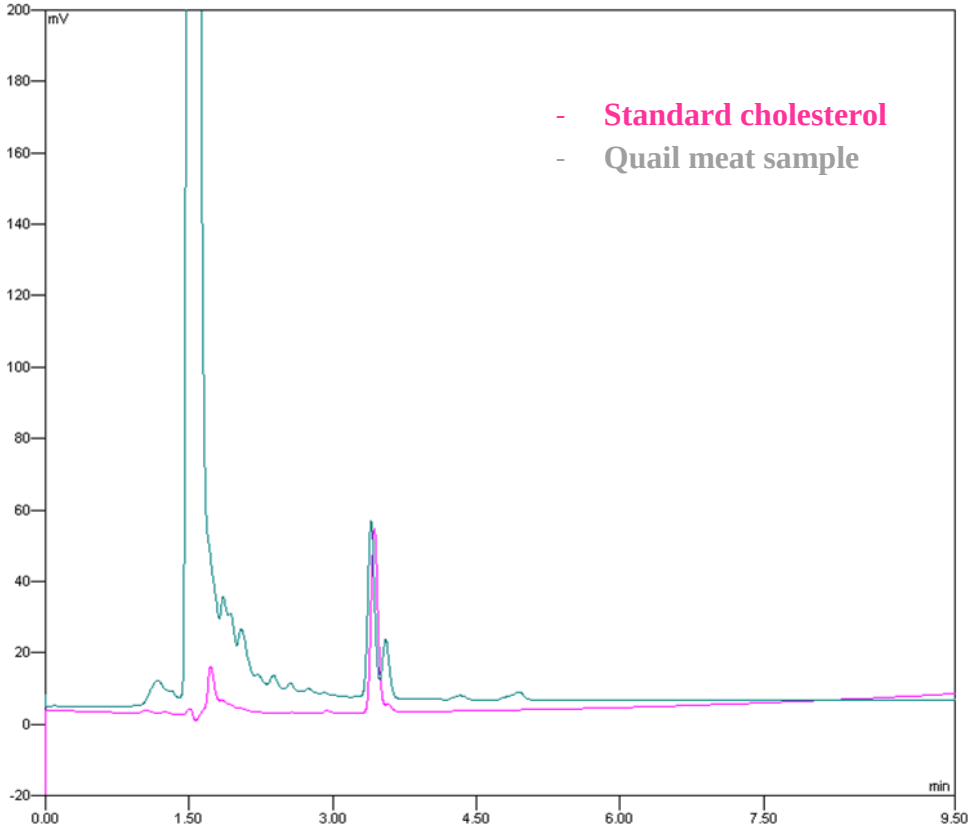
Figure 5.5. Example of chromatogram: a) Pyridoxamine; b) HLP



5.4 Measurement of muscle cholesterol

The muscle cholesterol content was determined using the method by Maraschiello et al. (1996). All analyses were carried out in duplicate. The breast muscle sample (100mg) was saponified with 2 ml of 0.5 N KOH in methanol for 1 hour at 80 °C. After cooling, 2 ml of distilled water saturated with NaCl was added. The tubes were vortexed followed by addition of 3 ml ether/hexane (1 : 1, *v/v)* and centrifuged for 10 min at 3000 g. The upper phase was recovered and the hexane/ether extraction step was repeated twice. The extracts were combined and evaporated to dryness and re-dissolved in 1ml of acetonitrile/isopropanol (1:1) for HPLC analysis. A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Kinetex 5µ C18 reverse-phase column (150 x 4.6mm x 5 µm; Phenomenex, Torrance, CA), was used. The HPLC mobile phase consisted of acetonitrile:2-propanol (55:45, v/v) at a flow rate of 1.0 ml/min. All solvents used were LC grade. The detection wavelength was 210 nm (Figure 5.6). The quantitation of muscle cholesterol content was based on the external standard method using a pure cholesterol standard Sigma, St. Louis, MO).

Figure 5.6. Example of chromatogram for cholesterol determination



5.5 Fatty acid analysis

Lipid extraction from breast muscle samples was performed by modification of Bligh and Dyer (1959) and Folch et al. (1957) methods. Bligh and Dyer extracts most classes of lipids and this system requires proportionally smaller volumes of chloroform and methanol than the Folch method.

Lyophilized breast muscle sample (100mg), added of tridecanoic acid methyl ester (C13:0) as internal standard, was treated with chloroform/methanol/water (1:2:0.8) and stirred for 4 hours. Then to obtain a better separation between the two phases, chloroform, 2N KCl/0.3 N HCl, and H_2O were added consecutively, and centrifuged for 5 min at 3000 g. The upper phase consisting of methanol, water, water soluble compounds such as sucrose or salts and very small amount of chloroform was discarded; while the lower lipid-containing phase was separated from the upper phase, and retained for use. Then, another extraction with chloroform was repeated.

The extracted lipids were esterificated and then analyzed by gas chromatography (GC). Analysis was performed using a HRGC 5300 Fisons (Rodano, Milan, Italy),

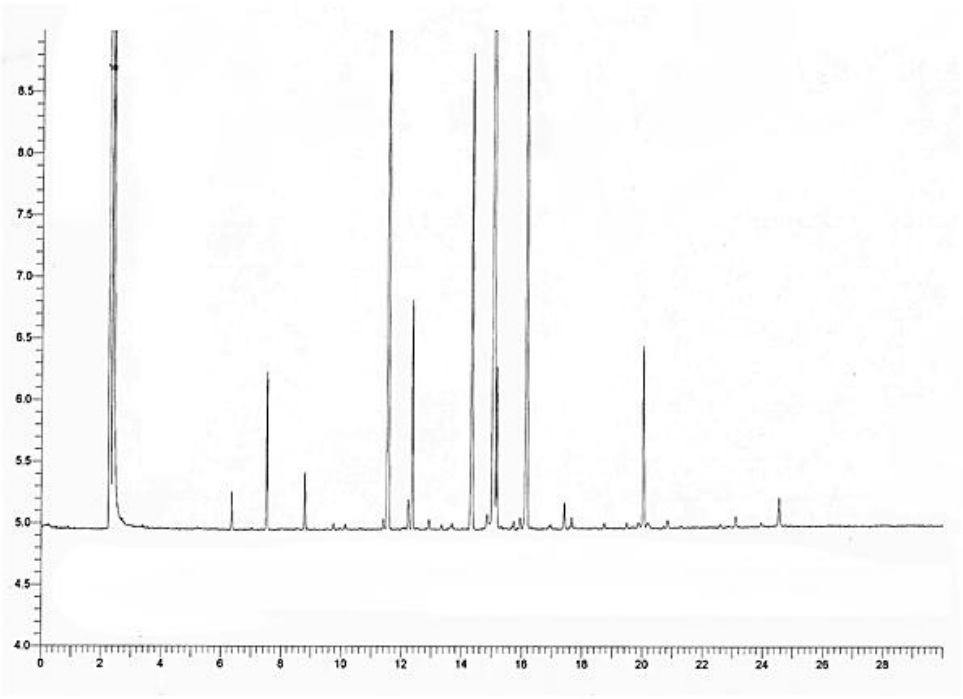
equipped with a flame ionization detector and a fused silica capillary Column (CP-Sil RTX 2330), 30 m x 0.25 mm x 0.5 μm film thickness (Restek, Bellefonte, PA, USA). The carrier gas was helium. The oven temperature program was 120°C for 1 min then increasing at 5°C/min up to 230°C where it was maintained for 20 min. The individual fatty acid peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (FAME, Sigma) run under the same operating conditions. Quantification of individual fatty acids was based on the internal standard method using tridecanoic acid methyl ester. Results were expressed as percentage of the total fatty acids analyzed (Figure 5.7).

To assess the nutritional implications, the n-6 fatty acids/n-3 fatty acids and the PUFA/SFA ratios 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), as follows:

$$AI = \frac{C12{:}0 + 4 \times C14{:}0 + C16{:}0}{\Sigma MUFA + \Sigma(n-6) + \Sigma(n-3)}$$

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n-6) + 3 \times \Sigma(n-3) + \frac{\Sigma(n-3)}{\Sigma(n-6)}}.$$

Figure 5.7. Example of chromatogram for fatty acid determination



5.6 Statistical analyses

One way analysis of variance (ANOVA) was performed for performance, carcass traits and IMC properties (SPSS Inc., 2010). Scheffé's test was applied to compare the mean values among the three generations.

PART 4

Chapter 6

RESULTS

In this section, in addition to the results regarding the effect of cross-breed of meat and egg line on productive performance and meat quality in Japanese quail from different generations, are reported some of the results obtained by the workgroups of the Department of Animal Biotechnology and Histology, University of Technology and Life Sciences (Bydgoszcz, Poland) and of the Department of Biological Basis of Animal Production, University of Life Sciences (Lublin, Poland), regarding the genetic mapping of QTLs in Japanese quails.

6.1 Effect of cross-breed of meat and egg line on performance, carcass traits, pH and IMC properties in Japanese quail from different generations

Growth, slaughter traits, pH and IMC properties of meat line (F-33) and egg line (S-22) Japanese quail are presented in Table 6.1. Quails of meat line had higher ($P < 0.01$) final BW, carcass weight and carcass yield (+ 8.3 %) than those of the egg line. Breast muscle and legs yield did not differ significantly between the two lines; giblets percentage was higher ($P < 0.01$) in egg type quails, while abdominal fat percentage was higher ($P < 0.01$) in meat type quails. Ultimate pH of meat type quails was lower ($P < 0.05$) than that of the egg type quails. The IMC amount was similar ($P > 0.05$) between the two quail lines. Compared with the quails of meat line, those of the egg line had a slower ($P < 0.01$) collagen maturation (HLP/collagen).

Gender effect on BW, slaughter traits, and meat quality (pH and IMC properties) of F_1 generation is shown in Table 6.2. Females were heavier than males ($P < 0.01$) and had a higher ($P < 0.01$) giblets percentage, while males showed a higher ($P < 0.01$) carcass yield (+ 6.2 %) and abdominal fat percentage. Breast and legs yields, ultimate pH and IMC amount were not significantly influenced by gender. The meat from females had a slower ($P < 0.01$) collagen maturation (HLP/collagen).

Table 6.1. Least squares means and SE for BW, slaughter traits, pH, and intramuscular collagen properties of Pectoral muscle from Japanese quails of F_0 generation

Item[1]	Group[2]		*P*-value
	F-33	S-22	
n	22	22	
Final BW (g)	166.21±2.62	149.00±3.82	0.001
Carcass weight (g)	115.65±2.41	91.26±2.58	0.001
Carcass yield (%)	69.52±0.71	61.24±0.67	0.001
Breast yield (%)	33.45±0.50	33.04±0.44	0.538
Legs yield (%)	24.67±0.61	25.52±0.69	0.358
Giblets (%)	5.35±0.11	7.52±0.16	0.001
Abdominal fat (%)	7.53±0.86	2.56±0.40	0.001
pH_{24}	5.67±0.02	5.79±0.05	0.033
IMC (μg/mg[3])	14.14±0.61	13.22±0.42	0.221
HLP (mol/mol of collagen)	0.121±0.005	0.098±0.003	0.001

[1]IMC = intramuscular collagen; HLP = hydroxylysylpyridinoline; [2]F-33= Meat line males; S-22= Egg line females; [3]of lyophilized muscular tissue.

Table 6.2. Least squares means and SE for BW, slaughter traits, pH, and intramuscular collagen properties of Pectoral muscle from Japanese quails of F_1 generation

Item[1]	Group		*P*-value
	Male	Female	
n	22	22	
Final BW (g)	163.00±2.78	184.58±3.52	0.001
Carcass weight (g)	101.98±1.59	104.30±2.15	0.391
Carcass yield (%)	62.71±0.75	56.54±0.58	0.001
Breast yield (%)	33.12±0.52	33.50±0.66	0.652
Legs yield (%)	22.90±0.34	23.30±0.27	0.363
Giblets (%)	5.74±0.18	7.90±0.16	0.001
Abdominal fat (%)	7.35±0.42	4.13±0.32	0.001
pH_{24}	5.78±0.02	5.74±0.05	0.535
IMC (μg/mg[2])	10.99±0.23	11.58±0.37	0.185
HLP (mol/mol of collagen)	0.144±0.008	0.119±0.005	0.008

[1]IMC = intramuscular collagen; HLP = hydroxylysylpyridinoline; [2]of lyophilized muscular tissue.

Gender effect on BW, slaughter traits, and meat quality (pH and IMC properties) of F_2 generation is shown in Table 6.3. Except for IMC properties, that were not significantly affected by gender, and the ultimate pH that was higher for males in comparison to females ($P < 0.01$), the same trend as in the F_1 generation was found for the BW and slaughter traits (carcass weight; carcass, breast and legs yields; giblets and abdominal fat percentages). Considering the hatch effect, it influenced significantly only the IMC amount (8.51 *versus* 10.15 μg/mg for hatch 1 and hatch 2, respectively; $P < 0.01$). Data regarding slaughter performance and pH are not shown, because are not statistically significant ($P < 0.05$).

Table 6.3. Least squares means and SE for BW, slaughter traits, pH, and intramuscular collagen properties of Pectoral muscle from Japanese quails of F_2 generation

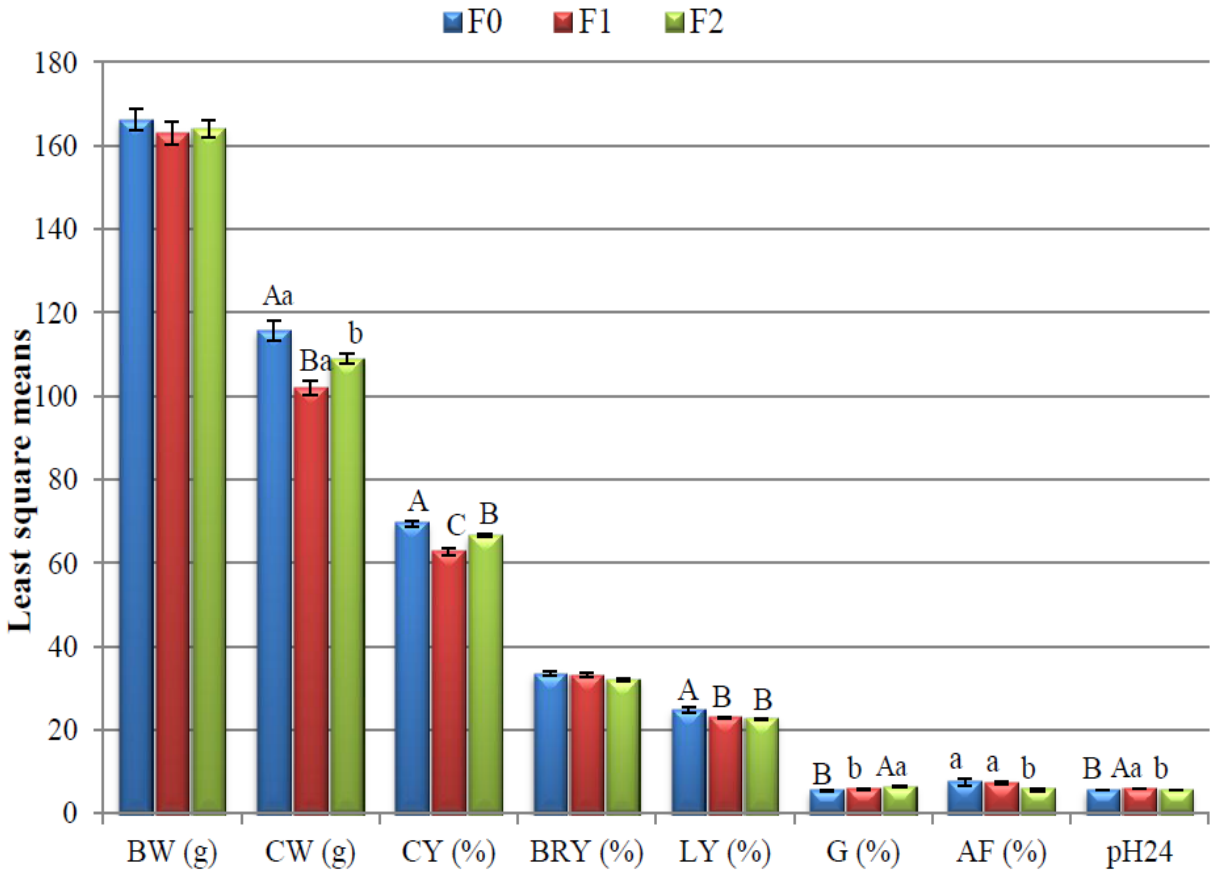
Item[1]	Group		*P*-value
	Male	Female	
n	84	152	
Final BW (g)	164.0±2.60	195.8±1.80	0.001
Carcass weight (g)	108.86±1.64	114.4±1.12	0.003
Carcass yield (%)	66.77±0.37	58.49±0.25	0.001
Breast yield (%)	32.37±0.50	32.35±0.34	0.610
Legs yield (%)	22.48±0.37	22.15±0.25	0.195
Giblets (%)	6.38±0.16	8.43±0.11	0.001
Abdominal fat (%)	5.73±0.30	4.30±0.21	0.001
pH_{24}	5.69±0.01	5.62±0.01	0.001
IMC (μg/mg[2])	9.69±0.18	9.37±0.12	0.799
HLP (mol/mol of collagen)	0.219 ± 0.012	0.221 ± 0.012	0.907

[1]IMC = intramuscular collagen; HLP = hydroxylysilpyridinoline (n = 22 males and 22 females); [2]of lyophilized muscular tissue.

The comparison of performance traits and pH among the three generations, according to the gender, are shown in Figures 6.1 (males) and 6.2 (females). The BW of F_1 and F_2 males compared to males of parental line (F_0) was not affected ($P > 0.05$) by the crosses. However, the carcass weight of F_0 males was higher than that of F_1 ($P < 0.01$) and F_2 ($P < 0.05$) males. On the contrary, the F_1 and F_2 females were heavier ($P < 0.01$) than parental line (S-22). F_1 quails (males and females) showed the lowest carcass

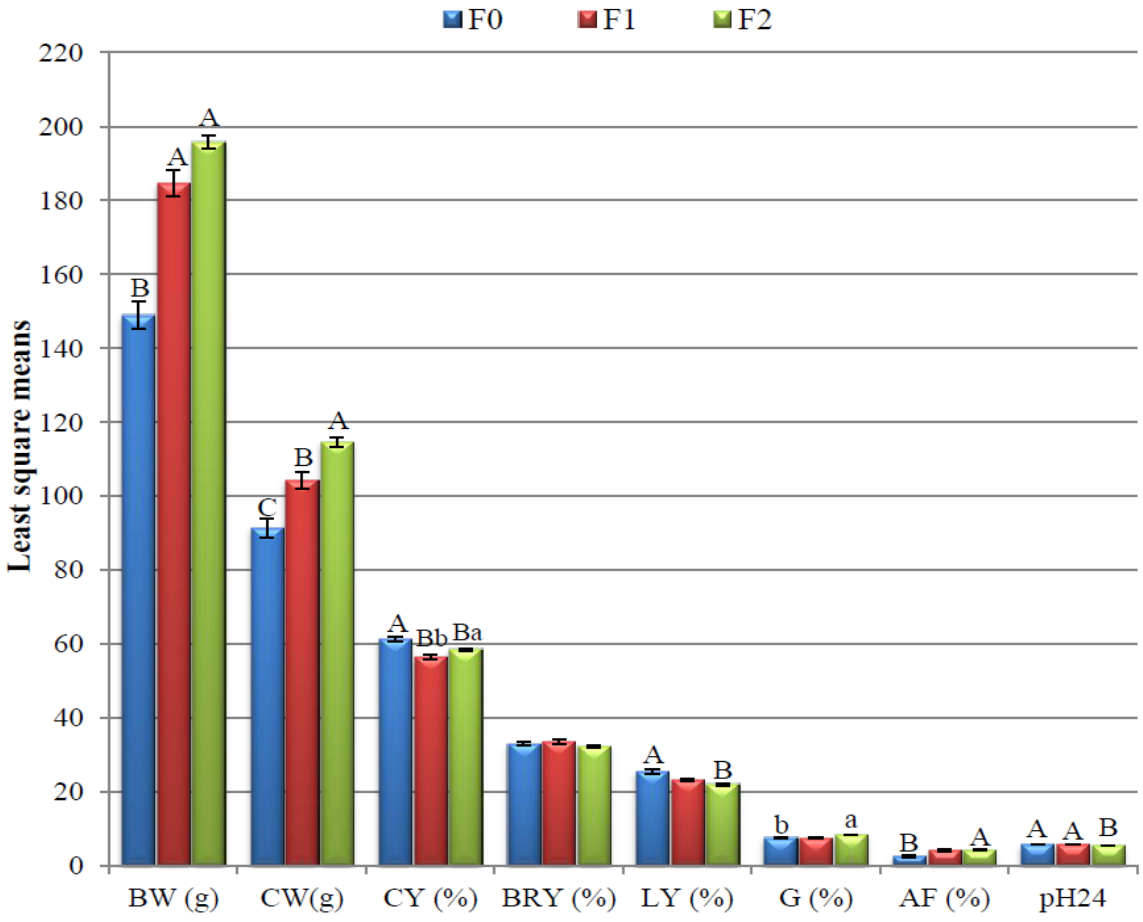
yield ($P < 0.01$) and F_2 quails had intermediate value ($0.05 > P < 0.01$). Abdominal fat was lower in F_2 males compared to F_0 and F_1 males ($P < 0.05$); on the contrary, it slightly increased ($P = 0.067$) in F_1 females and markedly ($P < 0.01$) in F_2 females compared to F_0 ones. Legs yield was lower ($P < 0.01$) in both F_1 and F_2 males and in F_2 females compared to parental lines. Differently, the giblets percentage of males and females was higher for F_2 in comparison to F_0 quails ($0.05 > P < 0.01$) and, moreover, the giblets percentage from F_2 males was higher ($P < 0.05$) than that of the F_1 birds. Breast yield did not differ ($P > 0.05$) among the three bird groups (males and females). In general, pH was significantly ($0.05 > P < 0.01$) affected by crosses.

Figure 6.1. Least square means and SE for BW, slaughter traits and pH of Japanese quail males of F_0, F_1 and F_2 generations.



CW = carcass weight; CY = carcass yield; BRY = breast yield; LY = legs yield; G = giblets; AF = abdominal fat. A, B, C: $P < 0.01$; a, b: $P < 0.05$.

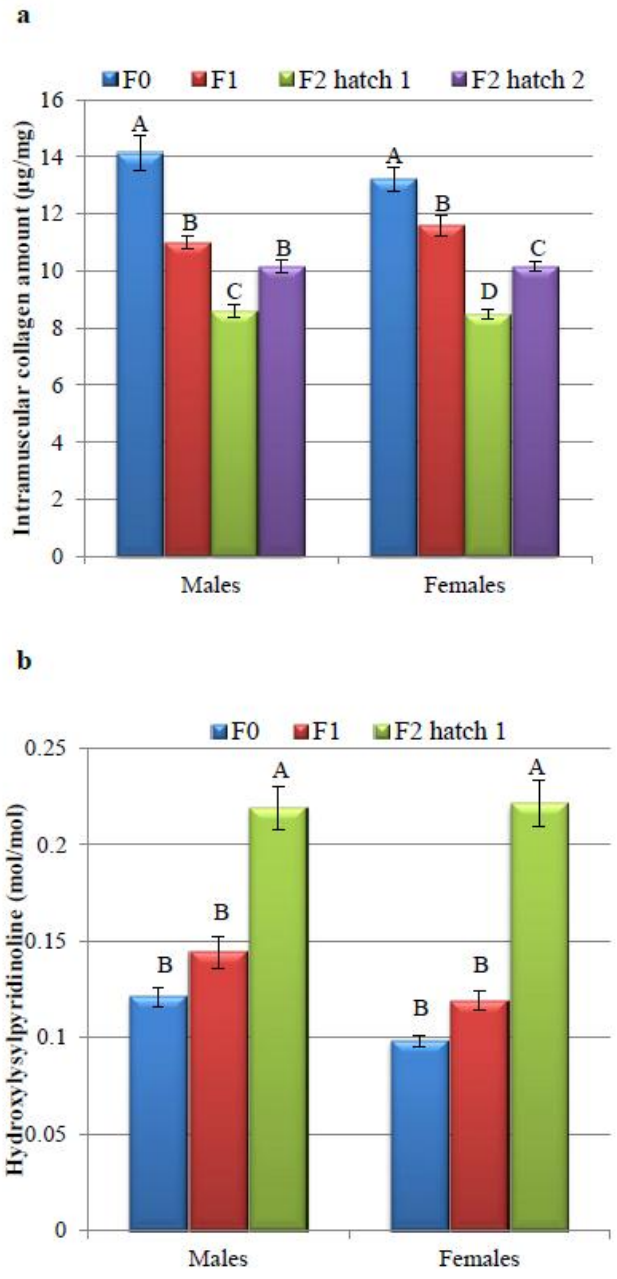
Figure 6.2. Least square means and SE for BW, slaughter traits and pH of Japanese quail females of F_0, F_1 and F_2 generations.



CW = carcass weight; CY = carcass yield; BRY = breast yield; LY = legs yield; G = giblets; AF = abdominal fat. A, B, C: $P < 0.01$; a, b: $P < 0.05$.

The comparison of IMC properties among the three generations, according to the gender and hatch, are shown in Figures 6.3a and 6.3b. Collagen amount, in both males and females, decreased ($P < 0.01$) from F_0 to F_1 and from F_1 to F_2 (this decrease was evident only in females of hatch 1 and hatch 2), while an increase of collagen maturation (HLP/collagen) with $F_0 < F_1 < F_2$, in both males and females, was apparent; however, the HLP values significantly ($P < 0.01$) differed only between F_2 and the other two generations.

Figure 6.3. Least square means and SE for Intramuscular collagen amount (a) and Hydroxylysylpyridinoline concentration (b) of Pectoral muscle from Japanese quail males and females of F_0, F_1 and F_2 generations; A, B, C, D: P < 0.01.



6.2 Fatty acid composition and cholesterol content of breast muscle of Japanese quail from different generations

Fatty acid composition and cholesterol content of breast muscle of meat line (F-33) and egg line (S-22) Japanese quail are presented in Table 6.4. Meat from egg line quails displayed a higher (+ 7 %) amount of total saturated fatty acids (SFA) compared to meat line quails ($P < 0.01$), due to the higher ($P < 0.01$) proportions of lauric (C 12:0; twofold higher than in meat type males), myristic (C 14:0), palmitic (C 16:0), heptadecanoic (C 17:0) and stearic (C 18:0) fatty acids. On the contrary, breast muscle of meat line quails had higher (+ 11.5 %; $P < 0.01$) amount of total monounsaturated fatty acids (MUFA), mainly due to the significantly higher ($P < 0.01$) proportion of palmitoleic acid (C 16:1 n-9; twofold higher than in egg type females), oleic acid (C 18:1 n-9; + 8.39 %) and C 14:1 ($P < 0.05$); while only the C 20:1 was higher ($P < 0.05$) in egg line quails.

Meat from the egg type females showed a higher (+4.4 %; $P < 0.05$) content of total polyunsaturated fatty acids (PUFA) compared to meat line quails. This is due to the higher ($P < 0.05$) proportion of linoleic (C 18:2 n-6), linolenic (C 18:3 n-3; $P = 0.051$) and docosatetraenoic (C 22:4 n-6) fatty acids in breast muscle of egg line quails. Also the total amount of n-6 fatty acids were higher ($P < 0.05$) in egg line quails. No significantly difference were found between groups for docosapentaenoic (DPA, C 22:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) fatty acids. Other PUFA such as eicosapentaenoic acid (EPA; C 20:5 n-3), C 20:2 n-6 and C:20:3 n-6 were observed at only trace levels.

Regarding the selected fatty acid ratios, neither the ratio of PUFA to SFA (P/S), nor the ratio of n-6 fatty acids to n-3 fatty acids (n-6/n-3) were significantly different between experimental groups. Contrarily, the atherogenic index (AI) and the thrombogenic index (TI) were lower ($P < 0.01$) in meat line quails compared to those of egg line quails. Instead, no significant difference was found for muscle cholesterol content between the two lines of Japanese quail.

Gender effect on fatty acid composition and cholesterol content of breast muscle of quail from F_1 generation is shown in Table 6.5. Except for a higher ($P < 0.05$) proportion of lauric acid (C 12:0) and a slightly higher ($P = 0.068$) proportion of C 20:1, displayed by hybrid females, no differences were found for other FA and for the nutritional indices and fatty acid ratios, being similar between sex.

Table 6.4. Least squares means and SE for fatty acid composition (% of total fatty acids) of breast muscle from Japanese quails of F_0 generation

Item[1]	Group		*P*-value
	F-33	S-22	
n	11	11	
C 12:0	0.25±0.02	0.59±0.05	0.001
C 14:0	0.62±0.03	0.93±0.06	0.001
C 14:1	0.12±0.01	0.08±0.01	0.036
C 16:0	17.95±0.22	20.86±0.41	0.001
C 16:1n-9	6.21±0.32	3.09±0.32	0.001
C 17:0	0.10±0.01	0.17±0.01	0.001
C 18:0	9.34±0.32	12.75±0.79	0.001
C 18:1n-9	44.91±1.08	36.52±1.93	0.001
C 18:2n-6	14.24±0.55	17.11±0.80	0.008
C 18:3n-3	0.31±0.02	0.40±0.04	0.051
C 20:1n-9	0.18±0.01	0.23±0.01	0.004
C 20:4n-6	4.16±0.42	5.26±0.90	0.281
C 22:1	0.10±0.01	0.09±0.01	0.693
C 22:2	0.18±0.02	0.26±0.04	0.096
C 22:4n-6	0.17±0.02	0.35±0.06	0.013
C 22:5n-3	0.16±0.02	0.12±0.02	0.167
C 22:6n-3	1.01±0.11	1.21±0.24	0.472
ΣSFA	28.26±0.37	35.28±0.71	0.001
ΣMUFA	51.51±1.16	40.01±2.13	0.001
ΣPUFA	20.24±0.99	24.70±1.76	0.039
Total n-6	18.57±0.87	22.72±1.50	0.027
Total n-3	1.48±0.13	1.73±0.28	0.441
n-6:n-3	13.15±0.82	14.78±1.19	0.274
P/S	0.72±0.03	0.70±0.04	0.771
Atherogenic index (AI)	0.29±0.01	0.39±0.01	0.001
Thrombogenic index (TI)	0.71±0.01	0.95±0.03	0.001
Cholesterol (mg/100g)	75.33±2.47	80.26±2.18	0.142

[1]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n-6:n-3 = PUFA n-6:PUFA n-3 ratio; P/S ratio is the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA). *n* − 3 fatty acids included 18:3, 22:5, and 22:6, and *n* − 6 fatty acids included 18:2, 20:3, and 20:4; Cholesterol (n = 22 F-33 quails and 22 S-22 quails).

Table 6.5. Least squares means and SE for fatty acid composition (% of total fatty acids) of breast muscle from Japanese quails of F_1 generation

Item[1]	Group		*P*-value
	Male	Female	
n	11	11	
C 12:0	0.14±0.03	0.23±0.02	0.019
C 14:0	0.61±0.05	0.67±0.04	0.329
C 14:1	0.13±0.02	0.13±0.04	1.000
C 16:0	20.15±0.27	19.79±0.51	0.540
C 16:1n-9	6.32±0.49	6.09±0.41	0.730
C 17:0	0.11±0.01	0.13±0.01	0.223
C 18:0	8.52±0.54	7.75±0.59	0.346
C 18:1n-9	46.85±1.34	46.94±1.07	0.960
C 18:2n-6	15.04±0.88	16.32±0.86	0.311
C 18:3n-3	0.36±0.05	0.38±0.03	0.769
C 20:1n-9	0.19±0.01	0.23±0.02	0.068
C 20:4n-6	0.98±0.17	0.81±0.10	0.389
C 22:1	0.07±0.01	0.06±0.01	0.352
C 22:2	0.21±0.02	0.18±0.02	0.397
C 22:4n-6	0.06±0.01	0.07±0.01	0.629
C 22:5n-3	0.07±0.01	0.05±0.01	0.197
C 22:6n-3	0.19±0.03	0.17±0.02	0.564
ΣSFA	29.53±0.73	28.57±1.04	0.458
ΣMUFA	53.56±1.33	53.45±1.20	0.951
ΣPUFA	16.91±1.01	17.98±0.90	0.438
Total n-6	16.08±0.95	17.20±0.88	0.397
Total n-3	0.62±0.07	0.59±0.04	0.715
n-6:n-3	28.15±2.39	29.65±1.54	0.604
P/S	0.58±0.03	0.64±0.04	0.281
Atherogenic index (AI)	0.33±0.01	0.32±0.01	0.784
Thrombogenic index (TI)	0.80±0.03	0.77±0.04	0.496
Cholesterol (mg/100g)	64.97±2.42	67.06±2.46	0.547

[1]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n-6:n-3 = PUFA n-6:PUFA n-3 ratio; P/S ratio is the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA). *n* − 3 fatty acids included 18:3, 22:5, and 22:6, and *n* − 6 fatty acids included 18:2, 20:3, and 20:4; Cholesterol (n = 22 males and 22 females).

Gender effect on fatty acid profile and cholesterol content of breast muscle from quails of F_2 generation is shown in Table 6.6. In the F_2 generation a sex-effect was more evident compared to F_1 generation. A slightly higher ($P = 0.085$) amount of total SFA was found in males, mainly due to the contribution of stearic acid ($P = 0.063$). F2 females had higher ($P < 0.01$) amount of lauric acid and slightly higher content of myristic acid ($P = 0.058$) and heptadecanoic acid ($P = 0.055$).

No significant sex effect was found in the composition of monounsaturated fatty acids and consequently on the total MUFA content.

On the contrary, the total PUFA amount was significantly affected by gender being higher ($P < 0.05$) in females (+ 4 %) compared to males, due to the higher ($P < 0.01$) proportion of linoleic (+ 4.09 %) and linolenic fatty acids. Also, females had higher ($P < 0.05$) total amount of n-6 fatty acids and consequently a lower ($P < 0.05$) thrombogenic index compared to males. However, P/S ratio was lower ($P < 0.05$) in males.

Muscle cholesterol content was affected by gender being lower ($P < 0.05$) in females.

Table 6.6. Least squares means and SE for fatty acid composition (% of total fatty acids) of breast muscle from Japanese quails of F_2 generation

Item[1]	Group		*P*-value
	Male	Female	
n	22	22	
C 12:0	0.01±0.00	0.02±0.00	0.008
C 14:0	0.40±0.01	0.44±0.02	0.058
C 14:1	0.06±0.01	0.06±0.01	0.755
C 16:0	19.65±0.47	18.97±0.41	0.278
C 16:1n-9	4.71±0.32	4.28±0.23	0.279
C 17:0	0.13±0.01	0.15±0.01	0.055
C 18:0	9.78±0.37	8.82±0.34	0.063
C 18:1n-9	42.77±1.12	40.72±1.29	0.237
C 18:2n-6	17.81±0.73	21.90±0.81	0.001
C 18:3n-3	0.46±0.03	0.69±0.04	0.001
C 20:1n-9	0.19±0.00	0.20±0.01	0.390
C 20:4n-6	2.87±0.35	2.58±0.28	0.533
C 22:1	0.09±0.01	0.08±0.01	0.157
C 22:2	0.14±0.01	0.16±0.01	0.084
C 22:4n-6	0.10±0.02	0.12±0.01	0.407
C 22:5n-3	0.18±0.02	0.10±0.01	0.012
C 22:6n-3	0.65±0.10	0.70±0.09	0.700
ΣSFA	29.97±0.73	28.40±0.50	0.085
ΣMUFA	47.83±1.30	45.34±1.28	0.180
ΣPUFA	22.20±1.13	26.26±1.13	0.015
Total n-6	20.78±1.00	24.60±1.02	0.011
Total n-3	1.28±0.15	1.49±0.12	0.287
n-6:n-3	19.28±1.68	17.97±0.95	0.498
P/S	0.75±0.04	0.93±0.04	0.004
Atherogenic index (AI)	0.31±0.01	0.29±0.01	0.240
Thrombogenic index (TI)	0.79±0.03	0.72±0.02	0.033
Cholesterol (mg/100g)	72.71±2.17	66.51±1.75	0.032

[1]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n-6:n-3 = PUFA n-6:PUFA n-3 ratio; P/S ratio is the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA). *n* − 3 fatty acids included 18:3, 22:5, and 22:6, and *n* − 6 fatty acids included 18:2, 20:3, and 20:4.

The comparison of fatty acid composition and cholesterol content of quail breast muscle among the three generations according to the gender are shown in the Table 6.7 (females) and 6.8 (males). Differences related to sex and generation were observed on the proportion of several fatty acids in the muscle.

Breast muscle from hybrid females (F_1 and F_2 generations) had a lower ($P < 0.01$) amount of total SFA compared to that of females of parental line. In particular, hybrid females of both generations displayed a lower ($P < 0.01$) amount of stearic acid (C 18:0) and only F_2 females had lower content of palmitic acid (C 16:0; $P < 0.05$), compared to females of parental line. Also the decrease ($P < 0.01$) from F_0 to F_1 and from F_1 to F_2 of lauric acid (C 12:0) and myristic acid (C 14:0) had an effect on the reduction of total SFA in hybrids females, even if the amount of these fatty acids is relatively low. On the contrary, in males no significant differences in the total SFA amount were found among the three generations. In particular, stearic acid amount was similar between groups, while palmitic acid was lower ($P < 0.05$) in hybrid males (F_1 and F_2). Lauric and myristic fatty acids were lower ($P < 0.01$) in hybrids compared to males of parental line, with $F_2 < F_1 < F_0$ for lauric acid and $F_2 < F_1$ and F_0 for myristic acid.

The total content of MUFA in breast muscle was higher ($P < 0.01$) in F1 females compared to that of F_0 and F_2 females, with $F_2 > F_0$ ($P < 0.05$). The increased proportion of total MUFA in F1 females corresponded mainly to the higher amount of C 18:1 (+ 10.42 % compared to F_0, $P < 0.01$; + 6.22 compared to F_2, $P < 0.05$) and of C 16:1 (+ 3 % compared to F_0 and +1.81 % compared to F_2; $P < 0.01$). Respect to oleic and palmitic acids amount, females of F_2 generation had intermediate value ($P < 0.05$). A similar trend for MUFA was found in males among the three generations. In fact, the total muscle content of MUFA were higher ($P < 0.05$) in F_1 males compared to F_2 males, but similar to those of parental line. In particular, both F_0 and F_1 males had higher amount of palmitoleic acid ($P < 0.05$) and of C 14:1 ($P < 0.01$) compared to F_2 males. Oleic acid amount was slightly higher ($P = 0.067$) in F1 males, with the lower content in F_2 males, even if not significant.

Regarding the total amount of PUFA, both males and female of the three generations displayed a similar trend. Indeed, in both sexes, F_2 quails were characterized by higher ($P < 0.01$ and $P < 0.05$ for females and males, respectively) content of total PUFA compared to F_0 and F_1 quails; this is mainly due to the greater contribution of linoleic acid ($P < 0.01$) and α-linolenic acid ($P < 0.01$ and $P < 0.05$ in females and males,

respectively). On the other hand, F_0 quails (males and females) displayed significantly higher content of long-chain fatty acids (C ≥ 20) compared to the other two generations. In particular, both females and males of parental line had higher ($P < 0.01$) content of arachidonic acid (C 20:4), while F_1 quails were characterized by the lowest content of that fatty acid. Significantly higher ($P < 0.01$) amount of docosahexaenoic acid (DHA, C 22:6) were found in F_0 quails (males and females) compared to that of F_1 and F_2 quails. Consequently, the total amount of n-3 and n-6 fatty acids was affected by the crosses. Both males and females of F_2 generation showed higher ($P < 0.01$ and $P < 0.05$ for females and males, respectively) amount of total n-6 fatty acids, while F_1 quails showed the lowest amount of total n-3 fatty acids. As a consequence, F_1 quails (males and females) had the highest ($P < 0.01$) ratio between n-6 fatty acids and n-3 fatty acids.

The ratio between PUFA and SFA (P/S) was lower ($P < 0.01$ and $P < 0.05$ for females and males, respectively) in F_1 quails compared with those of the other two generations.

No significant differences were found in males among the three generation for the atherogenic index (AI) and thrombogenic index (TI). On the contrary, hybrid females (F_1 and F_2) had lower ($P < 0.01$) indices (AI and TI) compared to parental line.

Muscle cholesterol content was significantly lower ($P < 0.01$) in both hybrid females compared to females of parental line; instead, only F_1 males showed the lowest ($P < 0.05$) muscle cholesterol content compared to the other two generations.

Table 6.7. Least squares means and SE for fatty acid composition (% of total fatty acids) of breast muscle from Japanese quails females of F_0, F_1 and F_2 generations

	Group			
Item[1]	Females F_0	Females F_1	Females F_2	*P*-value
n	11	11	22	
C 12:0	0.59±0.05^{A}	0.23±0.02^{B}	0.02±0.00^{C}	0.001
C 14:0	0.93±0.06^{A}	0.67±0.04^{B}	0.44±0.02^{C}	0.001
C 14:1	0.08±0.01^{B}	0.13±0.01^{A}	0.06±0.01^{B}	0.001
C 16:0	20.86±0.41^{a}	19.79±0.51	18.97±0.41^{b}	0.020
C 16:1n-9	3.09±0.32^{Bb}	6.09±0.41^{A}	4.28±0.23^{aB}	0.001
C 17:0	0.17±0.01	0.13±0.01	0.15±0.01	0.100
C 18:0	12.75±0.79^{A}	7.75±0.59^{B}	8.82±0.34^{B}	0.001
C 18:1n-9	36.52±1.93^{Bb}	46.94±1.07^{Aa}	40.72±1.29^{b}	0.001
C 18:2n-6	17.11±0.80^{B}	16.32±0.86^{B}	21.90±0.81^{A}	0.001
C 18:3n-3	0.40±0.04^{B}	0.38±0.03^{B}	0.69±0.04^{A}	0.001
C 20:1n-9	0.23±0.01	0.23±0.02	0.20±0.01	0.156
C 20:4n-6	5.26±0.90^{A}	0.81±0.10^{Bb}	2.58±0.28^{Ba}	0.001
C 22:1	0.09±0.01	0.06±0.01	0.08±0.01	0.072
C 22:2	0.27±0.04^{a}	0.18±0.02	0.16±0.01^{b}	0.016
C 22:4n-6	0.35±0.06^{A}	0.07±0.01^{B}	0.12±0.01^{B}	0.001
C 22:5n-3	0.12±0.02^{a}	0.05±0.01^{b}	0.10±0.01	0.022
C 22:6n-3	1.21±0.24^{Aa}	0.17±0.02^{Ba}	0.70±0.09^{b}	0.001
ΣSFA	35.28±0.71^{A}	28.57±1.04^{B}	28.40±0.50^{B}	0.001
ΣMUFA	40.01±2.13^{Bb}	53.45±1.20^{A}	45.34±1.28^{aB}	0.001
ΣPUFA	24.70±1.76^{a}	17.98±0.90^{Bb}	26.26±1.13^{A}	0.001
Total n-6	22.72±1.50^{aB}	17.20±0.88^{b}	24.60±1.02^{A}	0.001
Total n-3	1.73±0.28^{A}	0.59±0.04^{B}	1.49±0.12^{A}	0.001
n-6:n-3	14.78±1.19^{B}	29.65±1.54^{A}	17.97±0.95^{B}	0.001
P/S	0.70±0.04^{B}	0.64±0.04^{B}	0.93±0.04^{A}	0.001
Atherogenic index (AI)	0.39±0.01^{A}	0.32±0.01^{B}	0.29±0.01^{B}	0.001
Thrombogenic index (TI)	0.95±0.03^{A}	0.77±0.04^{B}	0.72±0.02^{B}	0.001
Cholesterol (mg/100g)	80.26±2.18^{A}	67.06±2.46^{B}	66.51±1.75^{B}	0.001

[1]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n-6:n-3 = PUFA n-6:PUFA n-3 ratio; P/S ratio is the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA). *n* − 3 fatty acids included 18:3, 22:5, and 22:6, and *n* − 6 fatty acids included 18:2, 20:3, and 20:4; Cholesterol (n = 22 F_0 females, 22 F_1 females and 22 F_2 females).

$^{a-b}$ 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).

Table 6.8. Least squares means and SE for fatty acid composition (% of total fatty acids) of breast muscle from Japanese quails males of F_0, F_1 and F_2 generations

Item[1]	Group			*P*-value
	Males F_0	Males F_1	Males F_2	
n	11	11	22	
C 12:0	0.25±0.02^{A}	0.14±0.03^{B}	0.01±0.00^{C}	0.001
C 14:0	0.62±0.03^{A}	0.61±0.05^{A}	0.40±0.01^{B}	0.001
C 14:1	0.12±0.01^{A}	0.13±0.02^{A}	0.06±0.01^{B}	0.001
C 16:0	17.95±0.22^{b}	20.15±0.27^{a}	19.65±0.47^{a}	0.008
C 16:1n-9	6.21±0.32^{a}	6.32±0.49^{a}	4.71±0.32^{b}	0.004
C 17:0	0.10±0.01^{b}	0.11±0.01	0.13±0.01^{a}	0.031
C 18:0	9.34±0.32	8.52±0.54	9.78±0.37	0.120
C 18:1n-9	44.91±1.08	46.85±1.34	42.77±1.12	0.067
C 18:2n-6	14.24±0.55^{B}	15.04±0.88	17.81±0.73^{A}	0.004
C 18:3n-3	0.31±0.02^{b}	0.36±0.05	0.46±0.03^{a}	0.019
C 20:1n-9	0.18±0.01	0.19±0.01	0.19±0.00	0.297
C 20:4n-6	4.16±0.42^{A}	0.98±0.17^{B}	2.87±0.35	0.001
C 22:1	0.10±0.01	0.07±0.01	0.09±0.01	0.140
C 22:2	0.18±0.02^{A}	0.21±0.02	0.14±0.01^{B}	0.002
C 22:4n-6	0.17±0.02^{Aa}	0.06±0.01^{B}	0.10±0.02^{b}	0.001
C 22:5n-3	0.16±0.02^{b}	0.07±0.01	0.18±0.02^{a}	0.009
C 22:6n-3	1.01±0.11^{Aa}	0.19±0.03^{Ba}	0.65±0.10^{b}	0.001
ΣSFA	28.26±0.37	29.53±0.73	29.97±0.73	0.260
ΣMUFA	51.51±1.16	53.56±1.33^{a}	47.83±1.30^{b}	0.013
ΣPUFA	20.24±0.99	16.91±1.01^{b}	22.20±1.13^{a}	0.010
Total n-6	18.57±0.87	16.08±0.95^{b}	20.78±1.00^{a}	0.009
Total n-3	1.48±0.13^{A}	0.62±0.07^{Bb}	1.28±0.15^{a}	0.002
n-6:n-3	13.15±0.82^{Bb}	28.15±2.39^{A}	19.28±1.68^{a}	0.001
P/S	0.72±0.03	0.58±0.03^{b}	0.75±0.04^{a}	0.014
Atherogenic index (AI)	0.29±0.01	0.33±0.02	0.31±0.01	0.096
Thrombogenic index (TI)	0.71±0.01	0.80±0.03	0.79±0.03	0.081
Cholesterol (mg/100g)	75.33±2.47^{a}	64.97±2.42^{b}	72.71±2.17^{a}	0.007

[1]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n-6:n-3 = PUFA n-6:PUFA n-3 ratio; P/S ratio is the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA). *n* − 3 fatty acids included 18:3, 22:5, and 22:6, and *n* − 6 fatty acids included 18:2, 20:3, and 20:4; Cholesterol (n = 22 F_0 males, 22 F_1 males and 22 F_2 males).

$^{a-b}$ 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).

6.3 Main results from the genetic mapping of quantitative trait loci in three generations population of Japanese quail (*Coturnix japonica*)

Regarding the genotypic data, the effective number of alleles in the parental generation ranged from 1.0 in monomorphic loci GUJ0063 and GUJ0095 to 4.57 in the most polymorphic locus GUJ0077. All of the analyzed marker loci were characterized by a lower effective number of alleles compared to the total number of alleles. According by Botsein's scale (1980), classifying the level of markers' informativeness based on PIC values, 13 microsatellite markers were characterized by a high level of variation (PIC > 0.5) and 11 markers by a moderate level of variation (0.25 < PIC < 0.5). Markers GUJ0063 and GUJ0095 (monomorphic loci) and GUJ0064, GUJ0084, GUJ0073 are classified as low informative (PIC < 0.25).

Regarding QTL results two statistically significant (P < 0.05) QTLs have been detected on quail chromosome two. First, QTL with additive effect (0.50) for intramuscular collagen has been detected at 39 cM in the marker bracket GUJ0037 and GUJ0093. Second QTL with additive (1.32) and dominant (1.91) effects for breast muscle weight has been detected at 163 cM in the marker bracket GUJ0084 and GUJ0073.

PART 5

Chapter 7

DISCUSSION

According to our best knowledge, no research has yet been conducted to study the effect of crossing two strains of Japanese quail on meat quality of the F_1 and F_2 generations, including QTL detection, and only one study on *in vivo* performance (Esmalizadeh et al., 2012), and few studies have been conducted to compare meat quality of different lines or breeds of quail (reviewed in Maiorano et al., 2009, 2011). In addition, very scarce are the studies conducted to evaluate the carcass and meat quality traits of adult or spent quails at the end of their productive life (Boni et al., 2010).

The choice to slaughter the quails at 20 weeks of age is subordinated to the main aim of the research project of developing three generations population for the QTLs study. However, the evaluation of the carcass and meat quality traits of 20 weeks old Japanese quail is not only related to the QTLs analysis, but it has also the purpose to increase the knowledge about quail meat and especially meat from adult quails. 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). Oftentimes, the old breeding birds are slaughtered and sold on commercial market without any distinction being made on age (Shanaway, 1994). In light of this, the goal of the new developing quail production system is to obtain along with the primary production of eggs a quality meat production conforming to market's standards, from unnecessary males and removed females. (Genchev et al., 2005)

7.1 Performance and carcass traits

The results of growth and slaughter traits observed in the present study, comparing meat line and egg line (F_0 generation), agree with the findings of Boon et al. (2000) and Maiorano et al. (2009), who reported significant differences in body weight gain, carcass weight and carcass yield between fast growing Japanese quails bred for meat production (broilers) and normal growing ones bred for egg production (layers): broilers

grew faster than layers. The obtained differences in the live body weight between the two different lines were logical, rendering into account the different production purpose of the studied breeds of Japanese quail. Also Genchev et al. (2005) reported that Japanese quail of the Faraon breed (meat type) were heavier (+ 5.9 % for females and + 7.9 % for males) than the those of the White English (general purpose, meat + egg) breed. They also found that the feed conversion of the White English quails was 9.5 % less effective compared to those of Faraon quails, which were consequently characterized by higher growth rate. Moreover, Moran (1977) reported that the fattening performance and carcass characteristics of quails are affected by the length of growth period, genotype, sex, age, selection, and nutritional content of the ration used especially during the growing period.

The carcass yield values found in the present study are higher (+ 7.72 % and + 2.54 % for meat and egg type, respectively) that those found by Maiorano et al. (2009) in agreement with Caron et al. (1990); these latter authors reported a lighter increase in carcass yield with an increase in mean BW of Japanese quails. A comparable carcass yield was reported by Attia et al. (2013) for 84 d old quails (males and females) of the same breed, as well as for giblets percentage.

The breast muscle and leg yields, not affected by genotype, were similar to those found by Al Daraji et al. (2011) for 20 wk old Japanese quail fed with different dietary fat. Genchev et al. (2008a) reported similar incidence of breast (30.6-31.3 %) but lower legs percentage (17.8-18.1 %) in 35 days old Japanese quail.

The significantly higher abdominal fat percentage measured in meat type (+ 5 %) was also reported by Genchev et al. (2005) who observed that the content of abdominal fat was significantly higher in the Faraon breed (specialized for meat production) than in the White English breed (good for meat and egg production). Abdominal fat is being regarded as the main source of waste in poultry and it is highly correlated (0.6 to 0.9) with the total carcass fat; it is used as the main criterion reflecting excessive fat deposition in birds (Chambers, 1990).

Ultimate pH values, lower in meat type than that of egg type, are close to the normal values for breast muscles in broiler chickens (Maiorano et al., 2012). It is well known that the ultimate pH of the muscle is an important contributing factor to meat quality expressed as tenderness, colour, and storage live (Van Laack et al., 2000). The ultimate pH of the meat is largely dependent on the initial glycogen reserves of the muscle at the

time of slaughter and it is likely that the glycogen reserve at the time of death is higher for fast growing birds compared with slow growing birds (reviewed in Zerehdaran et al., 2012). Sibut et al. (2008) showed that fat chickens exhibited greater glycolytic potential and lower ultimate pH than lean chickens. Le Bihan-Duval et al. (1999, 2001) reported a negative genetic correlation between ultimate pH and abdominal fatness in broiler chickens. This accords with our observations.

The analyzed data from F_1 cross-breed between the two genetic lines (meat and egg) show an evident sexual dimorphism and an additional effect could be due to hybrid heterosis. In fact, the females had greater final live weight (+ 13.2 %) and giblets percentage (+2.16 %), while the males had higher carcass yield and abdominal fat percentage. However, the effect of sex on slaughter and carcass characteristics is well known in quail (reviewed in Alkan et al., 2013). Large reproductive organs in females, such as ovary and oviduct, and heavier liver are the main reason behind lower carcass yield in females (Lotfi et al., 2011; Sari et al., 2011). The lower abdominal fat showed from females, which is consistent with other studies (Banerjee, 2010; Alkan et al., 2013), may be attributed to the fact that the abdominal fat occupies space which would otherwise be available for the development of the yolk needed for egg production (Banerjee, 2010). Furthermore, Le Bihan-Duval et al. (1998) reported that the differences in fatness between sexes are probably due to the greater competition between males, different nutritional requirements and greater effects of hormones for fatness in females. The abdominal fat values, found in the present study, are higher than those obtained by Banerjee (2010) in Japanese quails slaughtered at 50 d of age (4.04 and 0.75 % for males and females, respectively), the same can be attributed to the genetic makeup of the strain and also due to different age or better nutritional regime; that fact is of great importance for the modern quality and dietary assessment of quail meat. Crespo and Esteve-Garcia (2002) found that the size of separable fat depots in chicken can be modified by dietary fatty acid profile because fats rich in polyunsaturated fatty acids produce smaller fat depots than those rich in saturated or monounsaturated fatty acids; however, this effect is not produced in the rest of body lipids. The growth pattern of fat depots can be modified by dietary fat, suggesting that distribution of dietary fat within adipose tissues depends on the fatty acid profile. Thus, birds fed diets rich in saturated fatty acid from animal origin tend to deposit

proportionally more fat in abdominal and mesenteric fat depots than in the rest of the body adipose tissues.

The analyzed data from F_2 cross-breed, obtained from males and females of the F_1 generation, had the same trend as in the F_1 generation, with a more marked sexual dimorphism. In the present study, we did not observe any effect of the hatch on growth, carcass traits and pH. These results disagree with those reported in literature (Vali et al., 2005; Lotfi et al., 2011). Vali et al. (2005) observed that quails of fourth hatches at 35, 42 and 49 d of age were heavier than those of previous hatches. Lotfi et al. (2011) reported that hatch has a significant effect on growth and carcass traits in Japanese quail at 42 and 91 d of age. The authors observed that the mean values of studied traits were higher in birds with older mothers than those with younger mothers. Older hens compared with younger ones lay larger eggs that hatch into larger chickens, and egg weight and hatching weight of chickens is correlated to final BW and carcass traits (Peebles et al., 1999). Our inconsistent results with the literature may be due to the short period between the two hatches (14 d) and to the age of the mothers that was almost similar.

The comparison of performance traits among the three generations (Figures 6.1 and 6.2) showed an evident phenotypic variation. The cross between two genetically distant lines as well as the cross between full siblings hybrids did not influence the BW of hybrid males but had a negative effect on their carcass weight. Instead, hybrid females were heavier than parental line (S-22): F_1 hybrids had an increase of BW of 23.9 %, while F_2 showed an increase of 31.4 %. Also the carcass yield was negatively affected by first and second generation crosses. Legs and giblets percentage were also affected by crosses but no significantly influence was observed on the breast yield. However, the breast weight (data not showed) was lower in both F_1 and F_2 males than in males of parental line (33.78 ± 0.73 g and 34.74 ± 0.38 g *versus* 38.53 ± 0.64 g, respectively; $P < 0.01$). Conversely, the breast weight of F_1 and F_2 females was heavier compared to female of parental line (34.88 ± 0.91 g and 36.87 ± 0.52 g *versus* 30.06 ± 0.79 g, respectively; $P < 0.01$). Hybrid combinations increased the abdominal fat percentage in F_2 females (+ 1.7 %), whereas it decreased in F_2 males (- 1.83 %). Excessive fat has been recognized as an undesirable correlate of selection for rapid growth and high live BW (Deeb and Lamont, 2002). These results therefore provide evidence of a significant positive heterosis in the F_2. The present findings are partially consistent with Deeb and

Lamont (2002) who found a high level of heterosis only for the abdominal fat percentage in the F_2 chicken population. As suggested by Sohrabi et al. (2012), a possible explanation for the higher level of heterosis in the F_2 generation might be related to the genetic structure of the F_2 birds as regards the sex chromosomes.

Ultimate pH of breast muscle was different among the three experimental groups, ranged from 5.61 to 5.79. However, contradictory data regarding pH exist. Narinc et al. (2013) and Genchev et al. (2008a) reported higher pH values (5.94 and 6.17, respectively). Genchev et al. (2008a) suggest that the main reason behind the high pH values found in quail breast muscle were due to the morphology of muscle which is mostly composed by aerobic - red muscle fibers. On the contrary, other studies (Remignon et al., 1998; Ribarski and Genchev, 2013), according to our findings, reported low pH_u value. Nevertheless, poultry meats with pH_u between 5.7 and 6.1 are called normal and do not reveal any quality problems (Zhang and Barbut, 2005; Zhang et al., 2005).

7.2 IMC properties

Meat is a complex, composite substance. It consists of myofibers, connective tissue and lipids. It has been established that collagen, the major component of the intramuscular connective tissue, plays a key role in determining the background toughness of meat from different domestic animals including birds (reviewed in Maiorano et al., 2012). Furthermore, a marked difference in collagen maturity could affect meat tenderness (McCormick, 2009; Maiorano et al., 2011, 2012) and technological yield (Boutten et al., 2000). The results of IMC properties showed a similar collagen content (μg/mg) of the breast muscle between the two lines (F-33 and S-22), while a higher degree of collagen maturation (mol HLP/mol of collagen) was found in the meat type quails. These findings are in contrast with the results of Maiorano et al. (2009, 2011) that reported no effect of genetic lines on intramuscular collagen properties in the breast muscle of young quails.

The comparison among the three experimental groups showed interesting results regarding the IMC properties of the breast muscle in both males and females (Figures 3a and 3b). To our knowledge, no information is available from current literature on the effect of sex or hatch on IMC properties in quails. Differences in collagen content and maturity between the gender of animals, attributed to hormonal effect, has been reported

in findings on pork, beef, lamb and deer (reviewed in Maiorano et al., 2013). However, the question of sex differences in terms of IMC seems to vary with species. Differently, it is not easy to explain the higher amount of IMC found in quails of hatch 2 (+ 18.85 %) compared with those of hatch 1. Although, according to Laurent et al. (1978), this trend could reflect the relationship between the synthesis of total muscle protein and intramuscular collagen synthesis; in fact, we found a positive correlation between muscle total protein (data not published) and IMC amount ($r = 0.193$; $P < 0.01$).

In general, HLP values were inversely proportional to the amount of collagen (Maiorano et al., 2009). The steady increase in mature collagen crosslinking is due to progressive and ongoing crosslinking reactions that occur within fibrillar collagen with the slowing of collagen synthesis rates as animals reach maturity. Less collagen synthesis and turnover provide existing fibrillar collagen time to progressively crosslink or mature (McCormick, 1994).

The quality of collagen gives toughness to the meat (Corò et al., 2003). Lepetit (2007) analyzed various studies in which collagen crosslinks in muscle tissue were measured. He suggested that measurement of crosslinks (pyridinoline) is a reasonable predictor of tenderness. According to HLP crosslinks values, the meat produced from F_0, F_1 and F_2 could be different in background toughness. In other words, results of HLP crosslink indicate that meat from F_0 quails could be tender than that from the F_1 and F_2 groups and that from F_2 quails could be tough than that from F_1 quails. The comparison between the IMC properties of the present study and those reported by Maiorano et al. (2011) for pectoral muscle of Japanese quail (meat line and egg line) and for other quail breeds, slaughtered at 35 d old, revealed that the collagen content in the muscle of quails used in this study is lower (may be due to a slower collagen synthesis) and, on the contrary, the average of collagen maturation (0.146 and 0.161 HLP/collagen for females and males, respectively) is comparable to the degree of collagen stability observed in above mentioned birds.

Moreover, values for collagen content (ranging from 8.48 to 14.14 µg/mg) and HLP crosslinks (ranging from 0.098 to 0.221 mol HLP/mol collagen) are lower and higher, respectively, to the values reported in pectoral muscle of 6 wk old control broiler chicken (Ross 308) (Maiorano et al., 2012). It is known that collagen synthesis and maturation differ between species, muscles, animal age, growth rate and management practices (McCormick, 1994; Purslow, 2005).

7.3 Fatty acid composition of quail breast muscle

The composition of tissue triacylglycerols (the storage form of fatty acids) is a combination of dietary fat composition, absorption efficiency of those dietary fatty acids, post-absorptive modification of dietary fatty acids, *de novo* synthesis, and potentially other process such as preferential mobilization and preferential oxidation of certain fatty acids by muscles (reviewed in Price, 2010).

Very few studies on the lipid profile of Japanese quail meat have been published, and available data are not directly comparable because they refer to animals of different age at slaughter, fed different diets, or because they are determined on using different analytical methods. However, the obtained data from the present study are partially consistent with those reported in literature. The results on fatty acid composition of F_0 generation showed a marked difference on the proportion of several fatty acids in the breast muscle between meat line and egg line quails. In particular, egg line quails supply meat with higher total SFA amount compared to meat line quails, probably due to differences in lipid metabolism mainly associated with egg production activity of females. In fact, several sex-related differences may be explained by the physiological changes in metabolism in female birds due to egg laying: during the laying period, the hepatic synthesis of triglycerides, phospholipids, and cholesterol is increased (reviewed in Scholtz et al., 2009). These results are consistent with other studies conducted on Japanese quail slaughtered at different ages. Genchev et al. (2008a) found an amount of 34.1 % of total SFA in breast muscle from 35 d old Japanese quail. Similar values were reported by Rule et al. (2002) on chicken breast muscle (34.7 %). On the contrary, Boni et al. (2010) found lower value in young quail (8 weeks old, 25.8 %) and in spent quail (8 months old, 29.7 %), compared to the values found in this work and also in the above mentioned studies. These differences could be due to the different anatomical part analyzed which consisted in a bulk samples mechanically deboned. Moreover, meat line quails displayed a total content of muscle SFA lower than that reported by Genchev et al. (2008a), but similar to that found in spent quails by Boni et al. (2010).

The most abundant saturated fatty acids found in quail breast meat were the palmitic acid (C 16:0), followed by stearic acid (C 18:0) and lower amount of lauric (C 12:0) and myristic (C 14:0) acids. The proportions of the single SFA found in this study were consistent with those reported in literature on quails (Botsoglou et al., 2004; Genchev et al., 2008a; Boni et al., 2010) and on chicken (Rule et al., 2002). Myristic and palmitic

acids are the main fatty acids behind the cholesterol elevating effect. Stearic acid is partially converted to oleic acid *in vivo* and has not been shown to elevate blood cholesterol. Myristic and palmitic acids are common fatty acids in dairy products and meat. In meats, myristic acid contributes usually around 3–6 % of the total fatty acids (Valsta et al., 2005). However, chicken meat as well as quail meat is characterized by significantly lower content of myristic and stearic fatty acids compared to other meat animals (Rule et al., 2002; Valsta et al., 2005).

Higher muscle MUFA content was found in meat line quails (+11.5 %) compared to that of egg line, due to a significantly higher amount of oleic (C 18:1) and palmitoleic (C 16:1) acids, which are the desaturation products of stearic and palmitic acids, respectively. The amount of total MUFA and of single MUFA detected in meat line quails were higher than those reported in literature; while the amount detected in egg line quails were consistent with data in literature (Rule et al., 2002; Botsoglou et al., 2004; Genchev et al., 2008a; Boni et al., 2010).

Total PUFA content detected in quails of F_0 generation, higher in egg line quails, was similar to the results reported by Genchev et al. (2008a) and Botsoglou et al. (2004), differently, Boni et al. (2010) reported a significantly higher content of PUFA (41.9 % and 42.7 % for young and spent quails, respectively), probably due to the presence of skin in the bulk samples which is particularly rich in PUFA or due to a different nutritional regime of quails. In fact, the PUFA content in chicken tissues depends more on the variation in dietary fatty acid content than the SFA and MUFA contents in these tissues (López-Ferrer et al., 2001). The behaviour in the deposition of MUFA and SFA content in tissues showed an inverse relationship to the one shown for PUFA when dietary PUFA was modified (Cortinas et al., 2004).

The amount of linolenic acid, the most abundant PUFA detected in quail breast muscle, was lower than those reported in literature (Botsoglou et al., 2004; Genchev et al., 2008a) but similar to those reported in chicken breast muscle by Rule et al. (2002). Muscle contains also significant proportions of long chain (C20-22) PUFAs which are formed from 18:2 n-6 and 18:3 n-3 by the action of Δ5 and Δ6 desaturase and elongase enzymes. Important products are arachidonic acid (20:4 n-6) and eicosapentaenoic acid (EPA, 20:5 n-3) which have various metabolic roles including eicosanoid production (Wood et al., 2008). Breast muscle of the studied quails contained higher content of

arachidonic acid than those reported in literature (Botsoglou et al., 2004; Genchev et al., 2008a; Boni et al., 2010), while EPA was detected only in trace amount.

The implication for human nutrition is that meat from quails present a good fatty acid profile: high P/S ratio, as well as low atherogenic index (AI) and thrombogenic index (TI).

The analyzed data from F_1 cross-breed between the two genetic lines (meat and egg) didn't show significant differences in fatty acid composition and in the relative ratios, except for C 12:0 and C 20:1 fatty acids. On the contrary, in the F_2 generation females were characterized by higher PUFA content.

The comparison among the three experimental groups showed interesting results regarding the fatty acid composition of the breast muscle in both sexes. In fact, the total content of SFA was lower in muscle hybrid females (- 7 % in F_1 and F_2) compared to female of parental line; while the breast of males of three generations had a similar amount of SFA. The total MUFA amount was higher in both F1 males and females suggesting a positive heterosis in the F1 generation, especially for females. In pigs, it was reported that the major presence of oleic acid in adipose tissues suggests a major activity of stearoyl-CoA Δ9 desaturase (Kouba et al., 1997; Kouba and Mourot, 1998) that corresponds to a lower content of stearic acid in the same tissues.

On the contrary, the total PUFA content, as well as the total n-6 fatty acids amount, were negatively affected by first generation cross, being higher in F_2 hybrids (males and females). 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 the first and second generation crosses. In particular, the P/S ratio was highest in F_2 quails due to the greater contribution of linoleic acid. Compared with muscles of the other species, quail and chicken breast had higher linoleic and arachidonic acids and lower stearic acid, which were largely responsible for the highest P/S ratios observed in these species (Rule et al., 2002; Wood et al., 2003). From nutritional point of view, a higher P/S ratio is recommended, indeed it should be increased to above 0.4. Infact, because some meat naturally have a P/S ratio of around 0.1, meat has been often implicated in causing the imbalanced fatty acid intake of today's consumers (Wood et al., 2003). Regarding the n-6/n-3 ratio both F1 males and females were characterized by the highest ratio, due to the lower content of both n-3 and n-6 PUFAs. Moreover, the n-6/n-3 ratio found in breast meat of F_0 and F_1 generations was similar to that reported for chicken breast meat by

Rule et al. (2002), but significantly higher to that reported for ostrich meat (Girolami et al., 2003). In general, poultry meat is characterized by the highest n-6/n-3 ratio compared to other meats, essentially due to the higher amount of n-6 fatty acids than muscles of the other species (Rule et al., 2002; Wood et al., 2003). Infact, linoleic acid is the predominant essential fatty acid (EFA) in poultry and as a result the n-6 PUFA are the primary products found in tissue lipids. Consequently, the ratio n- 6/n-3 in poultry meat is well away from the ideal value of 1 and above the recommended maximum of 4. However, the fatty acid profile of poultry meat can be altered by inclusion of n-3 fatty acid in the diet of the birds (López-Ferrer et al., 2001; Cortinas et al., 2004; Bou et al., 2009; Al Daraji et al., 2011; Kouba and Mourot, 2011). Infact, when significant amounts of α-linolenic acid (C 18:3 n-3) are fed to chickens, the n-3 PUFA increase. Although desaturase activities regulate tissue concentrations of fatty acids, especially for PUFA, dietary lipid can dictate fatty acid composition in poultry. Varying the type and amount of dietary unsaturated fat dramatically modifies the fatty acid composition of lipids in hen egg yolk and in the tissues of growing chicks. Feeding linseed oil, which is rich in α-linolenic acid, to chicks depresses the amounts of arachidonic acid, but concomitantly raises levels of eicosapentaenoic acid in organ lipids, presumably by enhancing n-3 PUFA formation (reviewed in Watkins, 1991).

In spite of the high n-6/n-3 ratio found in Japanese quails, the atherogenic index (AI); and thrombogenic index (TI) were very low. These indices take in account the different effects that the single fatty acid might have on human health and in particular on probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation (Ulbricht and Southgate, 1991). Hybrid females (F_1 and F_2) showed a significant lower indices compared to parental line. The data of this study are lower than those reported by Castellini et al. (2006) in chicken meat, confirming that quail meat could be an interesting meat also from the nutritional point of view.

7.4 Muscle cholesterol content

In addition to total lipids, cholesterol is a nutritionally important component of meats, varying between about 38 and 123 mg/100 g of edible portion, or even higher in offals (Chizzolini et al., 1999). There has been growing interest over recent years in the modulation of the fatty acid composition and cholesterol content of poultry products, because high saturated fatty acid and cholesterol intakes are considered as risk factors in

the aetiology of cardiovascular disease (Skřivan et al., 2000). Infact, much research has been focused to reduce fat, cholesterol, and SFA contents of poultry meat by dietary supplementation of garlic, copper, α-tocopherol, and n-3 fatty acid (reviewed in Salma et al., 2007). However, the modulation of the fatty acid composition of poultry meat by dietary means is relatively easy, but the reduction of cholesterol concentration is more difficult (Skřivan et al., 2000).

We were able to find limited information in literature on the cholesterol content of the meat from Japanese quail (Genchev et al., 2008a; Maiorano et al., 2009, 2011). According to our best knowledge, no research has yet been conducted to study the effect of crossing two strains of Japanese quail on muscle cholesterol content of the F_1 and F_2 generations.

In the F_0 generation genotype did not significantly affect muscle cholesterol content, being similar between meat and egg line quails. Similarly, Maiorano et al. (2009) didn't find any statistically significant difference among the birds of egg line and meat line. In addition, Maiorano et al. (2011) did not report any significant effect due to genotype, studying quails of different genetic groups. Also in the F_1 generation muscle cholesterol content was not influenced by sex. Moreover, the obtained values from breast muscle of quails of F_0 and F_1 generations were higher than those reported in literature by Maiorano et al. (2009, 2011). In fact, Maiorano et al. (2011) recorded a cholesterol level of *Pectoralis superficialis* (PS) muscle, in quail slaughtered at 35 days of age of different lines, ranging from 23.57 to 37.20 mg/100 g, which in turn was lower than the cholesterol level found by Maiorano et al. (2009) in breast muscle of 35 days old Japanese quail, ranging from 27.83 to 43.38 mg/100 g. The higher muscle cholesterol content found in the present study could be due to the greater slaughter age and/or to different nutritional requirements. Moreover, the muscle cholesterol content determined in the present study was similar to those reported by Genchev et al. (2008a) in 42 days old Japanese quail (ranging from 68 to 75 mg/100 g). Comparing quail meat with other poultry meat, we found that the results from the present study are similar to those reported by Maiorano et al. (2012) for 42 days old broiler chickens (ranging from 70.45 to 78.12 mg/100 g), but higher than those reported by Pilarski et al. (2005) in breast muscle of 42 days old broiler chickens (ranging from 49.3 to 54.7 mg/100 g). On the contrary, Salma et al. (2007) observed higher cholesterol values (93.6 mg/100 g) in *Pectoralis major* muscle of 56 days old broilers. Furthermore, significant and

interesting differences have been reported in cholesterol content between muscle types. Infact, Smith et al. (1993) reported that duckling pectoralis muscle have more cholesterol (99.11 mg/100 g) than broilers pectoralis (47.41 mg/100 g). Duckling pectoralis muscle (likewise quail pectoralis muscle) contains a high proportion of red fibers that are smaller in transverse area than white fibers. Therefore, a duckling pectoralis muscle of similar weight to that of a broiler would be composed, proportionally, of more (red) fibers. An increase of fiber number within a muscle would increase the total sarcolemma perimeter to fiber per volume ratio and, therefore, cholesterol content. In addition, the oxidative muscles are richer in phospholipids which in turn are rich in cholesterol. The relationship between cholesterol and phospholipids would be largely explained by the physical effects of cholesterol on the ordering of phospholipid chains contributing to maintain the membrane fluidity in a narrow range (reviewed in Chizzolini et al., 1999).

Anyway, differences in muscle cholesterol content depend in great extent to slaughter age, diet, sex and breed (Wang et al., 2005), but also on the use of different methodologies for cholesterol quantification or for sampling (Bragagnolo and Rodriguez-Amaya, 2002). Komprda et al. (1999) also found that the cholesterol content and fatty acid composition of chicken tissues were influenced by rate of growth. Cholesterol in breast and thigh muscles, however, tended to decrease with increasing growth rate.

The analyzed date from F_2 cross-breed showed a sex-related differences on muscle cholesterol content, which was higher in F_2 males; anyway we didn't observe any hatch effect on muscle cholesterol content in quails of F_2 generation.

The comparison among the three experimental groups showed interesting results regarding the cholesterol level in breast muscle in both males and females. To our knowledge, no information is available from current literature on the effect of first and second generation cross on muscle cholesterol. In light of this, it's not easy to elucidate the reason why hybrids (both F_1 and F_2 females and only F_1 males) showed lower muscle cholesterol content compared to parental lines. A possible explanation for the decreasing amount of cholesterol level in hybrid quails maybe related to differences in total muscle fat content but also on the level of heritability of muscle cholesterol. Anyway, additional studies such as QTL study are required to understand the genetic basis of variability of such trait.

7.5 QTL results

To our knowledge, QTL for intramuscular collagen is the very first report of a QTL in quail and other poultry species for this trait. Intramuscular collagen is composed mainly of different proportions of Type I and Type III collagen. When proportions of Types I and III collagen from different muscles have been compared, increased proportions of Type III collagen have been associated with, in some cases, diminished tenderness or in others with increased tenderness (McCormick, 2009; reviewed in Maiorano et al., 2011). A single report of QTL for collagen content concerns limousine cattle. This QTL has been detected on BTA2 and is attributed to myostatin gene (Lines at al., 2009). Quail linkage maps contain mostly microsatellite (Roussot et al., 2003) and AFLP markers (Kayang et al., 2004). Therefore to look for biological and positional candidate genes we have used alignments of Japanese quail microsatellite and AFLP maps linked to assembled chicken sequence (Kayang et al., 2006). A QTL region linked with intramuscular collagen in the quail experimental population shows synteny with chicken chromosome 2 (GGA2, region: 14.8 – 46.4 Mbp). The most probably candidate gene in this region is *COL1A2* (LOC396243), collagen type I alpha 2, chain precursor. According to Gene Cards (www.genecards.org) this gene encodes the pro-alpha2 chain of type I collagen whose triple helix comprises two alpha1 chains and one alpha2 chain. Type I is a fibril-forming collagen found in most connective tissues. *COL1A2* gene has been reported in other studies in chicken (Sun et al., 2013) and pig (Li et al., 2010; Lobjois et al., 2008). Sun et al. (2013) proposed *COL1A2* as a candidate gene for meat color lightness based on the association study of a single SNP located 58.0 Kb away from the collagen type I alpha 2 gene. However, in the study by Sun and co-authors intramuscular collagen was not included on the list of meat quality traits. Lobjois et al. (2008) proposed *COL1A2* as a positional and biological candidate gene for meat tenderness in the study of complex traits in pig. A QTL for intramuscular collagen is in agreement with differences in phenotypic values between two parental lines (Table 6.1). A positive value for the additive effect implies that the Pharaoh (F-33) meat type male parent allele results in an increase in phenotype. Lack of QTL for other traits (final body weight, carcass yield, giblets, abdominal fat) under the study in current experimental cross might be explained by the limited number of chromosomes genotyped.

To our knowledge, QTL for breast weight is the very first report of such a QTL in Japanese quail. There are three QTL for breast muscle weight reported on chicken

chromosome 2 (Chicken QTL data base), however their location doesn't agree with comparative region between quail and chicken genomes. The higher dominant compare to additive effect for this QTL suggests that heterozygous individuals have bigger breast muscle weight.

PART 6

Chapter 8

CONCLUSION

This work contributes to existing knowledge on Japanese quail (*Coturnix japonica*) by providing new data regarding three generation cross of two Japanese quail lines (meat line and egg line) with respect to analysis of growth, carcass and meat quality traits.

This study has shown that quails of meat line (F-33) were significantly heavier than those of the egg line (S-22) and they had higher carcass weight, carcass yield and abdominal fat percentage; differently, giblets percentage and meat pH were higher in egg type quails. Breast muscle and legs yield, as well as the IMC amount did not differ significantly between the two lines. However, meat of the egg line had a slower collagen maturation (HLP/collagen) leading to a variability in meat tenderness. A marked difference on the proportion of several fatty acid was found between the two lines. Egg line quails has supplied meat with higher total SFA amount but also with a higher total PUFA content compared with meat line quails; on the contrary, the latter had higher total MUFA amount. No difference were found for P/S ratio and muscle cholesterol content between lines, even if meat line quails has supplied meat with lower atherogenic and thrombogenic indices.

The F_1 and F_2 generations showed an evident sexual dimorphism and an additional effect could be due to hybrid heterosis. Both females of F_1 and F_2 generations were heavier than males and had higher giblets percentage, while males showed a higher carcass yield and abdominal fat percentage. In the F_2 generation none hatch-effect was observed on growth, carcass traits and meat pH. The IMC amount was not influenced by gender in both generations, even if meat from F_1 females had higher degree of collagen maturation. In addition, in the F_2 generation, a significant hatch-effect was found for the IMC amount and the degree of collagen maturation. In the F_1 generation the fatty acid composition and the relative ratios, as well as the muscle cholesterol content, were similar between sexes. On the contrary, in the F_2 generation females were characterized by higher total PUFA content and consequently higher P/S ratio, but

lower muscle cholesterol content compared to males. None hatch effect was found on muscle cholesterol content in quails of F_2 generation.

The comparison of performance traits among the three generations showed an evident phenotypic variation. The cross between two genetically distant lines, as well as the cross between full siblings hybrids did not influence the body weight of hybrid males but had a negative effect on their carcass weight. Instead, hybrid females were heavier than parental line (S-22): F_1 hybrid had an increase of body weight of 23.9 %, while F_2 showed an increase of 31.4 %. Our results provide an evidence of a marked positive heterosis in the F2. Observed effect might be a parent of origin sex chromosome effect, which unfortunately cannot be fully investigated due to experimental design, which didn't account for a reciprocal cross. According to HLP crosslinks values, the meat produced from F_0, F_1 and F_2 could be different in background toughness. In addition, first and second generation cross affected the proportion of several fatty acids and the relative ratios. The total content of SFA was lower in muscle hybrid females (F_1 and F_2) compared to female of parental line, while the SFA amount between males was similar among the three generations. The total MUFA amount was higher in both F_1 males and females, suggesting a positive heterosis in the F_1 generation, especially for females. On the contrary, the total PUFA content, as well as the total n-6 fatty acids amount were higher in F_2 hybrids in both sexes. The P/S ratio was highest in F_2 quails, while both F_1 males and females were characterized by the highest n-6/n-3 ratio. Hybrid females (F_1 and F_2) showed a significant lower AI and TI compared to parental line. Interestingly, both F_1 and F_2 females and only F_1 males showed a considerably lower muscle cholesterol content compared to parental lines.

The findings of this study suggest that Japanese quail produced meat that is nutritionally comparable to that from chicken, with a good fatty acid profile, a high P/S ratio and very low atherogenic and thrombogenic index. In addition, even if the muscle cholesterol content reported in this study was not so low, meat from hybrid showed an interesting decreasing of muscle cholesterol content.

In light of these first results, in order to extend the knowledge, today still very scarce, on this alternative meat which in recent years has been gaining popularity among consumers, further experimental investigations are needed to:

i) better understand the genetic basis of variability of these traits among different generations;

ii) increase knowledge regarding the effect of different lines (meat type and egg type quails), cross (meat type x egg type quails; F_0 x F_1) and gender (males and females) on growth performance, carcass traits and IMC properties in adult Japanese quails.

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