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PRESERVATION AND DIFFUSION OF SOME NATIVE ITALIAN CHICKEN BREEDS

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CONTENTS

SUMMARY

The safeguard of animal biodiversity is a strong objective in developed countries. The genetic variability gives the chance to select individuals more adapted to climatic changes, diseases and potential market variations. Because of the different environments, up to decades ago, Italy showed a considerable biodiversity in native livestock breeds and populations. Within the last one hundred years, the number of endangered native breeds dramatically increased, leading to an irreversible loss of genetic resources. About 60% of chicken breeds reared in Italy until some decades ago are currently disappeared. The reason of this trend is mainly due to the selection of specialized breeds very efficient in converting feed into egg and meat. Contemporarily, the abandoning of rural breeding in favour of intensive farming system which uses few selected chicken lines exacerbates such negative trend. The extant breeds, excluded from commercial selection process, represent an important source of variability. The main critical point of local breeds is generally the low productivity which implies that a large part of body resources are used for maintenance (kinetic activity, forage behavior, immune response) and only the residual are assigned to production traits. This fact implies a low productivity but in the same time renders the animal adaptable to poor environment; often such breeds have a distinct metabolic pathways (digestive, fatty acid) with an inference on muscle and eggs characteristics. In particular seems that such pure breed had more aptitude to elongate and desaturate essential fatty acids (linoleic acid, n-6 and α-linolenic acid, n-3) in their long chain derivates (arachidonic acid-AA, eicosapentaenoic acid-EPA, docosahexaenoic acid-DHA), with a consequently greater accumulation on products. This would represent an important goal for the livestock word, since it provides a compromise: between rusticity and economic sustainability and designs a healthier chicken meat for nutritional-conscious consumers, also in accordance with a big problem of the safeguard of the biodiversity.

The **aim of the present PhD work** was to study the adaptation response of local chicken breed to extensive farming conditions. The experimental activity is showed in three chapters where we analyzed the behavior and welfare of different poultry breeds in extensive farming system and the quality of the products (meat and eggs) with a focus on lipid content and the metabolism of Long Chain Polyunsaturated Fatty Acids (LC-PUFA). It was also studied the metabolic pathways of LC-PUFA and how different genotypes respond to feed changes testing the hypothesis that nutrition can influence the ontogenetic development.

In detail the effect of extensive farming systems which implies large availability of pasture on different chicken genotypes (local breeds *vs* commercial hybrids) was compared. The main traits that have been evaluated are: productive performance, health and welfare, immune response and qualitative characteristics of product. Different physiological (IMMUNITY: lysozyme, complement, serum bactericidal activity; OXIDATIVE STATUS: ROS, antioxidant power), and ethological indicators (behavior, tonic immobility, body lesions) have been used for this purpose. Nutritional characteristics of eggs and meat, with particular attention to oxidative and fatty acid profile were studied through the evaluation of TBARS, tocopherols, carotenoids, polyphenols, PUFA n-3 and n-6 content. Great emphasis has been paid to the study of LC-PUFA since local strain seemed more efficient in the synthesis of these fatty acids.

The **first chapter** includes experiment 1 and 2 for assessing the adaptive response of local chicken breeds in comparison to commercial strains. Welfare, behavior and physiological state of two egg-type hens (Ancona *vs* Brown Hy-line), and three meat-types chicken (slow-, medium-and fast-growing) have been evaluated.

In the **second chapter** (experiment 3 and 4) the quality of eggs and meat were analysed in different experimental groups, as described in 1st chapter.

Finally, in the **third chapter** (experiment 5, 6 and 7) the response of different genotypes to feed changes has been studied, testing the hypothesis that diets can regulate the expression of key genes (Δ6-desaturase) in the PUFA biosynthetic pathway.

In particular in the experiment 1 the adaptive response of laying hens to extensive farming system has been analyzed. Welfare, behavioral and production performance of Ancona laying hens and Brown Hy–line hens reared under organic system have been evaluated. Ancona birds showed a specific ethological profile associated with very good welfare conditions, better immune system and oxidative status. The Ancona breed seems more adapted to less controlled environment and showed a higher percentage of "natural" behavior. Such behaviors are high in energy cost, allowing the more selected birds to save energy which could be reallocated to production traits. At the same time, the lower productivity of Ancona birds resulted in a better balance with the extensive environment, as evidenced by health status and mortality rate.

In the experiment 2 the meat traits of six hundred male chicks from slow-growing (Leghorn, Ancona, Cornish x Leghorn), medium-growing (Naked Neck, Kabir) and fast-growing (Ross) strains have been studied. The physiological state of the birds appears to be inversely correlated with the genetic selection, indeed the slow-growing showed more marked natural behaviors, followed by the medium-growing. The fast-growing genotypes showed an ethological and immune profile of pain. This trial shows that fast-growing birds, selected for intensive farming, have superior productive performance but the alteration of behavioral and physiological characteristics render these strains unusable in extensive rearing system since they are not consistent with the principles of good welfare, quality and biodiversity. On the contrary, slow- and medium-growing genotypes showed a good adaptation to extensive environment. Further, the trial showed that the slow-growth rate is only a prerequisite for adaptability to the extensive system; other traits as the grazing attitude, kinetic activity, body structure and the immune response greatly affect such adaptation.

It has been reported that the most relevant role of grass in organic poultry is represented by the intake of several bioactive compounds (i.e. PUFA, vitamins and pigments) with a direct effect on the quality of meat and eggs. For these reasons, it is important to determine the intake and the nutritional relevance of pasture to develop suitable free-range diets and to investigate the transfer of the above-mentioned compounds into the poultry products.

To this aim the **2nd chapter** studied the quality of eggs and meat products. In the experiment 3 eggs were collected during the four seasons in different phases of productive activity. Forty eggs per group were gathered at the same hour in winter, spring, summer and autumn. All the eggs were

stored at 5°C until the physical analyses (maximum 2 days after). Afterwards, the oxidative status, the fatty acids and bioactive compounds have been assessed. Ancona hens produced a lower number of eggs than commercial line but markedly different from the qualitative point of view. Indeed, Ancona eggs compared to Hy-line had higher carotenoid, polyphenol and tocopherol contents that in turn have a relevant effect on human health. Even the fatty acid profile improved (lower n-6/n-3 ratio; higher C18:3n-3 and LC-PUFA n-3); this latter fact confirms the ability of the hen to elongate/desaturate linolenic acid and to transfer n-3 LC-PUFA to the egg where exert important role during the chick growth. These differences were mainly due to the availability of green pasture and to the higher pasture attitude of Ancona hens.

In experiment 4 we analyzed the fatty acid and antioxidant profile of breast meat from chickens reared according to the organic system. The slow-growing strains are egg-type lines which seem to have a higher efficiency in LC-PUFA deposition respecting to meat-type, being that elongation is partly affected by the estrogen level. Concerning the content of saturated fatty acids (SFA), the highest value was observed in fast-growing genotype. However, the meat of slow-growing chickens had lower lipid stability despite higher antioxidant content probably due to the kinetic behavior and the resulting pro-oxidative metabolism. The results of this study indicate that in extensive farming chicken genotypes play an important role in the fatty acid composition of meat.

This finding assumes great importance because health concerns over human fat intake are one of the main factors contributing to the decline of meat intake. The observed differences among poultry genotypes indicate that a suitable compromise between rusticity and economic sustainability could be found and a healthier chicken meat for nutrition-conscious consumers could be designed.

The experimental design of the **3rd chapter** has been planned to verify the effect of genotypes on lipid metabolic pathways with the aim to improve the nutritional value of products. A relationship between genotype and desaturating ability was evidenced with a significant impact on the PUFA content in the meat. In the experiment 5 we have estimated the lipid indices of six chicken genotype organically reared. We can said that the differences in meat lipid content are affected by breed, and that the pure breed had a two time higher Δ5/Δ6-desaturase index value than medium and fast-growing strains.

To confirm our previously estimated, we have evaluated the direct measure of different enzymatic activity and gene expression of the above-mentioned complex in liver mitochondria in the experiment 6. Three groups of laying hens for each genotype (slow-, fast- and slow x fast-growing crossing) were fed with a standard diet. To hatch, 5 chicks/genotype were sacrificed and the liver was taken for enzyme activity and gene expression of Δ6-desaturase. Slow-growing chicken in comparison to fast-growing strain showed a higher desaturase activity. Data showed that the mRNA expression of FADS2 gene is strongly correlated with genetic selection. It seems to be higher in medium-growing strains although their enzyme activity was intermediate.

Finally, in the experiment 7 we have tested where possible to modified lipid metabolic pathway with dietary supplementation of precursor (linolenic acid) or directly LC-PUFA (EPA and DHA).

Three groups of laying hens for each genotype (slow-growing *vs* fast-growing) were fed with three different diets:

- control: standard diet *ad libitum,*
- LCPn-3: standard diet added with 3% fish oil (large amount of EPA/DHA - PRODUCTS)
- LNA: standard diet added with 10% of linseed (large amount of ALA - PRECURSOR).

To hatch, 5 chicks/dietary group/genotype were sacrificed and the liver was taken for enzyme activity and gene expression of Δ6-desaturase. The results showed that diet slightly affected lipid metabolism whereas the genetic effect was confirmed.

Data reported in this last chapter showed that the expression and activity of Δ6-desaturase is strongly correlated with the genotype, so reaching an important objective for the food industry, since dietary modifications do not seem able to change the lipid metabolism.

Concluding, my PhD work has the aim to assess the use of Italian local strains in extensive farming system, not just to safeguard the breeds from extinction, but also to exploit them economically, given the higher adaptability to poorer environment and the particular PUFA metabolism. They could represent an important source of gene bank, indeed selection processes and crosses of such strain with other more productive strains, could be a good alternative. In particularly, these genotypes could be exploited as suitable strains for the production of meat and eggs having higher nutritional value.

SOMMARIO

La salvaguardia della biodiversità rappresenta un importante obiettivo nei paesi industrializzati. La variabilità genetica rende possibile la naturale selezione di individui capaci di adattarsi ai cambiamenti climatici, più resistenti alle malattie e in grado di adeguarsi alle potenziali variazione di mercato. Grazie ai numerosi e differenti paesaggi ambientali, fino a pochi anni fa, l'Italia vantava un considerevole grado di biodiversità nelle razze da allevamento e nelle popolazioni. Nell'ultimo secolo, però, il numero delle razze autoctone in pericolo di estinzione è aumentato drammaticamente, portando ad una irreversibile perdita di risorse genetiche. Circa il 60% delle razze avicole allevate in Italia fino a pochi decenni fa, sono oggi estinte. Questo andamento è principalmente causato dall'eccessiva selezione di razze specializzate per l'elevata produttività. Allo stesso tempo, l'abbandono di sistemi di allevamento rurale in favore di quelli intensivi che utilizzano poche linee genetiche selezionate, ha contribuito al peggioramento. Perciò le razze esistenti, escludendo quelle ad uso commerciale, rappresentano un importante risorsa di variabilità.

Il principale punto critico delle razze autoctone è generalmente la bassa produttività, causata dal fatto che la maggior parte delle risorse alimentari vengono utilizzate dagli animali per il proprio mantenimento (attività cinetica, attività comportamentale, risposta immunitaria) e solo l'energia residua viene indirizzata ai caratteri produttive; allo stesso tempo però, tale comportamento, rende queste razze più adatte ad ambienti poveri. Spesso tali animali hanno un differente profilo metabolico (attività digestiva, composizione acidica) che influenza le caratteristiche qualitative dei prodotti. In particolare sembra che le razze pure abbiano una maggior attitudine ad elongare e desaturare acidi grassi essenziali (acido linoleico, n-6 e acido linolenico, n-3) nei loro derivati a lunga catena (acido arachidonico, AA; acido eicosapentaenoico, EPA e acido docosaesaenoico, DHA), con un ampio accumulo nella carne e nelle uova. Questo aspetto potrebbe rappresentare un importante obiettivo per il mondo dell'allevamento, dato che fornisce un buon compromesso tra rusticità, sostenibilità economica e richieste del consumatore-cosciente (carne più salature), in accordo con il grande problema della salvaguardia della biodiversità.

Lo scopo del presente lavoro di Dottorato è stato quello di studiare la risposta adattativa di razze avicole autoctone ad un sistema di allevamento estensivo. L'attività sperimentale è stata raggruppata in tre capitoli dove è stato analizzato il comportamento e lo stato di benessere delle razze in studio, in un sistema estensivo, nonché la qualità dei prodotti (carne ed uova) con particolare attenzione al contenuto lipidico e al metabolismo degli acidi grassi a lunga catena (LC-PUFA). È stato anche studiato il meccanismo metabolico degli LC-PUFA e come i differenti genotipi avicoli rispondono a cambiamenti alimentari testando l'ipotesi che l'alimentazione possa influenzare lo sviluppo ontogenetico.

Nel dettaglio, è stato valutato l'effetto di un sistema di allevamento estensivo che implica una grande disponibilità di pascolo, su differenti genotipi avicoli (razze autoctone e ibridi commerciali). Le principali caratteristiche analizzate sono state: performance produttive, salute e benessere, risposta immunitaria e caratteristiche qualitative di carne ed uova. A tale scopo sono stati usati indicatori fisiologici (immunità: lisozima, complemento, attività battericida sierica) ed indicatori etologici (comportamento, immobilità tonica, lesioni corporee). Sono state valutate, inoltre, le

caratteristiche nutrizionali di uova e carne, con particolare attenzione allo stato ossidativo e al profilo acidico , attraverso la valutazione di parametri quali TBARS, tocoferoli, carotenoidi, polifenoli, PUFA n-3 ed n-6. Grande attenzione è stata dedicata allo studio degli LC-PUFA dato che le razze pure sembrano avere una maggior efficienza nella sintesi di tali acidi grassi.

Il **primo capitolo** include l'esperimento 1 e 2 allo scopo di indagare la risposta adattativa di genotipi avicoli locali in comparazione con razze commerciali. Sono stati valutati il benessere, il comportamento e lo stato fisiologico di due razze di galline ovaiole (Ancona *vs* Hy-line marrone) e tre linee da carne (lento, medio e rapido accrescimento).

Nel **secondo capitolo** (esperimento 3 e 4) è stata analizzata la qualità delle uova e della carne in differenti gruppi sperimentali, come riportato nel primo capitolo.

Infine, nel **terzo capitolo** (esperimento 5, 6 e 7) è stata studiata la risposta di differenti genotipi avicoli ai cambiamenti alimentari, verificando l'ipotesi che la dieta possa influenzare l'espressione di alcuni geni (Δ6-desaturase) del metabolismo biosintetico dei PUFA.

In particolare, nell'esperimento 1 è stata analizzata la risposta adattativa di galline ovaiole ad un sistema di allevamento estensivo. Sono stati valutati il benessere, il comportamento e le performance produttive di galline ovaiole di razza Ancona e Hy-line marrone, allevate con sistema estensivo. La razza Ancona ha mostrato un profilo etologico associabile con buone condizione di benessere, una miglior risposta immunitario e un ottimo stato ossidativo. Questa razza sembra maggiormente adattabile a condizione ambientali poco controllate ed ha mostrato un'altra percentuale di comportamenti "naturali". Tali comportamenti hanno un elevato dispendio energetico, infatti, genotipi più selezionati preferiscono mantenere tale energia per distribuirla a livello delle caratteristiche produttive. Allo stesso tempo, però, la bassa produttività della razza Ancona, risulta in un miglior bilancio con l'ambiente estensivo, come dimostrato dal loro stato di salute e dal basso tasso di mortalità.

Nell' esperimento 2 sono state studiate le caratteristiche di seicento polli maschi da carne suddivisi in lento (Livorno, Ancona, incrocio Cornish x Livorno), medio (Collo Nudo, Kabir) e rapido accrescimento (Ross). Lo stato fisiologico degli uccelli è sembrato essere inversamente correlato con il grado di selezione genetica, infatti, i genotipi lenti, hanno mostrato comportamenti naturali più marcati, seguiti dai medi. Quelli a rapido-accrescimento invece, hanno mostrato un profilo etologico di sofferenza. Questa prova suggerisce che razze a rapido accrescimento, selezionate per l'allevamento intensivo, hanno performance produttive largamente superiori, ma le alterazioni del comportamento e lo stato fisiologico rendono queste razze inutilizzabili nell'allevamento estensivo, dato che ciò non è coerente con i principi di benessere, qualità e biodiversità, sul quale si basa questo tipo di allevamento. Al contrario, le razze a lento e medio accrescimento si sono meglio adattate al tipo di allevamento utilizzato. Inoltre, l'esperimento ha mostrato che il basso tasso di accrescimento è solo un prerequisito di adattabilità all'allevamento estensivo, altre caratteristiche come l'attitudine al pascolamento, l'attività cinetica, la struttura corporea e la risposta del sistema immunitario ne influenzano particolarmente l'adattabilità.

Molto rilevante nell'allevamento estensivo è anche il ruolo del manto erboso, in quanto rappresenta la fonte di molti composti bioattivi (i.e. PUFA, vitamine, pigmenti) con un effetto diretto sulla qualità della carne e delle uova. Per questa ragione, è importante determinare l'apporto e la rilevanza nutrizionale del pascolo per sviluppare un idonea dieta free-range e confermare il trasferimento dei composti precedentemente descritti, nelle produzioni avicole.

A tale scopo, il secondo capitolo riporta lo studio della qualità delle uova e della carne. Nell'esperimento 3, sono state campionate le uova durante le quattro stagioni, nelle differenti fasi di produzione delle galline, descritte nell'esperimento 1. Cinquanta uova per gruppo sono state raccolte alla stessa ora, in inverno, primavera, estate ed autunno. Tutte le uova sono state stoccate a 5°C fino all'espletamento delle analisi fisiche (massimo nei 2 giorni seguenti). Successivamente, sono stati valutati, lo stato ossidativo, la composizione in acidi grassi e la presenza di composti bioattivi. Le ovaiole di razza Ancona, hanno deposto un minor numero di uova rispetto alla linea commerciale ma marcatamente differenti a livello qualitativo. Infatti, tali uova avevano un elevato contenuto di caroteni, polifenoli e tocoferoli che hanno un effetto benefico sulla salute umana. Anche il profilo acidico è risultato migliore (bassi valori di n-6/n-3, alti livelli di C18:2n-3 e LC-PUFA n-3); quest'ultimo dato conferma l'abilità di queste galline di elongare/desaturare l'acido linolenico e trasferire i suoi derivati a lunga catena nell'uovo, dove esercitano un importante ruolo per lo sviluppo del pulcino. Le differenze qualitative delle uova, che si sono riscontrate, sono principalmente dovute alla disponibilità di pascolo verde, e all'alta attitudine pascolativa delle galline di razza Ancona.

Nell'esperimento 4 abbiamo analizzato la composizione acidica e il profilo antiossidante del petto di polli allevati secondo il sistema biologico, come descritto nell'esperimento 2. Le razze a lento-accrescimento, possono essere considerate linee adatte alla produzione di uova, sembrano, infatti avere un alta efficienza nella deposizione di LC-PUFA rispetto ai genotipi prettamente definiti da carne. Per quanto riguarda il contenuto in acidi grassi saturi (SFA), il maggior valore è stato riscontrato nelle razze pesanti. Tuttavia, la carne dei polli leggeri aveva una minor stabilità lipidica a dispetto dell'alto contenuto di antiossidanti, probabilmente a causa dell'elevata attività cinetica di questi animali e quindi del risultante equilibrio tra molecole pro e antiossidanti. I risultati di questo studio, indicano che nel sistema di allevamento estensivo, il genotipo avicolo utilizzato, gioca un importante ruolo sulla composizione acidica e sulla qualità della carne in generale.

Quanto riscontrato, diviene di grande importanza in rapporto al benessere umano. L'interesse crescente sull'effetto dell'assunzione di grasso per la salute umana, è infatti una delle principali cause della diminuzione dell'assunzione di carne. Le differenze osservate tra i genotipi avicoli, potrebbero suggerire un interessante compromesso tra rusticità e sostenibilità economica, permettendo al consumatore di assumere carne più salutare.

Il disegno sperimentale del **terzo capitolo**, è stato pianificato per verificare l'effetto del genotipo sul metabolismo lipidico con lo scopo di aumentare il valore nutrizionale dei prodotti. È stata evidenziata la relazione tra genotipo e abilità di desaturazione con un impatto significativo sul contenuto di PUFA della carne. Nell'esperimento 5, abbiamo stimato gli indici lipidici di sei genotipi allevati estensivamente. Abbiamo potuto affermare che le differenze nel contenuto

lipidico della carne sono strettamente correlate con la razza, e che le razze pure hanno valori di Δ5/Δ6-desaturasi tre volte maggiori rispetto ai genotipi a medio o rapido accrescimento.

Per confermare quanto detto, è stato necessario calcolare l'effettiva attività enzimatica e l'espressione genica del complesso enzimatica sopra stimato, nei mitocondri epatici, come riportato nell'esperimento 6. Tre gruppi di galline ovaiole per ogni genotipo (lento, rapido ed un incrocio lento x rapido accrescimento) sono stati alimentati con una dieta standard. Cinque pulcini per ogni gruppo, sono stati sacrificati alla schiusa, ed è stato prelevato il fegato per procedere con la valutazione dell'attività enzimatica e dell'espressione genica della Δ6-desaturasi. Il genotipo lento, in confronto a quello a rapido accrescimento, ha mostrato una maggior attività enzimatica. I dati hanno rilevato che l'espressione dell'mRNA del gene FADS2 è fortemente correlato con il grado di selezione genetica; sembra infatti essere molto maggiore nella razza incrocio, anche se l'attività enzimatica era risultata essere intermedia.

Infine, nell'esperimento 7, si è cercato di esaminare, se fosse possibile modificare il metabolismo lipidico, tramite l'arricchimento dietetico di PUFA precursori (acido linolenico) o direttamente con LC-PUFA (EPA a DHA). A tale scopo sono stati formati tre gruppi di galline ovaiole per ogni genotipo (lento e rapido accrescimento), ed alimentati con differenti diete:

- controllo: dieta tradizionale *ad libitum*,
- LCPn-3: dieta tradizionale arricchita con olio di pesce al 3% (ricco in EPA e DHA-PRODOTTI),
- LNA: dieta tradizionale arricchita con lino estruso al 10% (ricco in acido linolenico-PRECURSORE).

Alla schiusa, 5 pulcini per gruppo dietetico e genotipo, sono stati sacrificati e dal fegato è stata valutata l'attività enzimatica e l'espressione della Δ6-desaturasi. I risultati hanno confermato l'effetto del genotipo, mentre è stato riscontrato che la dieta influenza solo in piccola parte il metabolismo lipidico.

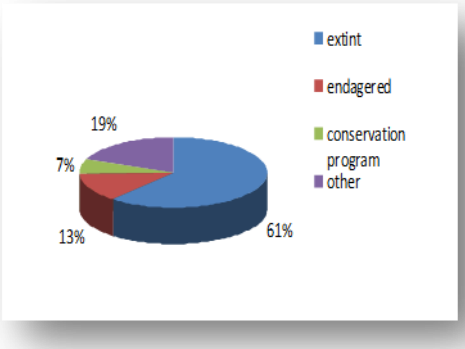
I dati ottenuti, in quest'ultimo capitolo, suggeriscono che l'espressione e l'attività della Δ6-desaturasi, sono fortemente correlata con il genotipo avicolo, raggiungendo così un importante obiettivo per il mondo industriale, dato che le modificazioni alimentari sembrano non comportare cambiamenti a livello metabolico.

In conclusione, il mio lavoro di Dottorato ha lo scopo di indagare il potenziale utilizzo di razze autoctone Italiane nell'allevamento di tipo estensivo, non solo per salvaguardare tali razze dall'estinzione, ma anche per valorizzarle a livello economico data l'elevata adattabilità ad ambienti poveri e, in particolare, alla maggior qualità dei prodotti. Esse potrebbero rappresentare un ottima banca genetica, infatti anche i processi di selezione ed incrocio di tali razze con altre a maggior produttività, potrebbe essere considerata una buona alternativa. In particolare, questi genotipi potrebbero essere valorizzati come linee adatte per la produzione di carne ed uova ad alto valore nutrizionale.

1. JUSTIFICATION AND OBJECTIVES

Animal genetic resources are an essential part of ecosystems and productive landscapes. Local breeds can be considered a part of the history of human populations as well as important biological materials. Such breeds have been subjected to natural selection imposed by endemic diseases, climate, nutrition and other factors. This has created a large diversity in morphological and physiological traits representing a source of genes for future breeding and research purposes (Bianchi et al., 2011). In Italy there are numerous native chicken breeds; however, 61% of breeds are extinct, 13.3% are endangered and only 6,7% are involved in conservation programmes (Figure 1., Castellini et al., 2011). Such decline is due to the abandoning of rural breeding and the diffusion of intensive farming system which uses only few chicken lines selected for productive performance. This selection was very effective but, in the meanwhile several alterations of behaviour, animal health and welfare emerged.

Figure 1. Risk status of Italian chicken breeds (Castellini et al., 2011).



Although the local breeds are not competitive for production traits, they could be used in extensive farming system (Castellini et al., 2005, 2008) which requires high kinetic activity and adaptation to less protected environments (Dal Bosco et al., 2011). Commercial chicken strains show a very low kinetic activity (< 20 m/day; Dal Bosco et al., 2010) in comparison to local chicks (about 1.5-2 km/day). Literature shows that broilers have severe muscle abnormalities (Sandercok et al., 2009) and the movement, which deeply affects metabolic, oxidative, and transcriptional response of muscle (Flück et al., 2005), enlarges these anomalies.
Although the organic system regulation (EC, 2007) and scientific papers (Hovi et al., 2003; Guéméné et al., 2009) suggest the use of indigenous breed, that have a slow-growing rate, however, fast-growing broiler genotypes, used in conventional rearing system, are mostly utilized in extensive poultry production system, due to economic reasons and limited chicks availability.

Nowadays the growth of "Conscious Consumption" is concerned not only with the quality of products but also to animal welfare. European consumers are increasingly looking for healthy lifestyles that imply eating healthy meals, with little fat content and good nutritional value but also they care for animal welfare.

Poultry is well placed to meet these qualitative demands. A recent U.S. survey data indicate that 63% of consumers are trying to consume less animal fat (International Food Information Council Foundation, 2009) and 41% of consumers decreased their consumption of beef (American Dietetic Association, 2008). The production of meat having a higher nutritional value and a healthy fatty acid profile is therefore a hot topic for the meat industry. Great attention is paid to essential fatty acid (EFA), linolenic (LA) and α-linolenic acid (ALA) which cannot be synthesized in the human and are therefore crucial components of our diet. Both LA and ALA may be converted by elongation and desaturation into their long-chain metabolites (LC-PUFA). The most important LC-

PUFA of the n-6 fatty acid series is arachidonic acid (AA, C20:4), whereas EPA (C20:5) and DHA (C22:6) are the major LC-PUFA n-3.
LC-PUFA n-3 may also derived from the diet and high contents are present mainly in fish and meat. AA and DHA are especially abundant in the brain and the retina and have relevant role in many physiological pathways and pathological disorder like: CHD, reproductive dysfunctions, depression, immune response. Human diets are retained unbalanced in term of total PUFA n-6 and n-3 with a too low proportion of the latter. Several studies assessed that humans are rather poor EPA and DHA synthesizers; as a result, seems that at least some LC-PUFA might be almost essential to humans (Burdge and Wootton, 2002; Burdge et al., 2002).

However, the increasing difficulty in finding renewable sources of LC-PUFAn-3, which are commonly obtained from fish are in contrast with its health benefits. Declining fish stocks caused by decades of overfishing makes urgent to find alternative dietary alternatives. Since LC-PUFA are usually absent from terrestrial higher plants, traditional agriculture can be excluded as viable sources of these fatty acids. Though this deficiency can be overcome by genetic engineering, transgenic foods are not always well accepted by the general public and thus food strategy supplementation and genetic selection should be developed to increase LC-PUFA n-3. It is known that fatty acids composition of meat and others animal products reflects the endogenous biosynthesis as well as the composition of the diet in every animal species. This relationship is stronger in monogastrics (pigs, poultry and rabbits) than ruminant. Several trials have demonstrated that it is possible to enrich poultry products (meat and eggs) with LC-PUFA through dietary strategies (Rossi et al., 2013; Fraeye et al., 2012; Woods and Fearon, 2009; Meluzzi et al., 2001). High dietary concentration of ALA increases the concentration of this FA in the poultry meat, whereas EPA and above all DHA have only a small increase (Rymer and Givens, 2005). These evidences indicate a relatively low efficiency of the desaturating enzymes in standard chicken, and this allow for further consideration on the selection of genotypes with enhanced desaturating activity.
In recent papers Sirri et al. (2010; 2011) evidenced that the chicken genotype dramatically affects the meat FA composition. In particular, comparing slow-growing (SG), medium-growing (MG) and fast-growing (FG) chickens, organically reared, they found higher n-6 and n-3 PUFA content in the breast muscle of SG suggesting a different expression of genes encoding for the desaturating enzymes (Δ6 and Δ5 desaturases).

On this basis, the major aim of this PhD work is to study adaptation response to extensive farming system of some native Italian chicken breeds in comparison with commercial hybrids, with particular attention to liver metabolism and essential fatty acids content of meat and eggs.

The specific goals were:

1st CHAPTER. THE ADAPTATIVE RESPONSE

- EXPERIMENT 1. Effect of genotype and husbandry system on blood parameters, oxidative and native immune status: welfare and implications on performance of laying hens extensively reared.

- EXPERIMENT 2. Behavior, welfare and adaptability of different meat-type chickens to extensive rearing system.

2nd CHAPTER. PRODUCT QUALITY

- EXPERIMENT 3. Testing of the effect of husbandry system on the grass intake and egg nutritive characteristics of laying hens.
- EXPERIMENT 4. Comparison of oxidative status and fatty acid composition of meat in different poultry genotypes reared under extensive system.

3rd CHAPTER. LIPID METABOLIC ACTIVITY

- EXPERIMENT 5. Fatty acid composition of meat and estimation of lipid metabolism indices in different poultry genotype reared under organic system.
- EXPERIMENT 6. Evaluating of the mRNA expression and Δ6-desaturase activity (capacity to desaturate LNA to EPA and DHA) in three chicken genotypes.
- EXPERIMENT 7. Effect of maternal dietary n-3 fatty acids supplementation on fatty acid composition, Δ6-desaturase expression and activity of chicken liver.

2. INTRODUCTION

2.1 The animal biodiversity

The general term "biodiversity" is used to describe the genetic variability of all form of life, including microorganisms, plants and animals (Davoli, 2011). Indeed, agricultural biodiversity refers to the diversity of the cultivated plants and domestic farm animals utilized by man for the supply of food and other benefits and services. The capacity of agro-ecosystems to retain, improve productivity and adapt to changing circumstances remain crucial and vital to global food security. On a worldwide scale, animal biodiversity is defined as the variability among organisms of different or same species with respect to the environment in which they live, giving special attention to genetic biodiversity (Lehman and Tilman, 2000). For the livestock sector, animal genetic diversity is a resource to be drawn upon to select stocks and develop new breeds. More generally, different livestock populations have to provide society with a greater range of option to meet future demands, so the management of the world's agricultural biodiversity has become an important aspect to the international community (FAO, 2007). The livestock sector, in particular, forms an essential component of agriculture output by producing high quality food. In developed countries the higher standard of living is generally accompanied with a greater consumption of animal products: meat, milk and eggs. In contrast, livestock in developing countries is an important component in the earning of livelihoods of some 70% of the world's poor rural people (Hoffmann and Scherf, 2005). The challenges for livestock production systems in effluent societies are food quality and safety to safeguard the human health, animal welfare in intensive systems and the sustainable use of resources. The utilization of farm animal genetic resources contributes to meet the different challenges in developed and developing countries. Between 1995 and 2004 global animal production increased (milk, 15 %; egg, 35 %; meat, 25 %) as reported by Rosati et al. (2005). The growth in production is predominantly realized in countries with a rapidly growing livestock sector like Brazil, China, Mexico, Thailand and several East European countries (The World Bank, 2005). In the analysis of 148 country reports by Oldenbroek (2006), it is evident that differences can exist between continents in the evolution of livestock production systems. In particular, in Europe the introduction of environmental and production restrictions has increased production cost, decreased the self-sufficiency and induced a worsening in livestock systems. A substantial amount of land is no longer used for agriculture and is surrendered back to nature. Less intensive systems like organic farming have been introduced and are growing in importance. At the same time a significant number of part-time farmers and hobbyists keep farm animals in rural areas (FAO, 2007).

2.1.1 Importance of genetic research in poultry

As already said, an increasing loss of genetic diversity has been observed for all zootechnical species, and more than half common livestock breeds are now endangered (Dohner, 2001). Thereby poultry genetic resources are considered to be one of the most endangered. Although the efficiency of the poultry industry is excellent under current conditions of agricultural development, there are several major concerns with regard to the losses of genetic diversity (Crawford, 1990; Crawford and Christman, 1992; Barker, 1994; Frankham, 1994; Hammond, 1994; Ollivier et al., 1994):

Genetic variation is the prerequisite for selection of desirable traits, for example, to cope with emerging disease agents and changes in consumer preferences or in the production management. The economic importance of single-purpose highly productive breeds and lines is distorting the perception of the value of multipurpose breeds that are adapted to local conditions. The replacement of these local breeds may lead to their extinction, and hence to an unrecorded loss of genetic variation.

- Taking into account the number of known breeds, commercial poultry lines have been developed on a relatively narrow base. Intensive selection for production traits and increasing concentration of breeding in the hands of a declining number of multinational companies may accelerate the loss of genetic variation in commercial poultry breeding. However, one should note that within commercial chicken lines breeders are careful to avoid strong inbreeding in their breeding programs.
- Genetically diverse breeds provide an indispensable source for research to improve our understanding of the genomic mechanisms that underlie biodiversity, physiological functions, disease resistance and performance traits. Highly differentiated strains are the basis to develop resource populations used in quantitative trait loci mapping, detection and utilisation for marker-assisted selection.
- Finally, from the historical and cultural points of view, old native breeds may be considered as living evidence of the achievements of many generations of breeders.

Nowadays, the general public does not understand well the reason or the need to preserve old breeds; nor it fully understands agriculture or the working relationship between farmers and their animal partners (Dohner, 2001). For conservation measures of farm animal genetic resources priorities have to be defined based upon a range of information: the degree of endangerment, adaptation to a specific environment, possession of traits of current or future economic importance or scientific interest, and cultural or historical value. An inventory of existing breeds and the risk status classification are some of the preliminary steps to conserve genetic diversity for the future, but it may provide important initial data on existing breeds within species, and the risk of losing this diversity in the near future.

2.1.2 Risk status determination

The risk status classification is an important element to define the genetic sustainability of livestock breeds or populations. It is an indicator and informs stakeholders on whether and how urgent, genetic conservation actions need to be taken. Different classification has been used by the European Federation of Animal Science (EAAP, 1998) or FAO (Scherf, 2000) to describe the various degree of risk, but the most widely reported is the one provided by FAO thought the Global Databank for Farm Animal Genetic Resources (DAD-IS). The risk status is classified into categories according to the number of available breeding males and females, the inbreeding rate (estimated from the effective population size), or population dynamics like increasing or decreasing population size. A framework to harmonise risk categories across institutions has been proposed (Gandini et al., 2005). DAD-IS monitors and classifies the world's breeds into the following risk categories:

- EXTINCT: when it is no longer possible to recreate a population of the breed. Extinction is absolute when there are no breeding males (semen), breeding females (oocytes), or embryos remaining.
- CRITICAL: if
    - the total number of breeding females is less than or equal to 100 or the total number of breeding males is less than or equal to 5;
    - the total breeding animals is less than or equal to 120 and decreasing and the percentage of females being bred to males of the same breed is below 80.
- ENDANGERED: if
    - the total number of breeding females is greater than 100 and less than or equal to 1,000 or the total number of breeding males is less than or equal to 20 and greater than 5;
    - the overall population size is greater than 80 and less than 100 and increasing and the percentage of females being bred to males of the same breed is above 80;
    - the overall population size is greater than 1,000 and less than or equal to 1,200 decreasing and the percentage of females being bred to males of the same breed is below 80.
- CRITICAL-MAINTAINED or ENDAGERED-MAINTAINED: identify specific populations for which active conservation programs are in place or populations are maintained by commercial companies or research institutions.
- NOT A RISK: if none of the above definitions apply and the total number of breeding females and males are greater than 1,000 and 20, respectively or the population size is greater than 1,200 and the overall population size is increasing.

2.1.3 Conservation strategies of genetic resources in livestock sector

The interest and awareness in livestock conservation has gradually increased over the last 25 years due to the drastic reduction of local populations that have been replaced by the expansion of more productive types (Hall and Bradley, 1995). From the FAO database, it is estimated that about 25% of all chicken breeds are included in some sort of conservation initiatives, but there is no information on the efficiency of these programmes. According to FAO country reports, only 15% of countries have poultry conservation programmes. An important step in the genetic resources sustainable management is the establishment of conservations measures. Theoretically, two types of conservation measures can be implemented: in situ conservation and ex situ, in detail the latter strategy expected another division: *in vivo* and *in vitro* conservation. The distinction between the different conservation programs can be rather uncertain, and Country Reports, do not usually make a clear distinction between the various types. Davoli (2011) proposes only two approaches to conservation of animal biodiversity namely: *in situ* and *ex situ*. *Ex situ* refers to conservation approaches outside of a breed's natural habitat, such as in zoos (*in vivo*) and gen banks (*in vitro*). This approach allows for easier handling by humans (research, genetic improvement), but nevertheless retains the genetic identity of the sample collected. Instead the *in situ* conservation of the breed occurs within its ecosystem and natural habitats. It involves the maintenance and recovery of viable populations in their natural surroundings where they have developed their

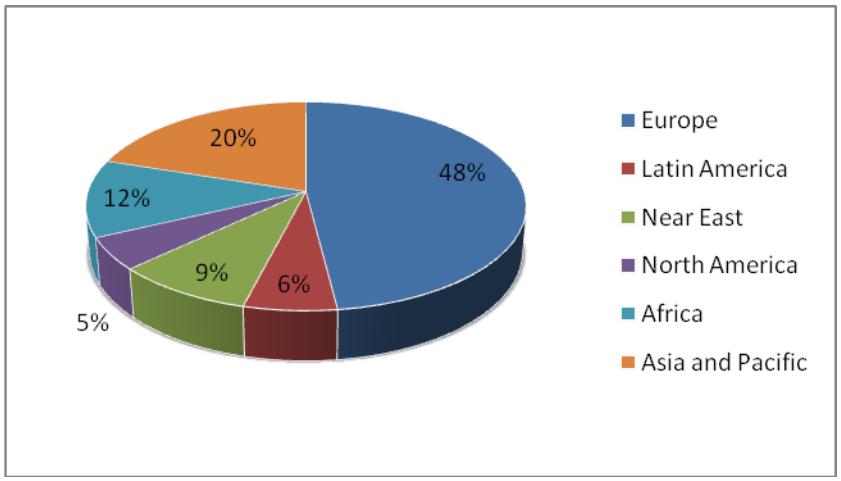
distinctive properties (Geerlings et al., 2002). This type of conservation can be performed both as hobby by small farmers, both on a large scale for the enhancement of typical product.
As previously mentioned, *in situ* conservation programmes can be divided in *in vivo* and *in vitro*. *In vivo* must include identification and registration of animals and monitoring of populations and population size. The majority of Country Reports indicate the presence of *in vivo* conservation measures, while only 37% indicate the presence of *in vitro* conservation. In the developed world, many people keep minor chickens breeds as a hobby and hence providing an opportunity for the *in vivo* conservation. In addition to *in situ* conservation, gene banks are being established for the *ex situ* conservation (Woelders et al., 2006). Many gene banks are present in Japan, India, the Nordic countries, France, the Netherlands, Poland, the Czech Republic and Hungary, while in other countries, the establishment of gene banks is being planned. The technology to store semen from all the livestock species and embryos of cattle, sheep and goats is easily available and widely utilized. In poultry is not easy to obtain oocyte or embryo (Blesbois et al., 2008). The only method currently feasible for management of reproductive cells for bird populations is therefore semen cryopreservation, mainly studied in the chicken to date (Bellagamba et al., 1993; Blesbois, 2007). Sometimes tissue DNA samples are also collected for the main species. In developing countries the implementation of in vitro conservation measures, is usually limited to the storage of semen from some local cattle and sheep breeds at private or governmental institutions. On the other hand only a few gene banks store poultry and horse semen. In Europe and North America many universities and research institutes try to conserve locally developed breeds of chicken that are no longer used by the industry. For chickens in vitro conservation of semen is a recent development. Cryoconservation is a proven technology and is an important complement to in vivo breed conservation (Woelders et al., 2006). There are several reasons for the conservation of genetic diversity in farm animals such as: rare or local breeds fulfill specific requirements with respect to local terrain or climate or may produce typical regional products. Efforts to conserve genetic diversity of farm animals include measures to stimulate the inclusion of indigenous and rare breeds by farmers. In many developing countries conservation programmes are necessary and these programmes should be encouraged and supported through external technical and financial assistance. Effective prioritization of breeds for conservation programmes is facilitated by phenotypic and genetic characterization and by knowledge of the size and structure of the population. The FAO definition of animal genetic resources eligible for conservation includes animal populations with economic potential, scientific use and cultural interest. There are several reasons why the implementation of conservation measures for a particular breed might be considered important: genetic uniqueness; high degree of endangerment; traits of economic and scientific importance; ecological, historical and cultural value. Conservation and development of local breeds is important because of their contribution to the livelihoods of farmers and biodiversity as well as their social and cultural importance (FAO, 2007).

2.2 Avian biodiversity

At the present, the Global Databank for Farm Animal Genetic Resources contained per-country records for 16 mammalian and 14 avian species including a total of 6,379 breeds. The latter number is likely to be an overestimate and does not reflect the actual amount of breeds because it involves

the similar breeds kept in different countries as well as breed varieties or commercial lines, strains and populations of the same breed or even breed/line crosses maintained as single units. Compared to 16 other livestock species, breed data recorded for the 14 avian species (1,049 breeds) constitutes only 16% of overall World Watch List for Domestic Animal Diversity information (Weigend and Romanov, 2002). Although FAO initiated recording of avian species in 1993 soon after collecting data from mammalian species, little more information has been added recently. Of the 1,049 avian breeds of all 14 species, population data are available for 77% breeds being a somewhat greater percentage than an average for all species. Of those breeds, 41% are classified into the risk categories "critical" or "endangered" that could be considered as indicative of a higher risk status of avian breeds. In comparison, a total of 32% of the breeds recorded with population data are classified at high risk of loss (critical or endangered without conservation program) across all livestock species. The majority of avian breeds (89%) recorded fits into one of the five major avian species: chicken, turkey, duck, Muscovy duck, and goose.

Figure 2. The world's livestock and poultry breeds recorded in the Global Databank of Farm Animal Genetic Resources as percentage by region. (Weighend and Romanov, 2002).



The largest number of breeds is recorded for chicken (734 breeds, or 71%), and much less for the other four species. That testifies to chicken's prevailing position among poultry species in global breeding and production systems and in human consumption. The remaining nine avian species account only for less than 11% of the total number of avian records. As illustrated in Figure 2, the largest proportion of breeds of all major avian species is listed for Europe, and Europe and Asia Pacific region together account for more than 80% of all breeds. In contrast, the least number of breed records is found for North America, maybe, because of shorter breeding history or lesser response from local experts. This proportion between regions is very similar for three of the five major avian species. However, for ducks (41%) and Muscovy ducks (62%) the largest number of breeds was registered in Asia and the Pacific region since duck breeding is known to be very popular there and duck meat and eggs are essential food components in this part of the world. Though the breed history is largely well documented, the actual genetic relationship between these various breeds in Europe is not adequately explored, and the number of breeds recorded might

overestimate existing diversity in the goose in Europe. The same could be true for other poultry species.

2.2.1 Poultry sector in the world

Chickens represent an important category (63% of all avian breeds) and the oldest type of poultry. Chicken breeds are divided according to type and are classified into: layers (used exclusively for eggs production), broilers (production of chicken meat), dual purpose breeds (meat and eggs), fighting breeds and ornamental breeds. In the developed countries, commercial synthetic strains dominate the production of meat and eggs, while local breeds are marginalized and restricted to the hobby sector. In the developing countries local breeds still play an major important role; and in some cases make up 70-80% of the (national) chicken population (Guèye, 2005; FAO, 2006). Chicken types found in the hobby sector may look very different from each other, but that does not necessarily mean they are genetically very diverse (Hoffmann and Scherf., 2005). The same may be true for some of the indigenous breeds in developing countries (FAO, 2006). The poultry livestock sector continues to grow and industrialize in many parts of the world. The interest in poultry and poultry products has grown tremendously over the last 20 years;. almost every country has a poultry industry of some sort. Japan is steadily increasing its domestic production of both broilers and eggs. Countries of the former Soviet Union always produced poultry and eggs and are still continuing to improve their output to meet the increasing domestic demand. China, the Middle East and Africa are all areas where over the last few years the demand for poultry has increased dramatically. In the period 2000 and 2010 poultry meat and egg production have shown remarkable dynamics. The trade volume of poultry meat varied between regions such that Asia and Africa recorded annual gains of around 4.5% during the decade, while less than 4 % was registered in the other continents. In Europe, there was a remarkable difference between countries within and outside of the European Union, in particular Russia and Ukraine. In the Europe community, the growth was less than 20 % as the total production climbed from 8.2 million tonnes (t) to 9.7 million t. In the non-EU countries a shift from 1.2 million t to 4.1 million t was observed (FAOSTAT). Generally speaking, poultry production falls in one of two main production systems: the commercial production system that generally utilizes the modern hybrids strains and the alternative (organic, free-range) system that employs different chicken breeds. Commercial patented strains purposely developed to fit into intensive production systems are used in the commercial system, while under the alternative system the more typical local breeds are popular.

2.2.2 Pure chicken breeds and commercial lines

The genomic sequencing of the chicken (Francham et al., 2004) showing that the commercial lines have lost 90% of their alleles in relation to local chickens. The low genetic variability of hybrids reduces the ability to adapt to new scenarios such as re-emerging diseases or less controlled environmental conditions.

Commercial meat-type hybrids are selected for precocity and have very fast growing performances. Such strains, selected for producing under highly controlled conditions, are unsuitable for extensive system because the environment is less controlled and animals are too heavy thus, health and welfare problems are common. However, economic reasons and limited chicks availability

render these animals widely used also in extensive poultry production (Hovi et al., 2003). The local breeds can provide an interesting alternative to commercial lines (De Marchi et al., 2005; Zanetti et al., 2010; Castellini et al., 2006). Local breeds have a high adaptation, marked rusticity, and exploratory attitude that allows to ingest large bioactive compounds (tocopherols, carotenoids, polyphenols and polyunsaturated fatty acids in especially those of the series n-3) contained in grass, insects and earthworms. Conversely, pure strains have too limited growth performances and must therefore be crossed with heavier breeds for improving productive traits maintaining his foraging behavior. Some authors (Castellini et al., 2002a; Lewis et al., 1997) showed that one aspect of free-range animals could be the lower oxidative stability of the meat due to the higher motor activity that improves the oxidative metabolism and the free radicals production. Different adaptation of genetic strains to outdoor environment greatly affect the animal equilibrium by modulating the intake of grass (Castellini et al., 2002a), and body metabolism (Branciari et al., 2009) with implications on the lipid profile and oxidative stability of meat (Castellini et al., 2005b). In order to assure a good welfare status, the EC Regulations and the final recommendation of Network for Animal Health and Welfare in Organic Agriculture (Hovi et al., 2003), suggest to utilize slow-growing birds (daily weight gain < 35g; Guéméné et al., 2009) for their higher adaptability to poorer environment (Table 1.).

Table 1. Farming Systems

intensive farming		extensive farming	
advantages	**disadvantages**	**advantages**	**disadvantages**
• Stable social order (the small groups). • No contact with manure, so minimal risk of infections by endoparasites. • Reduced risk of infestation by ectoparasites. • Low risk of cannibalism.	• Inhibition of many behaviors (walking, running, perch, fly, hide, stretch and flap its wings, etc.). • Prevention or modification of other behaviors (dust-bathing, nest building, scratch or peck at the litter or soil). • Lack of equipment for nesting and roosting. • Low light intensity to control pica and cannibalism. • Excessive growth of nails which might be reduced with abrasive strips. • Significant loss of feathers. • No chance to escape attacks. • Frail bones with increased risk of fractures.	• Scratching. • Making dust bath. • Choose where to nest. • Do exercises in many ways. • Perching. • To have space and freedom of movement • Eating grass and insects. • Have stronger bones due to increased activity and reduced productivity.	• The contact with the manure increases the risk of coccidiosis and ascariasis. • Ectoparasites represent a serious problem. • The predators can cause fear, injury and loss. • The mortality rate is usually higher. • Extreme temperatures can cause serious discomfort.

2.2.3 Native chicken breeds

Ancona and Livorno are two Italian autochthonous chicken breeds which represent a great resource in terms of specific genetic richness. Genetic surveys showed a lack of variability in Ancona and Livorno. In the meanwhile, microsatellites demonstrate the genetic uniqueness of Ancona breed given its genetic isolation (Bianchi et al., 2011). In spite of their endangered status, these chicken breeds are very appreciated for their ability to adapt themselves to extensive rearing systems. Besides that, they were proposed as egg layers models for an *en-plain air* rearing system (Castellini et al., 2006; Mugnai et al., 2009; Dal Bosco et al., 2011).

Ancona is a breed to light marked aptitude for production of eggs with white shell colour and 50-55 g weight. The average annual production of eggs is about 180, but can reach peaks of 250 hotels in F1 crosses with Livorno (Castellini et al., 1990). It is an Italian breed and owes its name to the city of Ancona. In 1880, some specimens were exported to England and the breed's productive attitudes were improved with a Minorca crossing. It has the same morphological characteristics of Livorno but it is less productive. As a meat-type, Ancona is a slow-growing bird, its weigh is on average 2 kg for chick, 3 kg rooster and the hen about 2.3 kg (Figure 4).

Livorno, or Leghorn, is an Italian breed of chicken known all over the world. The breed derives from crosses of chickens kept in the central Italy countryside, and took its name from the city of Livorno. It is a light and lively breed with a strong aptitude for production of eggs and little tendency to brood. Many industrial strains of hens derived from White Leghorn, which was used in Western countries for this purpose. Many foreign countries have made a careful selection starting from the original Italian chicken, so today there are also the American Livorno (Leghorn call), the German Livorno (called Italiener) and English Livorno (Leghorn). It has been created also a dwarf version of the breed retains the same characteristics, except that the size is greatly reduced. It is a light chicken the weight is 2.5-2.8 kg in the male and 1.8-2.1 in the hen. Its behavioral are the same of the Ancona breed. It is also considered a slow-growing meat type chicken as evidence by body weight (Figure 3).

Figure 3. Leghorn chicken breed

However in poultry livestock system, often it is preferred to use cross of these breeds in order to increase productivity and maintain high quality. Castellini et al. (2006) have studied the Leghorn x Sasso crossbreed (LC) in comparison to pure line, organically reared. We have showed that the productive performance and the carcass traits of Leghorn birds could be improved by crossing such birds with a more productive one. However such improvements did not limit their behavioral (kinetic activity) which remained very high. The same approach has been proposed by Dal Bosco et al. (2011), which have compared an Ancona x Cornish crossbreed with the pure Ancona strain. They have reported that all performance traits of Cornish x Ancona birds were better than Ancona birds, except for mortality rate. Even the meat characteristic were resulted similar. In both cases, the crossbreed provided good results. However this cross is not genetically defined, therefore it is not possible to know if these positive characters will be stable in subsequent generations. At the same time it is difficult to categorize these crossbreeds as commercial type. For

Figure 4. Ancona chicken breed

instance, their body weight in comparison with commercial line can be still considered as low, but in comparison with respective pure breeds, they are considered medium-growing birds. For these reasons further studies would be needed in order to define the use of crossbreed, in alternative farming system, as a junction point between animal welfare, environmental sustainability, food quality and market demand.

2.3 Quality of poultry livestock products in regard to genotypes used

Nowadays the livestock production system exhibit a particular attention to the needs of the consumer in all developed countries, who is increasingly looking for a high quality product. The major factors that influenced the quality parameters are below summarized.

- Genetics is giving the main contribution towards an improvement of the chemical, nutritional, sensorial and technological characteristics, above all for milk, but also for meat and eggs. Significant contributions from genetics have also allowed a positive approach towards infective diseases prevention by identifying some resistant genotypes.
- Nutrition is a powerful means to control several aspects of quality. It can have a positive effect on fat content and, within the level of the lipid fraction, on the specific level of essential fatty acids, low cholesterol levels, content of vitamins and trace minerals, targeting the product towards specific categories of consumers (infants, older people). It can also improve the organoleptic and technological qualities of products.
- Management can have different effects according to the product and the species. It can improve the hygienic qualities of products.

All the parameters previously described could be represented in poultry livestock by an extensive farming system (free range or organic) that uses pure chicken breeds. The effect of the organic system on some qualitative characteristics of the chicken meat or eggs has long been investigated. Such effect is mainly due to the greater physical activity of animals but it is largely modulated by the farming protocols used, as genetic strain and environment utilized. The availability of pasture, in fact, increases the motor activity of chickens, causing a modification on muscle fiber characteristics and enzyme functions only in animals adapted to the organic system (Branciari et al., 2011). Indeed many behavioral and physiological differences arise from the comparison of slow-growing strains with fast-growing strains (Castellini et al., 2003). Meluzzi et al. (2009) showed a lower n-6/n-3 ratio on meat of egg-type slow-growing stains than meat-type fast-growing in organically reared. Chicken meat quality is strongly affected by genotype whereas feeding exerts a minor effect. Layers nutrition and husbandry system significantly influences the qualitative characteristics and the chemical composition of eggs too (Matt at al., 2009; Spiteri et al., 2013). The pasture availability increased the vitamins and xantophylls contents in the yolk egg (Mugnai et al., 2009) as well as the unsaturated fatty acids content, at the same time improve the albumen and shell quality (Hammershøj, 1997).

Today, the consumer sees favorably the presence of long-chain fatty acids (LCP) in the food, for beneficial effect to human health (Patterson et al 2012), but the use of fish oil, notoriously rich in n-3 long chain derivates (EPA and DHA), has depressive effects on the sensory evaluation of eggs.

Furthermore, the over-fishing of the last decades, has led to a difficult finding this product. In this context, the situation of fish oil is particularly exacerbated in consideration of increased demand, declining production and rising commodity price (FAO, 2007; Tacon et al., 2006). Therefore, the replacement of dietary fish oil represents an important goal for the word food industry. More economical terrestrial alternatives, such as vegetable oils and animal product, is consequently a highly investigated research topic and an approach increasingly used.

The contribution of genetics in this field has been undoubtedly important. Specific selection programs have been adopted and in some European countries such as Germany, eggs with higher (2.5–4 times) n-3 unsaturated fatty acids content are sold with specific indication on the label (Nardone and Valfrè, 1999). Recently Sirri et al., (2010 2011) have demonstrated that slow-growing strains have a higher capacity to elongate and desaturate essential fatty acids (linoleic acid and α-linolenic acid) in their long chain derivates (LC-PUFA). These evidences indicate a relatively high efficiency of the desaturating enzymes in specific poultry genotype, and this allow for further consideration on the selection of genotypes with enhanced desaturating activity.

2.3.1 Lipid metabolism of LC-PUFA

Long chain n-3 and n-6 polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic (EPA, C20:5n-3), docosapentaenoic (DPA, C22:5n-3), docosahexaenoic acid (DHA, C22:6n-3) and arachidonic acid (ARA, C20:4n-6), are essential for normal growth and development of vertebrates. They are involved in the maintenance of cellular membrane integrity, cellular signalling, gene regulation and metabolism (Lands, 1992), ARA and EPA are also precursor of eicosaesanoic, which mediate a variety physiological processes such as infiammation, immunity, reproduction, and development (Levin et al., 2002). A number of studies have demonstrated that these PUFA, especially n−3 type, have health-promoting effects, including reductions in cardiovascular disease, neurological disorders, diabetes, arthritis, inflammation, autoimmune disorders and cancer, as well as the improvement of brain and visual development (Plourde et al., 2007; Russo, 2009). Because vertebrates are incapable of synthesizing PUFA *de novo*, they are entirely dependent on dietary sources to procure and maintain adequate peripheral and central tissue concentrations. Linoleic acid (LA; 18:2n−6) and α-linolenic acid (ALA; 18:3n−3) are two dietary precursors of long chain n−6 and n−3 PUFA, respectively. Vertebrate species possess the capacity to synthesize EPA, DPA and DHA from ALA, and ARA from LA, respectively, via two separate pathways involving desaturation and elongation processes (Figure 5). The extent to which animals can convert LA and ALA to long chain PUFA differs according to species and depends on their capacity for fatty-acyl desaturation and elongation. Two fatty acid desaturase enzymes, Δ5-desaturase and Δ6-desaturase, have been identified in the pathways for PUFA biosynthesis, which are distinctly encoded by fatty acid desaturase (FADS) 1 and FADS2 genes, respectively (Guillou et al., 2010). Of the seven fatty acid elongase subtypes identified in mammals, elongase 2 and 5 (or elongation of very long chain fatty acids protein 2 and 5), encoded by ELOVL2 and ELOVL5, respectively, play key roles in the biosynthesis of PUFA (Gregory et al., 2011). A recent study found high expression of ELOVL2 and ELOVL5 in liver of chickens (Gregory et al., 2013). The liver plays a central role in whole body lipid metabolism, which encompasses the synthesis and modification of fatty acids by way of desaturation, elongation and oxidation processes.

All fishes showed a Δ6 activity, required for the initial desaturation of LA and ALA, whereas Δ5 activity, necessary to desaturate 20:4 n−3 to EPA, have only been found in the diadromous or freshwater species (Tocher, 2010). In terrestrial species, the main monogastric species (pig, poultry, rabbit) show a certain conversion of ALA to EPA whereas DHA synthesis is generally much lower (Table 2).

Table 2. Estimated Δ5/Δ6-desaturase activity* and n-3 in different terrestrial and aquatic species.

	Estimated desaturase activity*	LCP n-3 (%)		DHA (%)
Pig	50.6	2.02	(Kouba et al, 2011)	0.44
Rabbit	54.5	1.80	(Dal Bosco et al. 2004)	1.00
Broiler	38.6	4.18	(Lo Zupone, 2009)	1.46
Laying hen (eggs)	66.5	2.44	(Oliveira et al. 2010)	2.06
Trout	88.7	34.80	(Dal Bosco et al. 2013)	17.57

*Δ5-Δ6=[(C20:2n-6+ARA+EPA+DPA+DHA)/(LA+ALA+C20:2n-6+ARA+EPA+DPA+DHA)] × 100

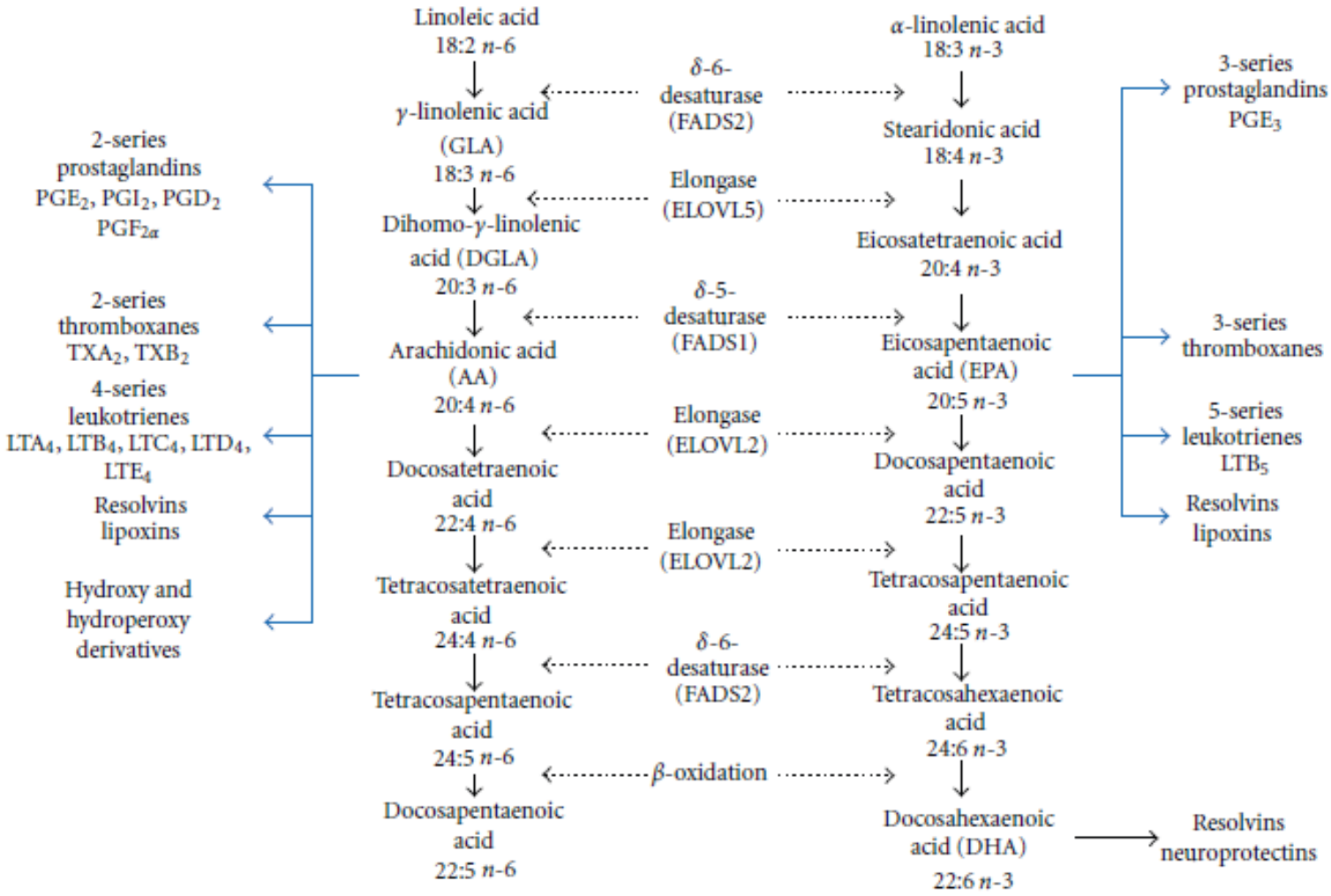
Within the same species, such conversion rate is deeply affected by sex, hormonal status, intestinal biota, genetic strain and feeding plan (amount of derivative and LCP, n3/n6 ratio) (De Meester, 2012).

It is usually retained that ALA is the preferential substrate for Δ6-desaturase (Huang et al., 1991; Garg et al., 1988; Portolesi eta al., 2007) the affinity for ALA is two to three times greater than for LA. Indeed, recent study highlighted that the adequate intake of LA depend of the concurrent intake of ALA (Kuipers et a., 2010). Because n−6 and n−3 fatty acids compete for a single set of desaturases and elongases. Gruffat et al. (2011) has showed that bovine liver was inefficient at converting ALA into long-chain n-3 PUFA, but actively converted LA in ARA. On the same time, our preliminary study has suggested that the preferentially substrate of Δ6-desaturase is breed-specific in rabbit species, indeed the New Zealand White breed seem to have a higher affinity to LA than ALA (Castellini et al., 2014). Even in the vegetable word the high selectivity of Δ6-desaturase for ALA in *Primula farinosa* is reported (Sayonava et al., 2003).

Nowadays n-6/n-3 ratio is higher in human dietary, (Kuipers et al., 2010) due to an unbalance dietary intake. Several studies assessed that humans are rather poor EPA and DHA synthesizers (Muskiet et al., 2004), despite ARA and DHA are especially abundant in the brain and the retina and have relevant role in many physiological pathways and pathological disorder like: CHD, reproductive dysfunctions and depression (Muskiet et al.,2004; Simopoulos, 1991). As a result, it seems that at least some LCP might be almost essential to humans (Robinson et al, 2013; Jeppesen et al, 2013). Such assessment could be corroborated by a high dietary LCPn-3 intake by our hominid ancestors might have precluded the need to conserve a highly sophisticated expression of genes coding for the enzymatic conversion of ALA to DHA (Kuipers et al., 2010). Further, some

data suggest that the conversion of ALA to EPA and DHA is also dependent on sex: greater in young women than in men possibly due to the estrogens (Burdge and Wootton, 2002; Burdge et al., 2002).

Figure 5. Metabolic pathways of n–6 and n–3 polyunsaturated fatty acids (PUFA). (Patterson et al., 2011)



3. MATERIALS AND METHODS

3.1 THE ADAPTATIVE RESPONSE (1st Chapter):

Planning of experiment 1(a). and experiment 2(b).

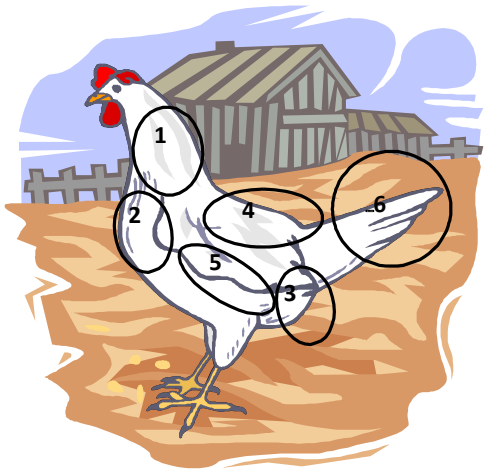
3.1.1a Animals, Housing and Feeding

One hundred 1-day-old chicks of the Ancona and one hundred 1-day-old female chicks of the Brown Hy-Line genotypes were reared during the first four weeks under identical conditions in floored pens, covered with wood shavings. The floored pens were located indoors in an environmentally-controlled building at the farm of the Department of Agricultural, Environmental and Food Science (University of Perugia). All animals were vaccinated against Marek and Newcastle diseases; the beaks were not trimmed and no other pharmacological treatments were given. At 4-weeks-of-age, both groups (Brown Hy-Line and Ancona) were reared in two covered, straw bedded houses (4 bird/m^2) with access to two pens with natural grazing (10 m^2/bird). Feed and water were provided indoors with manual feeders and automatic drinkers respectively. Inside the paddocks, there was a small hut with nests (1 per 6 hens) and perches. Environmental temperature, humidity and photoperiod were the natural ones. A conveyor belt running along the back of the nests collected the eggs. All the animals were reared according to Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes. A standard layer feed was given *ad libitum* to all groups.

3.1.1b Animals, Housing and Feeding

This trial was conducted at the experimental section of University of Perugia (Italy) from March to May. All animals were reared according to EU regulation 834/07 and Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes. Six hundred male birds were compared and categorized with regard to their growth rhythm: slow (<20 g/d), medium (20 = 35 g/d) or fast (>35 g/d) (RCE, 2007; RCE, 2008). The genotypes were the following: Ancona (A), Leghorn (L), crossbreed Cornish × Leghorn (CL), Kabir (K), Naked neck (NN), and Ross (R). The L and A genotypes are originated from a conservation flock at the Department of Agricultural, Environmental and Food Science in the 1960s.The CL chicks were produced by crossing Leghorn hens with Cornish fowl (from localfarmer), whereas K (strain KR4), NN (strain NN1), and Ross 308 were furnished by a commercial poultry farm (Avicola Berlanda, Italy). Chickens were kept separate after hatching until 20 of age in an environmentally controlled poultry hous with temperatures ranging from 20 to 32°C and with RH ranging from 65 to 75%. Incandescent light (30 lx) placed at bird level was used for heating and illumination. Chicks were vaccinated against Marek and Newcastle diseases. At 21 d of age, the chicks were transferred to straw-bedded indoor pens (0.10 m^2/bird) each equipped with feeders and drinkers and with free access to forage

Figure 6. Area evaluated for the plumage score



paddock (4 m^2/bird). Each genotype was represented in 4 replicates containing 25 chicks each. Birds were confined to indoor pens during night.

3.1.2a Behavioral Observations

Behavioural observations, Tonic Immobility Test (TI) and plumage evaluation were performed during four seasons, in a period of 5 days each. Ten animals per group were randomly selected and marked with different colours on the tip of the tail. Behavioural observations were recorded during three-hour periods in the morning (9.00-12.00) and afternoon (15.00-18.00) using the focal animal scan sampling method (Martin and Bateson, 1986). Before each observation session, 5 minutes were allowed for the animals to adapt to the presence of observers. The behavioural observations included: moving (walking, running and foraging), resting (standing, lying), feeding (food and water), comfort (dust bathing, self preening, scratching and starching), social relationships (allo-preening) and gentle and severe pecking others (Kjaer and Sørensen, 1997). Behaviours were recorded on a custom-designed table, and their respective frequencies were calculated as a percentage of the total observed behaviours. Since no differences were found between days and hours, all data were pooled to obtain a mean value. At the end of behavioural observation birds were caught and submitted previous to the TI test (Gallup, 1979) and then to plumage condition evaluation (Tauson et al., 2005). TI was induced by restraining the birds on their backs in a U-shaped wooden cradle for 10s (Gallup, 1979). A bird was defined as being in a state of TI if it remained immobile for a minimum of 10 s after restraint had ended (Figure 7). A maximum of three inductions and a test ceiling of 3 min in TI were applied. The total duration of TI, i.e. until the bird righted itself, was recorded. The plumage scoring system assigned values of 1 to 4 points for each trait, where a score of 4 implied the best and a score of 1 the worst condition. The 6 parameters (neck, breast, cloacae/vent, back, wings and tail) for plumage condition were summarized, implying a total score ranging from 6 to 24 points.

Figure 7 Tonic immobility (TI)

3.1.2b Behavioral Observations

Before slaughtering, the foot pad dermatitis (FPD) of a sample of 50 birds per group (17, 17 and 16 birds replication) was assessed by assigning them to 1 of 3 different classes: 0=no mark (no lesion), 1=mild lesions (superficial lesions, erosions, papillae, and discoloration of the footpad) or 2=severe lesions (deep lesions, ulcers and scabs) (Berg, 2002). The FPD score was calculated by applying the formula reported in the Proposal for a Council Directive of the European Commission (European Commission, 2005). The plumage condition was also assessed according to Tauson et al. (2005). Other welfare-related traits of carcasses, such as skin damage and the presence of breast blisters were also recorded.

3.1.3 Blood sample collection and analytical determinations

Blood samples were collected from each animals during the experimental period. 5mL blood samples from the brachial vein were collected in vacutainers and transported to the laboratory. Here, the samples were allowed to coagulate at room temperature for 2 hours, and then the collection tubes were rimmed and refrigerated at 4°C for a maximum of 24 h before analysis. Plasma was produced by the same procedure, except that blood was collected directly into tubes containing Na_2-EDTA and immediately centrifuged at 2,500 × *g* for 10 min at 4°C to measure innate immunity and oxidative status.

3.1.3.1 Immunity state

The leukocyte counts have been done on two drops of blood, and blood smears were made on duplicate glass slides. Both the slides were counted and the means were calculated for each bird. These smears were stained with Wright stain in 15 min. One hundred leucocytes, including heterophils, lymphocytes, monocytes and eosinophils werecounted on each slide. The H/L ratio was also calculated.

Serum lysozyme was measured with a lysoplate assay (Osserman and Lawlor, 1966), carried out in a moist incubator at 37°C for 18 min. The method is based on the lyses of *Micrococcus lysodeikticus* in 1% agarose. The diameter of the lysed zones was measured with a ruler and compared with the lysed zones of a standard lysozyme preparation (Sigma, Milan, Italy, M 3770). The value is expressed as μg/mL.

The Serum Bactericidal Activity (SBA) was performed according to a method previously validated for cattle (Amadori et al., 1997). The test is based on the challenge of serum with non-pathogenic *E. coli*. Its concentration is expressed as a percentage.

Haptoglobin (AP) was measured by a commercial kit (Phase Haptoglobin Colorimetric Assay, Tridelta Development Ltd, Kildare, Ireland) according to the manufacturer's directions. The test is based on the different peroxidase activity in acidic environment of haptoglobin-hemoglobin complexes and free hemoglobin, respectively. Briefly, a known amount of hemoglobin is added at low Ph to the serum sample; the residual peroxidase activity of hemoglobin is directly proportional to serum haptoglobin concentration.

The Haemolytic Complement Assay (HCA) (Barta and Barta, 1993) was carried out in microtitre plates. The complement titre is the reciprocal of the serum dilution causing 50% lyses of red blood cells of ram. Its concentration is expressed as CH50%.

3.1.3.2 Oxidative parameters

Reactive Oxygen Substances (ROS) of the plasma were evaluated with a commercial kit (Diacron, Grosseto, Italy) and are expressed as mmol H_2O_2.

The Antioxidant Power of plasma (PAO) was measured with a commercial kit (Diacron, Grosseto, Italy) that evaluates the ability of plasma to oppose the massive oxidative action of a hypochlorous acid (HClO) solution. PAO levels of the sample are expressed as μmol of neutralized HClO.

The extent of blood lipid peroxidation was evaluated by a spectrophotometer (set at 532 nm, Hitachi U-2000), which measured the adsorbance of thiobarbituric acid reactive substances (TBARS), and a tetraethoxypropane calibration curve in sodium acetate buffer (pH=3.5) (Dal Bosco et al., 2009). The results were expressed as malondialdehyde (MDA) nmol/mL of plasma.

The α-tocopherol level of plasma was assessed according to Schuep and Rettenmeier (1994) with HPLC method (Jasco, pump model PU-1580, equipped with an autosampler sistem, model AS 950-10, Tokyo, Japan) on a Ultrasphere ODS column (250 x 4,6 mm internal diameter, 5 μm particles size; CPS analitica, Milan, Italy). Tocopherols were identify using a FD deterctor (model Jasco, FP-1520) set at excitation and emission wavelength of 295 nm 328 nm respectively and were quantified using external calibration curves prepared with increasing amounts of pure tocopherols in etanol.

3.2 PRODUCT QUALITY(2nd CHAPTER):

Planning of experiment 3(a). and experiment 4(b).

3.2.1 Diet and pasture

3.2.1.1a Sampling of diet and pasture

The floristic (Table 3) and chemical composition in each pasture pen was estimated by sampling a 1m² fenced area by cutting with garden scissors (at 2 cm above the soil) before the onset of the trial. Samples of fresh grass (three pens × four seasons per group) were collected throughout the period of egg collection. The plants in the mixture were manually separated into groups, and the species were identified by macroscopic examinations. The characterisation was conducted in the laboratory of the Department of Agricultural, Environmental and Food Science where voucher specimens were stored. The grass intake was estimated according to the method of Lantinga et al. (2004) using a metallic frame (0.50 m × 0.50 m) per period with a cutting height of 4 cm. The herbage samples were collected before and after the outdoor period in each of the pens. The first batch of samples was collected the day before the hens were transferred to the outdoor fields at the beginning of every season, and the second batch was collected shortly after the hens were moved to the next pen (in the next season).

Table 3. Floristic composition of pasture in different seasons.

Pasture			
Winter	Spring	Summer	Autumn
Lolium perenne	*Lolium perenne*	*Lotus corniculatus*	*Sorgum halepense*
Lotus corniculatus	*Lotus corniculatus*	*Sorghum halepensis*	*Lotus corniculatus*
Trifolium pratense	*Trifolium pratense*	*Trifolium pratense*	*Trifolium pratense*
Daucus carota	*Dactilis glomerata*	*Lolium perenne*	*Lolium perenne*
Diplotaxis erucoides	*Diplotaxis erucoides*	*Diplotaxis erucoides*	*Diplotaxis erucoides*
Malva moscheta	*Malva moscheta*	*Malva moscheta*	*Malva moscheta*
Coniza canadensis	*Coniza canadensis*	*Coniza canadensis*	*Coniza canadensis*
Amaranthus retroflexus	*Amarantus retroflexus*	*Amarantus retroflexus*	*Amarantus retroflexus*
Convolvolus arvensis	*Convolvolus arvensis*	*Convolvolus arvensis*	*Convolvolus arvensis*
Ranunculus bulbosus	*Brassica oleracea*	*Kickia spuria*	-

3.2.1.1b Sampling of diet and pasture

Feed and water were provided ad libitum. Standard diets begin at 22% of crude protein (CP) and finish at 17 to 18% CP, organic rations begin at approximately 20 to 21% CP and finish at 15 to 16% CP. Such a low protein ration is also used to slow the rapid growth of meat-type broilers, which are the types used in the current study. The pasture lands were not treated with pesticides or herbicides during the 3 yr before organic production. The pasture area also contained mature trees, bushes, and hedges. Birds were fed starter (1 to 21 d) and finisher (22 d to slaughter, 81 d) diets that, as required by the European Union Regulation (EC 2008), contained 100% organic ingredients, certified by a national agency. (Table 4).

Table 4. Ingredients composition and calculated analysis of diets

	Starter	Finisher
Ingredients (%)		
Maize	52.0	46.0
Full fat soybean	30.5	12.5
Wheat	-	20.0
Soybean meal(1)	9.00	14.0
Alfalfa meal	2.80	2.80
Gluten feed	3.00	2.00
Vitamin-mineral premix(2)	1.00	1.00
Dicalcium phosphate	1.00	1.00
Sodium bicarbonate	0.50	0.50
NaCl	0.20	0.20
Chemical composition		
Dry matter (%)	90.9	90.8
Crude protein (%)	22.3	18.0
Ether extract (%)	7.95	4.98
Crude fibre (%)	4.67	4.01
Ash (%)	5.76	5.59
NDF - Neutral Detergent Fibre (%)	10.7	10.1
ADF – Acid Detergent Fibre (%)	5.58	5.06
Cellulose (%)	4.22	3.56
ADL – Acid Detergent Liquid (%)	1.03	1.11
Hemicellulose (%)	5.16	5.05
Metabolizable Energy (Mj/kg DM)	12.5	12.9

(1) From conventional crops.

(2) Amounts per kg: Vit. A 11.000 IU; Vit. D_3 2.000 IU; Vit. B_1 2.5 mg; Vit. B_2 4 mg; Vit. B_6 1.25 mg; Vit. B_{12} 0.01 mg; ◎-tocopheryl acetate 30 mg; Biotin 0.06 mg; Vit. K 2.5 mg; Niacin 15 mg; Folic acid 0.30 mg; Panthotenic acid 10 mg; Choline chloride 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.

3.2.1.2a Grass intake and productive performance

The grass intake (GI) was stimated using the following equation (Lantiga et al., 2004):

$$GI = (GMs - GMe) + 1 - (GMe/GMs)/ - \ln (GMe/GMs) \times (GMu - GMs)$$

where GMs was the herbage mass present at the introduction of the hens in each pen; GMe was the grass that remained at the end of the sub-period, and GMu was the undisturbed mass of grass from nearby ungrazed areas inside every sub-pen. Data for calculating the deposition rate and feed

intake were recorded per pen or cage/group throughout the productive cycle by the farm workers. Feed consumption per pen/cage was recorded weekly.

Data for calculating the deposition rate and feed intake were recorded per pen or group throughout the productive cycle by the farm workers. Feed consumption per pen was recorded weekly.

3.2.1.2b Productive performance

Individual body weight (BD) were recorded every week (50 birds/strain), and mean daily weight gain (DBW) and feed efficiency (FE) were calculated accordingly. Bird mortality and weight (of dead bird) were recorded daily. Chickens from first experiment groups were slaughtered at 81 d of age.

3.2.2 Analytical determination of products

3.2.2.1 a Eggs sampling

Eggs were collected for analyses during the four seasons in each of the different phases of productive activity. Forty eggs per group were gathered (at 07.50 h) on three consecutive Tuesdays in winter, spring, summer and autumn. All the eggs (120 per group/season) were stored at 5◦C until the analyses (maximum 2 days after) were performed in the laboratory of the Department of Agricultural, Environmental and Food Science.

3.2.2.1b Carcass dissection and sampling

At 81 days of age, 20 chickens per genotype were slaughtered in the processing plant of the farm, 12 h after feed withdrawal. Chickens were stunned by electrocution (110 V; 350 Hz) before killing. After killing, carcasses were plucked, eviscerated (non-edible viscera: intestines, proventriculus, gall bladder, spleen, oesophagus and full crop) and stored for 24 h at +4°C. Head, neck, legs, edible viscera (heart, liver, gizzard), and fat (perivisceral, perineal and abdominal) were removed in order to obtain the ready-to-cook carcass (Romboli et al., 1996). From the carcass, the *Pectoralis major* muscles were excised for successive analysis.

3.2.2.2 Analysis of diet and pasture

The chemical composition of the feed was determined according to AOAC (1995). The fatty acid profile of the feed, pasture and yolk was determined by gas chromatography after lipid extraction according to Folch et al. (1957) In particular, 1 mL of lipid extract was evaporated under a stream of nitrogen, and the residue was derivatised by adding 3 mL of sulfuric acid (3% in methanol). After incubating at 80◦C for 1 h, the methyl esters were extracted with petroleum ether, and 1 mL was injected into the gas chromatograph (Mega 2 - model HRGC; Carlo Erba, Milan, Italy), which was equipped with a flame ionization detector. The separation of the fatty acid methyl esters (FAMEs) was performed using an Agilent (J&W) capillary column (30 m × 0.25 mm I.D; CPS Analitica, Milan, Italy) coated with a DB-wax stationary phase (film thickness of 0.25 mm). The operating conditions upon column injection of the 1 mL sample volume were as follows: the temperatures of the injector and detector were set at 270°C and 280°C, respectively, and the

detector gas flows were H2 at 50 mL min^{-1} and air at 100 mL min^{-1}. The oven temperature was programmed to provide a good peak separation, as follows: the initial oven temperature was set at 130°C; this temperature increased at a rate of 4.0◦C min^{-1} to 180°C and was held for 5 min; the temperature was then increased at a rate of 5.0°C min−1 to 230°C; and the oven was held at the final temperature for 5min. Helium was used as a carrier gas at a constant flow rate of 1.1 mL min^{-1}. Individual fatty acid methyl esters were identified by reference to the retention time of FAME authentic standards. The relative proportion of each fatty acid in the fatty acid pattern of the egg yolks was expressed as a percentage. The mean value of each fatty acid was used to calculate the sum of the saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and long-chain polyunsaturated fatty acids (LCPs; ≥ 20C) of different series (*n*-3 and *n*-6). The peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai (1986):

PI = *(*% monoenoic × 0.025*)* + *(*% dienoic × 1*)* + *(*% trienoic × 2*)* + *(*% tetraenoic × 4*)* + *(*% pentaenoic × 6*)* + *(*% hexaenoic × 8*)*

The amount of each fatty acid was used to calculate the indexes of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991) and the hypocholesterolaemic/ hypercholesterolaemic ratio (HH), as suggested by Santos-Silva et al. (2002)

AI = (C12:0 +4xC14:0 + C16:0) / [(SMUFA + S(n-6) + S(n-3)]

TI = (C14:0 + C16:0 + C18:0) / [(0.5 x SMUFA + 0.5 x (n-6) + 3 x (n-3) + (n-3)/(n-6)]

HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)/(C14:0 + C16:0)

The *α*-tocopherol level was assessed according to Hewavitharana et al. (2004) using a high-performance liquid chromatography (HPLC) method (pump model PU-1580, equipped with an autosampler system, model AS 950–10; Jasco, Tokyo, Japan) and an Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 μm particle size; CPS Analitica). The tocopherols were identified using a fluorescence detector (Jasco model FP-1520) set at excitation and emission wavelengths of 295 nm and 328 nm, respectively, and were quantified using external calibration curves prepared with increasing amounts of pure tocopherols in ethanol. The extraction of individual carotenoids was performed using the aerial portions of the different plant species of the pasture and in the feed and yolk. Acetone extracts of the plants and feed were filtered through Millipore filters (0.2 mm). The carotenoid contents were quantitatively determined by HPLC (Jasco, pump model PU- 1580, equipped with an autosampler system, model AS 950–10, Tokyo, Japan) and a Ultrasphere ODS column (250 × 4.6 mm internal diameter, 5 μm particle size; CPS Analitica). The solvent system consisted of solution A (methanol–water–acetonitrile, 10:20:70) and solution B (methanol–ethyl acetate, 70:30). The flow rate was 1 mL min^{-1}, and the elution program was a gradient starting from 90% A in a 20 min step to 100% B and then a second isocratic step of 10 min. The detector was a UV–visible spectrophotometer (Jasco UV2075 Plus) set at a wavelength of 450 nm. The different carotenoids were identified and quantified by comparing the sample with pure commercial standards (Sigma- Aldrich, Steinheim, Germany; and Extrasynthese, Genay, France).

3.2.3a Egg sampling and analytical determination

Eggs were collected for analyses during the four seasons. Forty eggs per group were gathered (at 07.50 h) on three consecutive Tuesdays in winter, spring, summer and autumn. All the eggs (120 per group/season) were stored at 5◦C until the analyses (maximum 2 days after) were performed in the laboratory of the Department of Agricultural, Environmental and Food Science.

3.2.3.1a Physical analysis of eggs

Data recorded were:

- whole egg weight;
- integrity, weight and thickness of shell (Mueller and Scott, 1940);
- weight and colour of yolk (Roche scale);
- albumen height (Haugh unit) using an electronic gauge (Bukley et al., 1981).

After physical measuring, each egg yolk was frozen and lyophilized to proceed with the chemical analysis

3.2.3.1b Physical analysis of meat

Breast conformation was measured as follows: the maximal breast width and length were measured with a caliper, whereas the thickness was evaluated by inserting a metal needle in the fourth anterior of the sternum. Successively, the breast muscles and the thigh and drumstick (bone and meat) were excised to calculate the breast meat yield, the thigh and drumstick weight and the meat/bone ratio.

The ultimate pH (pHu) was measured 24 h after killing on refrigerated carcasses with a Knick digital pHmeter (Broadly Corp., Santa Ana, CA, USA). The colour parameters (L*, a*, b*) were measured using a tristimulus analyser (Minolta Chroma Meter CR-200, Azuchi-Machi Higashi-Ku, Osaka 541, Japan), with the CIELAB Colour System. The cooking loss was measured on samples of about 20 g placed in open aluminum pans and cooked in an electric oven (pre-heated to 200°C) for 15 min to an internal temperature of 80°C. The cooking loss was estimated as percentage of the weight of the cooked samples (cooled for 30 min to about 15°C and dried on the surface with a paper towel), with respect to the weight of the raw samples. The water holding capacity (WHC) was estimated (Nakamura and Katoh, 1985) by centrifuging 1 g of the muscles placed on tissue paper inside a tube for 4 min at 1,500g. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. WHC was calculated as: (weight after centrifugation-weight after drying)/initial weight* 100.

3.2.3.2a Chemical analysis of eggs

The chemical composition of the feed and eggs was determined according to AOAC (1995). The fatty acid profile of the feed, pasture and yolk was determined by gas chromatography after lipid extraction according to Folch et al. (1957) In particular, 1 mL of lipid extract was evaporated under a stream of nitrogen, and the residue was derivatised by adding 3 mL of sulfuric acid (3% in methanol). After incubating at 80◦C for 1 h, the methyl esters

were extracted with petroleum ether, and 1 mL was injected into the gas chromatograph (Mega 2-model HRGC; Carlo Erba, Milan, Italy), which was equipped with a flame ionisation detector. The separation of the fatty acid methyl esters (FAMEs) was performed using an Agilent (J&W) capillary column (30 m × 0.25 mm I.D; CPS Analitica, Milan, Italy) coated with a DB-wax stationary phase (film thickness of 0.25 mm). The operating conditions upon column injection of the 1 mL sample volume were as follows: the temperatures of the injector and detector were set at 270◦C and 280◦C, respectively, and the detector gas flows were H2 at 50 mL min−1 and air at 100 mL min−1. The oven temperature was programmed to provide a good peak separation, as follows: the initial oven temperature was set at 130◦C; this temperature increased at a rate of 4.0◦C min−1 to 180◦C and was held for 5 min; the temperature was then increased at a rate of 5.0◦Cmin−1 to 230◦C; and the oven was held at the final temperature for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.1 mL min−1. Individual fatty acid methyl esters were identified by reference to the retention time of FAME authentic standards. The relative proportion of each fatty acid in the fatty acid pattern of the egg yolks was expressed as a percentage. The mean value of each fatty acid was used to calculate the sum of the saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids and long-chain polyunsaturated fatty acids (LCPs; ≥ 20C) of different series (*n*-3 and *n*-6). The peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai (1986):

PI = *(*% monoenoic × 0.025*)* + *(*% dienoic × 1*)* + *(*% trienoic × 2*)* + *(*% tetraenoic × 4*)* + *(*% pentaenoic × 6*)* + *(*% hexaenoic × 8*)*

The amount of each fatty acid was used to calculate the indexes of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991) and the hypocholesterolaemic/ hypercholesterolaemic ratio (HH), as suggested by Santos-Silva et al. (2002) as previously indicated.

The tocopherol level was measured on 0.1 g of lyophilisate egg yolk. It was saponified in 1M KOH in ethanol in a thermostat bath at 50°C for 1 hour. Then the content was sonicated and extracted 2 times with 10 mL of *n*-hexane. The upper phase was collected and dried with N_2 to be then recostituited in 200uL of acetonitrile and injected into the HPLC system. The α-tocopherol content was measured by HPLC, using the method of Zaspel and Csallany (1983). The quantitative determination of carotenoids was carried out too. The acetone extracts were filtered through Millipore filters (0.2μm), and then analysed in HPLC (Jasco, pump model PU-1580, equipped with an autosampler sistem, model AS 950-10, Tokyo, Japan) on a Ultrasphere ODS column (250 x 4,6 mm internal diameter, 5 μm particles size; CPS analitica, Milan,Italy). The solvent system consisted of a solution A (methane/water/acetonitrile 5/10/85) and solution B (methanol/ethyl-acetate 70/30). The flow was 1mL/min and the elution program was a gradient starting from 90% A in a 20 min step to 100% B and then a second isocratic step of 10min. The detector was an UV-VIS spectrophotometer (Jasco 875-UV) set at λ 436nm. The different carotenoids were identified and quantified by comparing the sample with pure commercial standards (Sigma-Aldrich, Steinheim, Germany; Extrasynthese, Genay, France).

The total polyphenol content was also determined in the egg yolks, using the colorimetric method of Singleton and Rossi (1965) after extraction in methanol. The methanol extracts (200 μL) were mixed with 800μL of distilled water and 10mL of 0.2N Folin-Cicalteau reagent (Sigma®); 8mL of

saturated sodium carbonate (75g/L) were added and the mixture was shaked. After 2 hours the absorbency of the solution at 765nm was measured with a spectrophotometer. Quantification was based on the standard curve of 50, 100, 200, 300 and 500mg/L gallic acid prepared in the same way.

3.2.3.2b Chemical analysis of meat

Samples were immediately analyzed in duplicate to determine proximate composition and energy. Moisture, ash, and total nitrogen using AOAC methods N. 950.46B, 920.153, and 928.08, respectively (1995).

Total protein was assessed from Kjeldahl nitrogen using whole samples of both muscles (about 20 g) were placed in open aluminum pans and roasted in an electric oven (pre-heated to 200_C) for 15 min to an internal temperature of 80°C. The extent of muscle lipid oxidation was evaluated by a spectrophotometer set at 532 nm (Shimadzu Corporation UV-2550, Kyoto, Japan) which measured the absorbance of thiobarbituric acid-reactive substances (TBARS). Five grams of homogenized sample in water solution of TCA 7.5% containing 0.1% of diethylenetriaminepenta-acetic acid (DTPA). The mixture was filtered and 5 mL of the filtrate was reacted with TBA 2.88 g / L, at 95 ° C for 45min. At the end of the reaction the pink adduct formed was read in a spectrophotometer at 532nm (Ke et al., 1974). The quantification was done through a calibration curve with 1,1,3,3-tetraethoxypropane in sodiumacetate buffer (pH = 3.5). Oxidation products were quantified as malondialdehyde equivalents (MDA mg/kg muscle).

The fatty acid composition of diets, breast and drumstick was determined on lipids extracted from samples of about 5 g in a homogeniser with 20 ml of 2:1 chloroform/methanol (Folch et al.,1957), followed by filtration through Whatman No. 1 filter paper. Fatty acids were determined as methyl esters with a Mega 2 Carlo Erba Gas Chromatograph, model HRGC (Milano, Italy), using a D-B wax capillary column (25 mm ø, 30 m long). The fatty acid percentages were calculated using the Chrom-Card software.

Meat tocopherols was measured according to Schuep and Rettenmeier (1994). 2 g of muscle was accurately weighed and homogenized in 2 mL of water, and successively 5 mL of ethanol solution of BHT was added. The mixture was saponified with ethanol and KOH (60%) at 70°C for 30 minutes and exstracted with exane/ ethyl acetate (2:1). The homogenate was centrifuged and the supernatant was transferred in glass tubes and dried to 2 mL under N_2 The pellet was re-extracted two time. 50µL of filtrate was than injected into the HPLC/ FD (Jasco, pump model PU-1580, equipped with an autosampler sistem, model AS 950-10, Tokyo, Japan) on a Ultrasphere ODS column (250 x 4,6 mm internal diameter, 5 µm particles size; CPS analitica, Milan,Italy). Tocophrols were identify using a FD deterctor (model Jasco, FP- 1520) set at excitation and emission wavelength of 295 nm 328 nm respectively and were quantified using external calibration curves prepared with increasing amounts of pure tocopherols in etanol. Retinol was detected by UV (JASCO UV 2075 Plus) at 325 nm, and quantified using a external calibration curve like the vitamin E.

3.3 LIPID METABOLIC ACTIVITY (3rd CHAPTER):

Planning of experiment 5(a), experiment 6(b) and experiment 7(c).

3.3.1a Animals and diets

This trial was conducted at the experimental section of University of Perugia (Italy) from March to May 2010. All animals were reared according to EU regulation 834/07 and Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes. Six hundred male birds were compared and categorized with regard to their growth rhythm: slow (<20 g/d), medium (20 = 35 g/d) or fast (>35 g/d) (RCE, 2007; RCE, 2008). The genotypes were the following: Ancona (A), Leghorn (L), crossbreed Cornish × Leghorn (CL), Kabir (KR), Naked neck (NN), and Ross (R). The L and A genotypes are originated from a conservation flock at the Department of Applied Biology in the 1960s. The CL chicks were produced by crossing L hens with Cornish fowl (from local farmers), whereas KR (strain KR4), NN (strain NN1), and Ross 308 were furnished by a commercial poultry farm (Avicola Berlanda, Italy). Chickens were kept separate after hatching until 20 d of age in an environmentally controlled poultry house with temperatures ranging from 20 to 32°C and with RH ranging from 65 to 75%. Incandescent light (30 lx) placed at bird level was used for heating and illumination. Chicks were vaccinated against Marek and Newcastle diseases. At 21 d of age, the chicks were transferred to straw-bedded indoor pens (0.10 m^2/bird) each equipped with feeders and drinkers and with free access to forage paddock (4 m^2/bird). Each genotype was represented in 4 replicates containing 25 chicks each. Birds were confined to indoor pens during night. The pasture lands were not treated with pesticides or herbicides during the 3 yr before organic production. The pasture area also contained mature trees, bushes, and hedges. Birds were raised until the minimum slaughter weight (81 d); NN, KR, and R reached 2.50 kg, 2.38 kg, and 4.5 kg, respectively, whereas the crossbreed (1.8 kg) and the pure breeds (1.4 kg and 1.3 kg for A and L, respectively) were much lighter. Chickens were fed ad libitum the same starter (1–21 d) and grower-finisher (22 d to slaughter) diets (Table 1) containing 100% organic ingredients certified by a national agency. Access to feed and water was freely available, and all diets were formulated to contain adequate nutrient levels. The chemical composition in each pasture pen (n =24) was estimated by cutting a 1-m^2 fenced area using garden scissors (at 5 cm above soil) at the beginning and at the end of the trial. A sample of 20 birds per strain, each weighing between ± 10% of the population mean, were slaughtered in the department processing plant 12 h after feed withdrawal. Chickens were not transported and were electrically stunned (110 V; 350 Hz) before killing. After killing, the carcasses were placed in hot water (56.5°C for 1 min) and then plucked, eviscerated (nonedible viscera: intestines, proventriculus, gall bladder, spleen, esophagus, and full crop), and stored for 24 h at 4°C.

3.3.1b Animals and diets

A total of 60 lying hens belonging to the three experimental groups were studied: Leghorn (SG, n=20), a slow-growing strain selected for egg production; Leghorn female x Ross male (SFG, n=20) and Ross 408 (FG, n=20) medium and fast growing strains respectively at 21 days old. Chickens were housed in 3 paddock of the same poultry house at the Research Centre of the University of Perugia (IT). All birds were fed *ad libitum* the same starter and finisher diets (Table 3). Ten eggs per

group were incubated. To hatch, 5 chicks/group were killed and the liver was quickly excised with sterile scissors. The organs were weighted, minced with scissors, and approximately 1g was imbibed in 5 volumes RNAlater (Sigma, Milan, Italy) as indicated by the manufacturer. After 1 day at +4°C RNAlater was removed and samples placed -80°C until analysis. The rest was rinsed with ice-cold physiological saline for subsequent analysis enzyme activity. 5g was stored at -20°C for total lipid quantification.

3.3.1c Animals and diets

A total of 60 female chicks of 21 days old, belonging to the three experimental groups were studied: Leghorn (SG, n=30), a slow-growing strain selected for egg production and Ross 408 (FG, n=30) fast growing strains. Chickens were housed in different paddocks of the same poultry house at the Research Centre of the University of Perugia (IT). Three groups of laying hens for each genotype (slow-growing vs fast-growing) were fed with three different diets:

- control: standard diet *ad libitum* (Table 1 on experiment 7),
- LCPn-3: standard diet added with 3% fish oil (large amount of EPA/DHA - PRODUCTS)(Nordos®)
- LNA: standard diet added with 10% of linseed (large amount of LNA - PRECURSOR).

Ten eggs per group were incubated. To hatch, 5 chicks/group were killed and the liver was quickly excised with sterile scissors. The organs were weighted, minced with scissors, and approximately 1g was imbibed in 5 volumes RNAlater (Sigma, Milan, Italy) as indicated by the manufacturer. After 1 day at +4°C RNAlater was removed and samples placed -80°C until analysis. The rest was rinsed with ice-cold physiological saline for subsequent analysis of enzyme activity. 5 g was stored at -20°C for total lipid quantification. From the carcass, the pectoralis major muscle was excised for analysis and stored at -20°C.

3.3.2 Analytical determination

3.3.2.1 Lipid profile

The fatty acid profile of feed, muscle and liver were determined by gas chromatography after lipid extraction according to Folch et al. (1957) In particular, 1 mL of lipid extract was evaporated under a stream of nitrogen, and the residue was derivatised by adding 3 mL of sulfuric acid (3% in methanol). After incubating at 80◦C for 1 h, the methyl esters were extracted with petroleum ether, and 1 mL was injected into the gas chromatograph (Mega 2 - model HRGC; Carlo Erba, Milan, Italy), which was equipped with a flame ionisation detector. The separation of the fatty acid methyl esters (FAMEs) was performed using an Agilent (J&W) capillary column (30 m × 0.25 mm I.D; CPS Analitica, Milan, Italy) coated with a DB-wax stationary phase (film thickness of 0.25 mm). The operating conditions upon column injection of the 1 mL sample volume were as follows: the temperatures of the injector and detector were set at 270◦C and 280◦C, respectively, and the detector gas flows were H_2 at 50 mL min−1 and air at 100 mL min−1. The oven temperature was programmed to provide a good peak separation, as follows: the initial oven temperature was set at 130◦C; this temperature increased at a rate of 4.0◦C min−1 to 180◦C and was held for 5 min; the temperature was then increased at a rate of 5.0◦C min−1 to 230◦C; and the oven was held at the final temperature for 5 min. Helium was used as a carrier gas at a constant flow rate of 1.1 mL

min−1. Individual fatty acid methyl esters were identified by reference to the retention time of FAME authentic standards. The identification of individual fatty acids was carried out by using PUFA-2 fatty acid methyl ester standards and they were quantified by using nonadecanoic acid methyl ester (C19:0) as internal standard. The average amount of each fatty acid was used to calculate the sum of the total saturated (SFA), total monounsaturated (MUFA), and total polyunsaturated (PUFA) fatty acids. Peroxidability index (PI), AI and TI were calculated as previously indicated.

3.3.2.1a Indices estimation

Several indices were used to estimate desaturase and elongase activity of muscle tissue. Estimated desaturase activities are often used and, among many other authors, Vessbyet al. (2002) reported that the calculated activities of Δ9-, Δ5-, and Δ6-desaturase can be used as surrogates of the measure of the true desaturase activity. In particular, the activities of Δ9-desaturase and elongase were estimated by relating the percentage of product to the percentage of precursor (Okada et al., 2005). Specifically, the Δ9-desaturase index for the C18:1, which is the main MUFA of poultry meat, was calculated as 100 times the ratio of oleic acid (C18:1)to the sum of C18:1 and stearic acid (C18:0). The total Δ9-desaturase index (both 16 and 18) was calculated as 100 times the ratio of the sum of C16:1 and C18:1 to the sum of C16:1, C16:0, C18:1, and C18:0. The elongase index was calculated as the ratio of C18:0 to C16:0, whereas the thioesterase index was calculated as the ratio of C16:0 to myristic acid (C14:0; Zanget al., 2007). In order to evaluate the activity of both Δ5-desaturase and Δ6-desaturase, the enzymes catalyzing the formation of long-chain n-6 and n-3 PUFA starting from the precursor C18:2n-6 and C18:3n-3, the following equation was used (Sirri et al., 2010):

Elongase = C18:0/C16:0
Thioesterase = C16:0/C14:0
Δ9-desaturase (18) = [C18:1/(C18:1+C18:0)]x100
Δ9-desaturase (16+18) = [C16:1+C18:1/(C16:1+C16:0+C18:1+C18:0)]x100
Δ5+Δ6-desaturase = [C20:2n-6+AA+EPA+C22:5n-3+DHA/LA+ALA+C20:2n-6+AA+EPA+C22:5n-3+DHA]x100

3.3.2.2 b,c Preparation of total RNA, cDNA synthesis, fatty acid-2 gene (FADS2) cloning and sequencing

Total RNA was extracted from chicken liver using PureYield RNA Midiprep System (Promega, Italy), following the manufacturer's protocol as described in PureYield™ RNA Midiprep System Technical Manual #TM279, available online at: www.promega.com/tbs. This kit isolates intact, pure total RNA from essentially any sample type for use in a wide range of applications. The use of a novel Clearing Agent enables the rapid purification of total RNA with undetectable levels of genomic DNA contamination without using DNase. A novel combination of reagents, membranes and protocol leads to yields of up to 1 mg of total RNA without organic solvents, protease digestions or alcohol precipitations. The quantity of the extracted RNA was calculated spectrofotometrically by using the absorbance at 260 nm (NanoDrop, Thermo Scientific, Italy), whereas the integrity of RNA was assessed by agarose gel electrophoresis. Crisp 18S and 28S

bands, detected by ethidium bromide staining were indicator of the intact RNA. After extraction, total RNA was reverse transcribed into cDNA in a mix containing oligo dT16 primer, and dNTPs. This mix was heated, chilled on ice, and then reverse transcription buffer, DTT, RNaseOUT, and Moloney murine Leukaemia virus (M-MLV) reverse transcriptase were added, as described in the M-MLV Reverse Transcriptase kit (Invitrogen). To perform PCR, an aliquot of the resulting cDNA was amplified with GoTaq Polymerase (Promega) in a final volume containing buffer, dNTPs, and the primer set (FADS2 sense + antisense) designed by us (Table 5). To design the primers, we firstly performed a BlastN search (http://www.ncbi.nml.nih.gov/BLAST/) at the GeneBank database for FADS2 gene in *Gallus gallus*, finding a cDNA sequence with the accession number: NM_001160428.2. This sequence was used to design the primers FADS2-sense1+ antisense1 (Table 5; Figure 8). The PCR amplifications for FADS2 were performed using an automated Thermal Cycler (Mycycler, Biorad). An aliquot of each PCR reaction was then electrophoresed on agarose gel and bands were detected by ethidium bromide staining. The PCR product from primer amplification were then cloned using the pGEMT-Easy cloning vector system (Promega, Italy) and subsequently sequenced in both directions (T7 and SP6).

Table 5. Sequences of the primers used in the work.

Primer	Sequence 5′ – 3′	Purpose
FADS2-sense1	AGTACGGCAAGAAGAAGCTGA	RT-PCR
FADS2-antisense1	CACCACCTGTTCCCAACAATG	RT-PCR
FADS2_T3promoter	*caattaaccctcactaaaggga*CGGCAAGAAGAAGCTGA	mRNA std.curve
FADS2_antisense2	CTGGAGAGCCACTGGTTTGT	mRNA std.curve
FADS2 – sense 3	CCCGTGTATTTCCAAATCCAAATCAT	Real-time
FADS2 – antisense 3	AGCTACTACATGCGCTATTTCA	Real-time
Taqman® FADS2	GTTCTGGGCGGACCTGG	Real-time

Figure 8. *Gallus gallus* fatty acid desaturase 2 (FADS2), mRNA

NCBI Reference Sequence: NM_001160428.2
>gi|261878589:149-1483 Gallus gallus fatty acid desaturase 2 (FADS2), mRNA
ATGGGGAAGGGGGGCGAGAAAGGAGAGGAGTCCGGGGAGTGCAAGCCGCAGGTCCGCTCCTACACCTGGGAG
GAGATCCAGAAGCACAACCTGAGGACGGACAGGTGGCTGGTGATAGAGCGGAAGGTTTACAATGTCACCCAGT
GGGCGAGCAGGCACCCGGGCGGCCAGCGGGTCATCGGCCACTGCGCCGGCGAGGATGCCACGGATGCATTCCA
GGCCTTCCACATCAATCCCAGCTTGGTGCAGAAGTTTCTCAAGCCATTACTTATTGGAGAGCTTGCTCCAGGGGA
GCCCAGCCAGGACCGAGATAAAAATTCCCAGCTGGTGGAGGATTTTCGGACCCTGAGGAAGACAGCAGAGGA
CATGAACTTATTCAGAGCCAGTCCTTTGTTCTTCTCTCTTTACTTGGCCCATATCATTGCAATGGAAGCATTGGCT
TGGCTAATGGTTTCATACTTCGGTACCGGCTGGATCACAACTCTAATCCTTGCCTGCATCCTTGCAACTTCCCAGG
CCCAGGCAGGTTGGCTGCAACATGACTTTGGACACCTCTCTGTCTTTAAGAAGTCTTCCTGGAACCACATCGTCC
ACAAGTTTGTGATTGGACACCTTAAGGGTGCCTCTGCAAACTGGTGGAACCATCGTCACTTCCAACATCACGCT
AAGCCCAACATATTCAAGAAAGACCCAGATGTGAACATGCTGCATATTTTTGTCCTTGGCGAAAGTCAGCCTAT
TGAGTACGGCAAGAAGAAGCTGAAGTACCTGCCTTACAACCACCAGCATGAGTACTTCTTCCTCATCTTCCCAC
CTCTGCTCATCCCCGTGTATTTCCAAATCCAAATCATCTCAACCATGATCAAGCGCAGGTTCTGGGCGGACCTGG
CCTGGGCCATCAGCTACTACATGCGCTATTTCATCACATACATCCCATTCTATGGCATTCTGGGATCCCTGTTTCT
CCTCACTTTTGTCAGGTTTCTGGAGAGCCACTGGTTTGTATGGGTCACTCAGATGAATCACATTCCAATGGAAAT
TGATTGTGAGAAGCACAAAGACTGGCTTAGCTCTCAGCTGGCAGCCACCTGCAATATTGAGCAATCCTTTTTCA
ATGACTGGTTCACCGGGCACCTGAACTTTCAAATTGAGCACCACCTGTTCCCAACAATGCCACGGCACAATTTCT
GGAAAATCAAACCCTTGGTGAAGTCATTATGTGCCAAGTATGGAGTGCATTACGAAGAGAAGTCTCTTGGAAA
AGCATTTGTAGACATAGTTGGGTCACTAAAGAAATCTGGAGATTTATGGCTGGATGCTTACCTCCACAAGTGA

3.3.2.3 Quantitative real-time RT-PCR

3.3.2.3.1 Generation of in vitro-transcribed mRNAs for standard curves

The number of gene transcript copies of FADS2 gene was quantified by comparing them with a standard graph constructed using the known copy number of mRNAs of this gene. For this, a forward (FADS2_T3promoter) and a reverse (FADS2_antisense2) primer were designed based on the cDNA sequence of *Gallus gallus* (acc.nr. NM_001160428.2) (Table 5; Figure 8). The forward primer was engineered to contain a T3 phage polymerase promoter gene sequence at its 5′ end (Table 5) and used together with the reverse primer in a conventional RT-PCR of total chicken liver RNA. RT-PCR products were then evaluated on a 2.5% agarose gel stained with ethidium bromide, cloned using pGEM®-T cloning vector system (Promega, Italy), and subsequently sequenced.

This primer pair was used to create templates for the *in vitro* transcription of mRNAs. *In vitro* transcription was performed using T3 RNA polymerase and other reagents supplied in the Promega RiboProbe In Vitro Transcription System kit according to the manufacturer's protocol. The molecular weight (MW) of the *in vitro*-transcribed RNA for each gene was calculated according to the following formula:

MW= (n° of A bases × 329.2) + (n° of U bases × 306.2) + (n° of C bases × 305.2) + (n° of G bases × 345.2) + 159.

The mRNAs produced by *in vitro* transcription were then used as quantitative standards in the analysis of experimental samples using one-step TaqMan EZ RT-PCR Core Reagents (Life technologies, Italy). RT- PCR conditions were: 2 min at 50°C, 30 min at 60°C, and 5 min at 95°C, followed by 40 cycles consisting of 20 s at 92°C, 1 min at 62°C. The Ct values obtained by amplification were used to create standard curves for target genes.

3.3.2.3.2 Quantitation of FADS2 transcript by One-step Taqman RT-PCR

A hundred nanograms of total RNA extracted from the experimental samples was subjected, in parallel to 10-fold-diluted, defined amounts of standard mRNAs, to real-time PCR under the same experimental conditions as those used for the establishment of the standard curves. Real-time Assays-by-Design℠ PCR primers and Taqman gene-specific fluorogenic probes were designed by Life Technologies (LT). The sequences of primers (FADS2 sense3 + antisense 3) and of the Taqman probe are presented in Table 5. One Step TaqMan® PCR was performed on a StepOne Real Time PCR System (LT, Italy). Data from Taqman® PCR runs were collected with StepOne Sequence Detector Program. The reaction efficiency was in the range 90–92%. Furthermore, a minus-reverse transcriptase control ("No Amplification Control" or NAC) was included in qRT-PCR experiments. The NAC was a mock reverse transcription containing all the RT-PCR reagents, except the reverse transcriptase. No product was seen in the NAC, which indicates that contaminating DNA was not present in the sample.

3.3.2.4 Δ6-Desaturase activities

3.3.2.4.1 Preparation of microsomes from chicken liver

Microsomes were isolated from fresh chicken liver (approximately 2g) by standard methods. The liver was homogenized in 0.25M sucrose plus EDTA 0.1mM and 5mM Hepes, pH 7.4 (S/H buffer). The homogenate was centrifuged at 1,500g x 10min to remove cell nuclei, unbroken cells, and debris. The supernatant was centrifuged at 8,000g x 20min to eliminate crude mitochondrial fraction by pelleting. Lysosomes and other particles were removed with a 20,000g centrifugation for 30min. the resulting supernatant was centrifuged at 105,000g for 60min in a Beckman 60 Ti-rotor. Sedimented microsomes were resuspended in the correct amount of 0.15M KCl, 0.25M Sucrose and 10mM Hepes, oH 7.4 (Buffer A) and aliquots were dispensed into vials and stored at -80°C until use. Microsomal protein concentration was determined by Lowry assay (Lowry et al., 1951) with bovine serum albumin as standard.

3.3.2.4.2 Δ6-desaturase assay

The Δ6-desaturase enzymatic activity was estimated by measuring the amount of [1-^{14}C]18:3 n-6 (γ-linolenic) produced from [1-^{14}C]18:2 n-6 (linoleic acid) (Perkin Elmer) in buffer A. 0.5mL assay mixture contained the following concentrations of cofactors and reagents: 4mM ATP, 0.1mM CoA, 1.25mM NADH, 0.5mM nicotinmide, 5mM $MgCl_2$, 62.5mM NaF, 1.5mM GSH and 35 of unlabeled fatty acid nmol of substrate. For each sample, about 3nmol [1-^{14}C]18:2 n-6 were blended with 30nmol of unlabeled fatty acid and resuspended in buffer A complexed with 0.02% bovine serum albumin (free fatty acid). The specific radioactivity of substrate (~4nCi/nmol) was calculated by liquid scintillation counter. The reaction was started by addition of 2mg of microsomal proteins and assay mixture was incubated in shaking water bath at 37°C for 30min. Under these assay conditions the rate of desaturation of 18:2 n-6 fatty acid was linear with respect to the microsomal protein, substrate concentration and incubation time. After stopping the reaction by 10% KOH in methanol, total lipids were saponified by heating the methanolic KOH mixture for 60 min at 80°C. The mixture was then acidified with 8M HCl (pH 1-2) and fatty acid were extracted with hexane in a 3 steps extraction. The fatty acid were then converted to methyl ester by heating for 2min at 100°C with 0.5mL of 14% (w/w) BF_3-methanol, and extracted with hexane in a 3 steps extraction. The purified fatty acid methyl esters (FAME) were dissolved in hexane with butylated hydroxytoluene as an antioxidant, flushed with N_2, and kept in a -20°C freezer until analysis. The distribution of radioactivity between the 18:2 n-6 substrate and the 18:3 n-6 product of Δ6-desaturase activity was determined by thin-layer chromatography (TLC) with silica gel plates impregnated wiyh 10% (w/w) $AgNO_3$ (SiliCycle, Canada). Standard methyl esters of 18:2 n-6 and 18:3 n-6 were spotted near the labelled FAME to identify reaction product. Plates were developed in hexane/diethyl ether (2/3, v/v) for the separation of trienes from tetraenes. The spots were made visible under UV light by spraying with 2′,7′-dichlorofluorescein [0.2% (w/v) in ethanol]. Radioactive spots were also identified by Istant Imager (Packard) and scraped off directly into the scintillation vials and counted for radioactivity with 4mL of scintillation fluor by using a liquid scintillation analyzer (Tri-carb Packard, model 1600 CA).

3.4 Statistical analysis.

3.4.1a 1st Chapter

Several linear models (STATA, 2005; ANOVA procedure) were used to assess the effects of genotype and rearing system and their interactions in laying hens. For simplicity of exposition the season/age effect was not showed. Significance of the differences was assessed by the multiple t-tests and X-square was used for the not parametric variables.

3.4.1b 1st Chapter

A linear model (STATA, 2005; ANOVA procedure) was used to evaluate the interactive effect of genetic strains and grass intake. Significance of differences (P=0.05) were assessed with a Bonferroni multiple *t*-test. Differences in mortality rates, plumage conditions, percentage of FPD and breast blisters were evaluated by the X-square (FREQ procedure).

3.4.2a 2nd Chapter

A linear model (STATA, 2005) was used to analyse data to assess the effect of rearing system along the different seasons. Significance of the differences was assessed by the multiple *t*-test and X-square was used for the non-parametric variables.

3.4.2b 2nd Chapter

The data were analyzed with a linear model (STATA, 2005) to evaluate the effect of genotype. The significance of differences ($P<0.05$) was evaluated by multiple *t*-test and Fisher's Least Significant Difference test (LSD). Differences with at least a $P<0.05$ value were considered statistically.

3.4.3a 3rd Chapter

The data were analyzed with a linear model (STATA, 2005) to evaluate the effect of genotype. The significance of differences ($P<0.05$) was evaluated by multiple *t*-tests.

3.4.3b 3rd Chapter

The data were analyzed with a linear model (STATA, 2005) to evaluate the effect of genotype. Statistical analysis of gene expression and enzyme activity were performed using Turkey test and one-way analysis of variance (ANOVA) respectively. The level of statistical significance was set at $P<0.01$ and $P<0.05$.

3.4.3c 3rd Chapter

The data were analyzed with a linear model (STATA, 2005) to evaluate the effect of genotype and diets. The significance of differences ($P<0.05$) was evaluated by Bonferroni post-hoc test. Statistical analysis of gene expression and enzyme activity were performed using multiple *t*-tests and one-way analysis of variance (ANOVA) respectively. The level of statistical significance was set at $P<0.01$ and $P<0.05$.

4. RESULTS AND DISCUSSION

1st CHAPTER:

THE ADAPTATIVE RESPONSE

4.1 EXPERIMENT 1.

Effect of genotype and husbandry system on blood parameters, oxidative and native immune status: welfare and implications on performance of laying hens extensively reared.

Abstract: The aim of the present work was to evaluated the adaptative response to extensive farming system of pure breed (Ancona) *vs* commercial strain (Brown Hy-Line) of laying hens through a multifunctional approach (behaviour, tonic immobility, feathers score, antioxidant status, blood parameters, innate immunity and mortality). The trial was carried out in farm of the Department of Agricultural, Environmental and Food Science (University of Perugia), where 17-weeks-old females of Ancona breed (n=100) and Brown Hy-Line (n=100) were kept separately in a straw-bedded poultry house (0.10 m^2/bird), with free access to a grass paddock (4 m^2/bird). The genotype of the hens affected in a degree which depends on rearing system almost all the variables. The feeding and resting activities were higher in Hy-line birds while the main activity of Ancona hens was walking. The TI duration was affected by genotype and seasons being the immobility time longer in Brown Hy-Line especially during summer and autumn periods. The plumage condition showed a great adaptability effect, Ancona hens showed the better feathers condition than commercial line. Regarding native immune status serum bactericidal activity showed the highest values in Ancona. On the contrary, lysozyme and haptoglobin value showed greater value in Hy-line hens. In terms of production Ancona hens showed a lower production both at the level of intensity of deposition that as the total weight of eggs laid, except for the spring period. As was to be expected, such animals reared with the extensive system, were severely affected by the seasonality during the summer. They showed lower deposition rates and the least amount in weight of eggs produced. This result shows how the temperature and photoperiod significantly influence the deposition of eggs. In agreement with ethological profile, Ancona hens showed lower levels of stress, resulting in lower value of lymphocytes rate and H/L ratio. As expected, autochthones birds showed the worst feed efficiency and percentage of deposition, but the lowest mortality rate. In conclusion, on the basis of all these information together with the low mortality rate, Ancona hens showed the better welfare status on extensively reared .

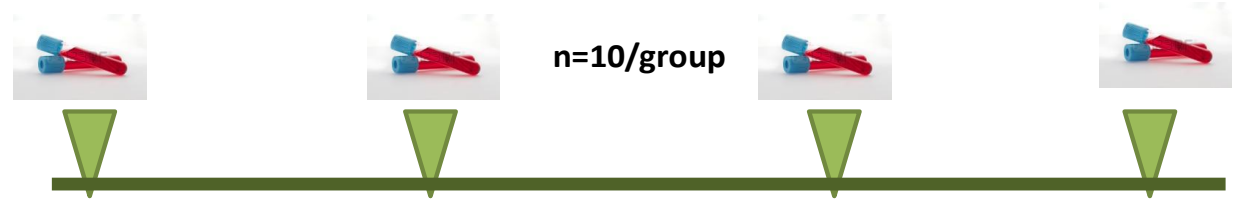
Figure1. Example of behavioral table

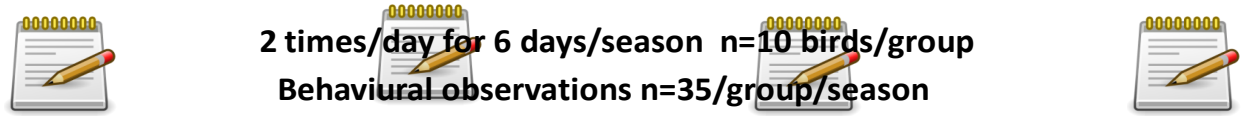
BEHAVIOURAL SCAN SAMPLING										
BREED										
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
EATING										
feed pecking										
other pecking										
drinking										
MOVE										
running/walking										
scratch										
rest										
stand										
roost										
sleep										
perching to feed peck										
perched to other peck										
COMFORT										
preening										
scratch										
shake										
stretching										
wings flapping										
swell										
INTERACTIONS										
attack										
escape										
allo-preening										
feather conditions										
POINTS										
0	Naked									
1	Bad									
2	half and half									
3	Good									
4	Perfect									
ANIMAL	1	2	3	4	5	6	7	8	9	10
neck										
breast										
wings										

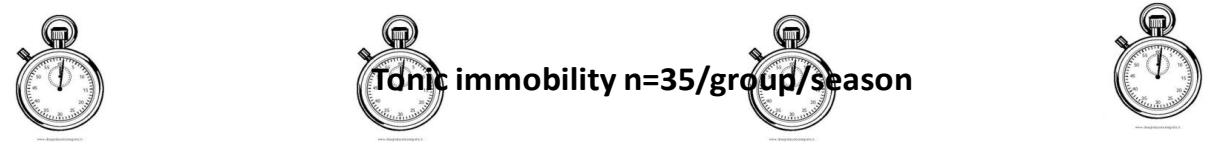
back										
tail										
LESIONS										
plantar lesions (foot-pad dermatitis, FPD)										
0 = no lesions										
1 = superficial lesions										
2 = ulcers										
sternal lesions										
0										
1										
ANIMAL	1	2	3	4	5	6	7	8	9	10
plantar lesions										
sternal lesions										
ANIMAL	1	2	3	4	5	6	7	8	9	
Tonic Immobility (sec.)										


100 Ancona hens


100 Brown Hy-line hens


n=10/group
Winter
Spring
Autumn
Summer


2 times/day for 6 days/season n=10 birds/group
Behaviural observations n=35/group/season


Tonic immobility n=35/group/season

1. Introduction

A correct assessment of animal welfare should involve multiple indicators such as behaviour, physiology, body injuries, disease and performance (Fraser and Broom, 1997). In recent years it has become evident that there is a strong correlation between animal behaviour, stress and the neuro-endocrine and immune systems (Yudkin et al., 2000; Marchetti et al., 2001). Therefore, unfavourable environmental conditions could lowered homeostatic functions, such as the immune response and in particular the innate immune system (Amadori et al., 1997) and the health status of animals (Moscati et al., 2003). Most of the intensive housing and management systems used in commercial poultry farms have been developed in order to reduce the production cost and are not the best with respect to the ethological needs of animals. The welfare of laying hens raised in commercial cages has been placed under intense scrutiny. The European Commission, has completely eliminated by January 1, 2012, the use of conventional cages. This directive stated that all existing cages must meet the 750 cm^2/bird space requirements and that each cage must be enriched with facilities that will allow birds to express their normal behaviours. Nowadays, the diffusion of alternative farming system (free-range or extensive) has underlined the problem of egg-type chickens housed in cages which have a negative effect on the welfare of hens (Appleby, 1993; Sossidou and Elson, 2009). However, not all hens genotype were adapted to extensive system and poorer environments. Commercial lines are unsuitable for extensive system because the environment is less controlled and animals are too heavy thus, health and welfare problems are common. However, economic reasons and limited chick availability render these animals widely used also in extensive production system. Among the different alternative farming systems organic egg production has increased during the last 15 years. In 2006, the market share in Italy of organically-produced eggs was 7.6% of all the organic products in retail and over a two year period the request for organic eggs increased by 4.6% (ISMEA, 2007). To date, there has been no clear evidence that welfare is improved when hens are raised under the organic production system. Few studies have been published on this subject and the results are conflicting, due to the great variation in the breeds used, production methods, diets used, pasture availability. Some studies (Casagrande et al., 2001; Minelli et al., 2007) and reviews (Van De Weerd et al., 2009; Berg, 2001) concerning the effect of conventional and extensive systems on productivity and product quality did not establish one system as being better than the other (Sauveur, 1991; Sundrum, 2001). The overall main opinion is that successful organic farming will depend on the right combination of layout and management of the henhouse and free range on the one hand, and on the choice of the right breed of hen on the other, thus the balance between these aspects will probably be of major importance. Regarding the genotype of the hens to be used in order to assure a good welfare status, the EC Regulation 1804/99 and the final recommendation of Network for Animal Health and Welfare in Organic Agriculture (2003), suggest utilizing local breeds for their higher rusticity. Hybrid birds selected to produce under highly controlled conditions, seem to be quite unsuitable for extensive systems, such us the organic one because the environment is less controlled and the rations are less equilibrated. The use of less selected strains, which still conserve natural behaviour, could also be a valuable alternative, particularly if they are in danger of extinction (Sponenberg, 1995). Italy is the country of origin of some egg-type chicken strains that have seen a drastic

decline in number; the Ancona breed which was widespread through Europe is an example. It has good productivity (about 280 eggs/year); the eggs are white and weigh 54-56 g (Castellini et al., 1990). As other pure breeds, Ancona has been progressively replaced by hybrids expressly selected for high egg production. Being animal welfare a "state" (Fraser and Broom, 1997) that encompasses many complex aspects of the animals it includes biological, psychological and ethical components. The biological components can be further divided into physical, physiological and behavioural. Most of the physical components of welfare are easy to determine, as it includes parameters traditionally used by the producers to evaluate performance and health. Behaviour is frequently used by experienced farmers to determine potential problems in animals (Kjaer and Vestergaard, 1999) and the status of birds' integument has recognized of considerable impact on the interpretation of bird health and welfare (Scott and Moran, 1993; Tauson et al., 2005). Physiological parameters which include hormone levels such as cortisol or corticosterone, heart rate or immune status (Jones and Faure, 1981; Craig et al., 1989) are frequently used as reliable indicators of the welfare status as well (Puvadolpirod and Thaxton, 2000a; Puvadolpirod and Thaxton, 2000b). Moreover the haematological stress indicator H/L is expected to increase if hens experience mild to moderate long-term stress (Maxwell and Robertson, 1998). Thus the aim of the present work was to study the adaptability response on the exestensive farming system of pure breed (Ancona) *vs* a commercial strain (Brown Hy-Line) of laying hens.

2. Results

2.1 Productive performance

Productive performance of laying hens (Table 1) have revealed differences and interactions due to the season, age and genotype. The live weight was mainly influenced by the genetic type. The Hy-line hens had a very fast growth rate even though increased locomotion (low animal density) and reduced quality of feed (intake of grass pasture).

Ancona hens showed a lower production both at the level of intensity of deposition that as the total weight of eggs laid, except for the spring period. They showed lower rates of deposition and the least amount in weight of eggs produced. The lay peak (around 80%) has occurred in the spring, when temperatures and photoperiod were optimal. The average mortality was lower in Ancona hens ($P<0.05$); the highest percentage occurred during the spring period in correspondence to the egg-deposition peak.

2.2 Behavioral observations

The time devoted to behavioral activities are significantly varied depending on the genotype and season examined (Figure 2). Compared to the percentage of movement, it should be noted that the Hy-line group, while having space available and thus a greater freedom behavioral, showed low percentages. In different seasons, these animals have committed 53.2, 42.7, 44.8 and the 49.9% of the total behaviors observed (respectively in winter, spring, summer and autumn) in walking/running. Hens have used almost all the space available to explore the whole area of the parquet floor. On the contrary, in the same subjects, it was found a lower tendency to take static positions (16.8, 21.2, 18.3 and 22.8% respectively in winter, spring, summer and autumn). Also the time spent to eating was lower (14.1, 11.4, 14.4 and 13.1%, respectively, in winter, spring, summer

and autumn). In Ancona,were absent social behaviors of fighting and fleeing, while the majority of social behavior were represented by *allo-grooming* (data not shown), while, the Hy-line hens, showed a higher percentage of social relations. Pure breed had also a better aspect (feather condition in Figure 3), then Hy-line and a better response to the test of tonic immobility (Figure 4).

2.3 Oxidative parameters and Immunity state

Regarding the blood parameters (Table 2), the Ancona breed showed significantly higher values of RBC ,HGB and HCT. Monocyte, eosinophiles and heterophils were lower in the pure breed. On the contrary lymphocytes had lower value in Hy-line hens. Ancona breed had a lower value of the H/L ratio and lymphocytes rate, resulting in lower levels of stress (Table 4).

Innate immunity traits (Lysozyme, SBA and HCA), TBARs and Tocopherol values are shown in Table 3. All animals enjoyed a good state of health, as demonstrated by the efficient non-specific immune system. It was observed that SBA showed a value greater than 40% inhibition of growth the bacterial strain (51.9% for Ancona). Regarding lysozyme, its highest value in commercial hybrids, indicates the presence of inflammatory states in this breeds (3.1 *vs* 1.4 μg/mL). The semi-quantitative titration of haemolytic complement, showed no significant changes, at the same time the AP value were lowest in Ancona hens (0.20 *vs* 0.46 mg/mL) demonstrating the highest standard of health enjoyed by these animals. The parameters related to oxidative status were strongly influenced by genotype (Table 4). Ancona birds showed a greater ROS value ($P<0.05$) than Hy-line in spring period (106.9 vs 87.8 mM H_2O_2) probably due to the higher productivity rate (80%). In the present case, all the hens have presented a smaller values of ROS at the beginning of experimentation, increasing the plasma levels of ROS with increasing age, in all groups. In comparison, the antioxidant capacity (PAO) was reduced under its consumption designed to hinder the peroxides. The importance of this physiological mechanism has been confirmed by the trend of plasmatic TBARs remained constant over time ($P>0.05$). It should be noted that the Ancona hens, in the autumn, probably due to the ingestion of grass, showed TBARs stable. Even plasma levels of tocopherols (manly α-Tocoferol) and carotenes were higher in the Ancona breed, reflecting their greater consumption of grass.

3. Discussion

Productive performance of laying hens have revealed differences and interactions due to the season, age and genotype. The Hy-line hens had a very fast growth rate even though increased locomotion (low animal density) and reduced quality of feed (intake of grass pasture). As was to be expected, animals reared with the extensive system, were severely affected by the seasonality during the summer. Ancona hens showed lower rates of deposition and the least amount in weight of eggs produced. This result suggested how the temperature and photoperiod significantly influence the deposition of eggs. Indeed the peak of lay has occurred in the spring, when temperatures and photoperiod were optimal. However we can assume, that the increased motor activity has diverted part of nutrients for coverage of increased need of maintenance, reducing its availability for the production of eggs. In fact, as previously mentioned Ancona breed showed a greater aptitude for movement. Even if the Ancona hens have showed a better adaptability to the environment conditions (lower mortality), it seemed to have a highest mortality during the spring

period in correspondence to the egg-deposition peak. The causes could have been due to ovarian diseases that affected the hens that showed the highest rates of deposition. In agreement, Lewis et al. (1996) reported that mortality increased with photoperiod, which corresponds to increases in deposition rate. In relation to time spent outside there was a greater tendency of Ancona breed to go out and to explore the available parquet. Indeed, if one must consider the constant presence of grazing livestock and sorghum which represented a safety for the animals, encouraging them to stay out of the shelters. The increased attitude to the pasture of Ancona breed, increased the kinetic activity. Compared to the percentage of movement, it should be noted that the Hy-line group, while having space available and thus a greater freedom behavioral, showed low percentages of movement. Hens have used almost all the space available to explore the whole area of the parquet floor. Such results can be explained by the effect of the genotype that, when selected for high performance production, tends to focus on activities with a low power output at the expense of those with high energy expenditure. In fact, they had intended primarily food energy for egg production (Rauw et al., 1998). These data as well as genetic factors, also be explained the higher level of welfare in Ancona hens which have not suffered from chronic stress and therefore have implemented behavioral mechanisms (abnormal behavior and aggression) in order to reduce it (Poderscek et al ., 1991). Even the response to the test of tonic immobility confirming when said.

Regarding immunity state, the Ancona breed showed significantly lower values of monocyte, eosinophiles and heterophils. A reduction in the percentage of monocytes in chickens subjected to stress (heat) was also observed by Altan et al. (2000). About the values of eosinophils, there are few and contradictory work for an avian species and the retrieval of basophils in the blood seems to be poor (Nemi, 2000). Searches performed on humans to accurately documenting the role of eosinophils and basophils in allergic diseases. Furthermore, clinical and experimental evidence suggest that they play a key role in the pathogenesis of allergic and immune diseases (Mukai et al., 2005). *In vitro* studies have shown, in patients exposed to specific allergens, histamine release from basophils and eosinophils is correlated with the severity of respiratory symptoms (Schreeder et al., 1964). In addition, basophils, eosinophils and Th2 lymphocytes are found at the starter of the inflammation in the case of allergic diseases in the skin and respiratory tract (MacFarlane et al., 1999). For the *Gallus gallus domesticus* species are reported ranges for heterophile from 15.0 to 40.0, lymphocytes from 45.0 to 70.0, monocytes from 5.0 to 10.0 and eosinophils from 1.5 to 6.0 (Nemi, 2000).

A further important parameter to be considered as an indicator of stress in poultry is the heterophil/lymphocyte ratio (Gross and Siegel, 1983). Numerous studies (Siegel, 1968; Thaxton et al., 1968, 1982; Siegel, 1984; Beuving et al. , 1989, Gray et al., 1989; Puvadolpirod and Thaxton, 2000a) have confirmed that heterophylly and lymphopenia are associated with chronic stress (constant production of ACTH). The data obtained have confirmed these findings. Under conditions of stress , these parameters rapidly changes, if the stressor doesn't stop to act, may have permanent and irreversible changes in homeostasis, resulting in the decline of resistance disease. The Ancona hens also, showed lower levels of stress, resulting in lower value of lymphocytes rate. In addition, some researchers (Akpa et al., 2007) have found a positive correlation between the duration of tonic immobility, stress severity and reduction of immune defenses (Campo et al., 2007). Although reference values on non-specific immune system of laying hens do not exist in the

literature, experiments carried out in our structures of animals without stated of disease and pathological lesions, it was observed that SBA showed a value greater than 40% inhibition of growth the bacterial strain (51.9% for Ancona). Even the lysozyme, its highest value in commercial hybrids, confirmed the presence of inflammatory states in this breeds. Lysozyme and complement are related with macrophage system function and indicates the presence of inflammation. Lysozyme is a strong antibacterial enzyme (against Gram+) that has a synergic action with immune humoral response and factors of the serum complement (Carroll and Martinez, 1979). The lack of complement indicates the risk of pathological forms or severity of infectious diseases already in place. The haptoglobin is a transporter of free hemoglobin (Hb) which is toxic and has a pro-inflammatory effect (Wagener et al., 2001). High levels of this substance are found in all situations associated with inflammation or tissue damage (Pfeffer et al., 1993). In our study, the values of haptoglobin were lowest in Ancona breed demonstrating the highest standard of health enjoyed by these animals, probably also due to smaller production rate. In other species, some authors have shown that transport stress (Jacobson and Cook, 1998) or sub-clinical infections (Melnick and Horstmann, 1947), increased levels of this parameter. Furthermore, even parameters related to oxidative status suggested an influence by genotype. It is widely documented that the motor activity increases the production of free radicals (Petersen et al., 1997) that organism tries to counteract activating an adequate antioxidant response (Alessio et al., 2000). It is also shown that even aging (Costantini et al., 2008) results an increase in the production of ROS. In the present case, all the hens have presented a smaller values of ROS at the beginning of experimentation, increasing the plasma levels of ROS with increasing age. In comparison, the antioxidant capacity (PAO) was reduced under its consumption designed to hinder the peroxides. The importance of this physiological mechanism has been confirmed by the trend of plasmatic TBARs remained constant over time. It should be noted that the Ancona hens, in the autumn, probably due to the ingestion of grass (Lopez-Bote et al., 1998), showed TBARs stable. It is likely that this phenomenon also contributes to a lower percentage of deposition (Table 1), which took place in organic chickens from the summer that could have resulted in a lower thrust oxidative these animals with the possibility of a greater balance of its oxidation state. In conclusion, on the basis of all these information (greater AP response to ROS production, lower lysozyme, higher SBA, lower AP concentration, lower H/L) together with the low mortality rate it is possible to affirm that the Ancona hens showed the better welfare status were extensively reared.

4. Conclusion

The Ancona breed seems more adapted to alternative farming system. It showed a higher percentage of walking, and an higher exploratory attitude (Dal Bosco et al., 2011) that results in a major grass ingestion. However the intensive kinetic activity of this breed, caused an increasing of ROS and oxidative status, that its balanced with antioxidant ingestion (plasmatic α-tocopherol and carotenes). Such behavioral activity caused a lower productivity, in Ancona hens respect to commercial hybrids, that resulted in a better health status and a higher resistance to disease, as evidenced by non-specific immune parameters. On the contrary the commercial line, showed a lower welfare and an really difficult to adapted on extensive housing (lesions, mortality rate), while productive performances were better. Then, the adaptability to the alternative system is not

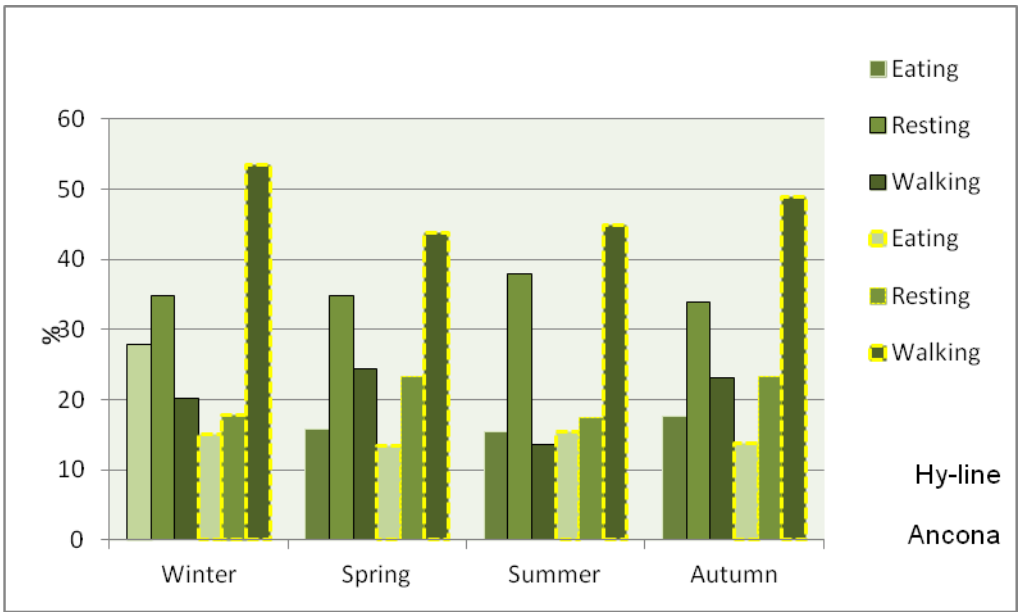
only attributable to the productivity rate, but also to the different grazing attitude, kinetic activity and body structure intrinsic to the genotype.

Table 1. Productive performance of laying hens

		Ancona				**Hy-line**				DSE
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	
Live weight*	g	1.890^{b}	1.693^{a}	1.600^{a}	1.895^{b}	1.965^{c}	1.950^{c}	1.940^{c}	1.995^{c}	155
Feed intake†	g/d	94.0^{a}	95.3^{a}	90.6^{a}	94.5^{a}	124^{c}	123.3^{c}	110.5^{b}	124.2^{c}	10.67
Feed /gain ratio†**		4.4^{c}	3.0^{a}	3.7^{b}	4.2^{c}	4.7^{c}	3.8^{b}	3.8^{b}	3.9^{b}	1.34
Deposition†	%	72.0^{c}	80.0^{d}	51.2^{b}	42.5^{a}	71.9^{c}	79.6^{d}	56.2^{b}	47.8^{a}	7.57
Eggs production†	g/d	23.5^{a}	34.8^{b}	26.9^{a}	35.0^{b}	45.2^{c}	50.4^{c}	49.1^{c}	51.4^{c}	3.8
Mortality†***	%	2.1^{b}	2.5^{b}	0.8^{a}	1.2^{a}	2.4^{b}	3.1^{C}	2.6^{b}	2.1^{b}	1.45

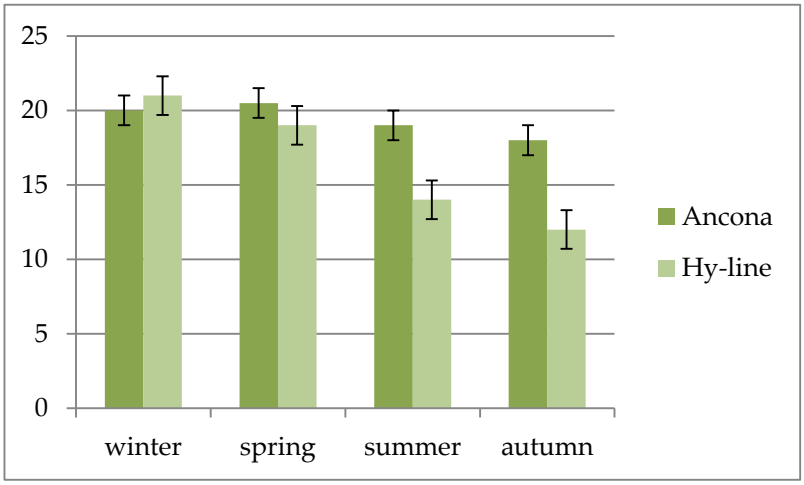
*: N = 35 per group and season; **: feed for egg laid $^{-1}$; ***: X^2; a..d.: P<0.05.

Figure 2. Main ethogram (%) of laying hens



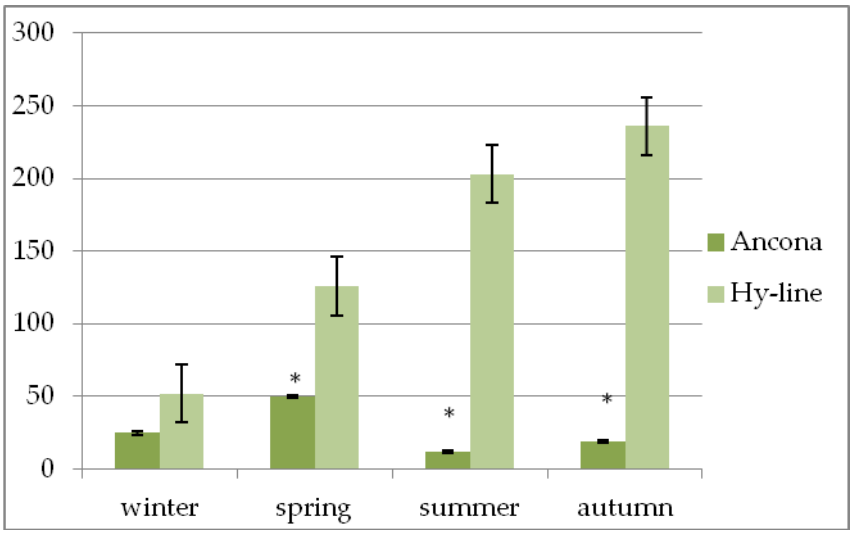
N = 35 per group and season

Figure 3. Feather condition of laying hens (average values of the different seasons).



N = 35 per group and season; *: P<0.05.

Figure 4. Tonic immobility (TI, s) of laying hens



N = 35 per group and season; *: P<0.05.

Table 2. Effect of genotype on innate immunity of laying hens (average values of the different seasons).

		Ancona	Hy-line	DSE
RBC	10^6/ml	2.7[b]	2.0[a]	0.62
HGB	g/dl	17.5[b]	14.5[a]	4.12
HCT	%	34.2[b]	30.5[a]	7.21
Heterophils	%	26.5[a]	31.3[b]	4.48
Lymphocytes	"	68.5[b]	66.2[a]	8.12
H/L ratio		0.36[a]	0.45[b]	3.15
Monocytes	%	5.8[a]	6.3[b]	3.10
Eosinophiles	"	1.5[a]	2.2[b]	0.96

N = 10 per group and season, a..b: P<0.05

RBC: red blood cells; HGB: Hemoglobin; HCT: Hematocrite; H/L: Heterophils/Lynphocytes ratio

Table 3. Effect of genotype on non-specific immune parameters (the mean of the different seasons)

		Ancona	Hy-line	DSE
SBA	%	51.9[b]	48.3[a]	4.8
Lysozyme	μg^{-1} ml	1.4[a]	3.1[b]	3.1
Complement	CH_{50}	102.4	108.2	29.1
AP	mg/ml	0.20[a]	0.46[b]	0.32

N = 10 per group and season; a..b: P<0.05.

SBA: serum bactericidal activity; AP: haptoglobin

Table 4. Plasmatic oxidative status and antioxidants of laying hens

		Ancona				Hy-line				DSE
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	
ROS	mM H_2O_2	48.8^{a}	106.9^{c}	135.9^{c}	115.3^{c}	39.8^{a}	87.8^{b}	125.8^{c}	153.8^{d}	11.4
TBARs	nmol ml^{-1}	47.9	39.7	39.9	40.0	42.8	44.8	55.5	54.2	5.7
PAO	µM HClO ml^{-1}	518.9^{d}	167.2^{b}	175.8^{b}	343.0^{c}	460.4^{d}	188.3^{b}	136.05^{b}	79.6^{a}	201.2
α-tocopherol	µg ml^{-1}	4.0^{b}	2.9^{a}	6.9^{b}	9.7^{c}	1.9^{a}	2.1^{a}	2.2^{a}	2.5^{a}	13.1
Carotenoids	µg ml^{-1}	646.7^{a}	1601.4^{d}	1298.7^{bc}	1401.8^{c}	439.8^{a}	1259.0^{bc}	1000.4^{b}	1002.5^{b}	304.8

N = 10 per group and season; a..b: P<0.05.

ROS: reactive oxygen species; TBARS: reactive substances of thiobarbituric acid PAO: antioxidant power of plasma

4.2 EXPERIMENT 2.

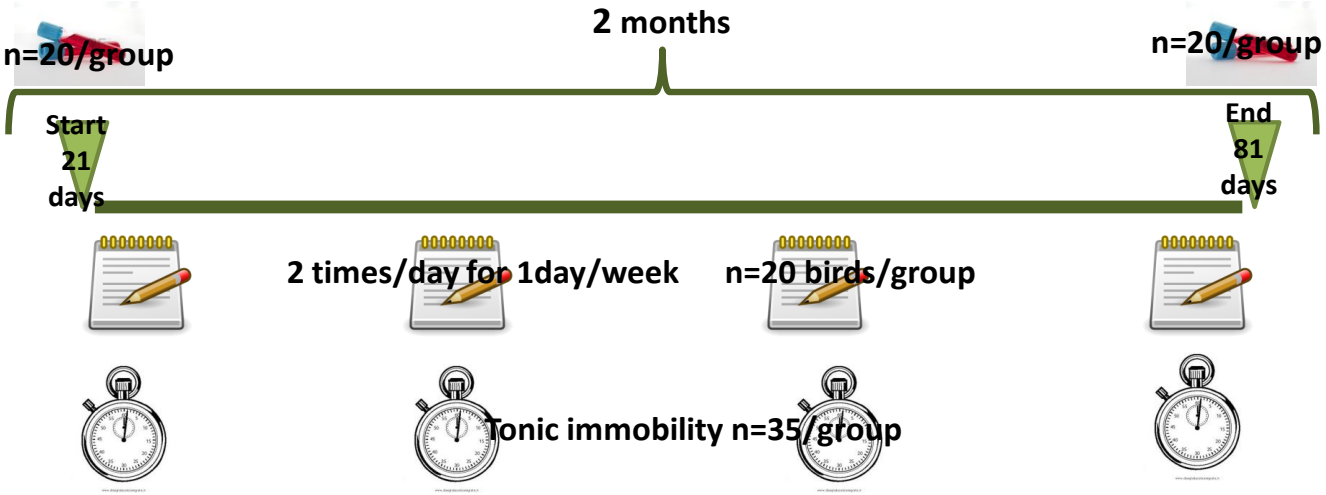
Behavior, welfare and adaptability of different meat-type chickens to extensive rearing system.

Abstract. The aim of this study was to define the adaptability of six different poultry genotype to extensive rearing system through the assessment of several endpoints (welfare, health, immune response). One hundred male birds of each genotype: Ancona (A), Leghorn (L), Cornish x Leghorn crossbred (CL) (slow-growing rate, A and L as pure breeds), Kabir (KR), Naked Neck (NN) (medium-growing rate) and Ross (R) (fast-growing rate) were extensively reared. The A and L genotypes displayed a higher reaction time on TI, a great variety of behavior patterns and the exploitation of all the available pasture area. The feather condition of slow-growing genotypes showed the best values for all body regions considered as well as an absolute absence of footpad dermatitis and breast blisters. The static behavior of fast-growing strains did not produce a significant plasma oxidative activity increase, whereas the active behavior of the slow-growing birds increased their oxygen demand. Accordingly, the plasma ROS and TBARS increased. The PAO, the response of the body to oxidative stress, was lower due to the intervention of comparison to antioxidant response. Plasma α-tocopherol followed the trend of kinetic and foraging activity and then decreases with increasing of genetic selection. Regard innate immunity, our results showed that HCT was higher in slow-growing birds and decreased in birds with moderate and fast-growth rate. Birds with moderate and fast growth also showed higher lysozyme levels, while it was lower in slow-growing strains, indicating a less incidence of acute and chronic inflammation. Accordingly, slow- and medium-growing birds showed lower H/L ratio. Although the fast-growing chickens had the best production, they appeared to be poorly adapted to the outdoor environment and had high losses (culling and mortality).

NATIVE BREEDS

CROSSBREED

COMMERCIAL LINES



1. Introduction

Art. 12 of Regulation (EC) n. 889/2008 provides that each Member State must specify criteria for defining slowly growing poultry genotypes and must evaluate the adaptability of these genotypes to organic rearing systems. This Regulation establishes that "*..in organic livestock production the choice of breeds should take account of their capacity to adapt to local conditions, their vitality and their resistance to disease and a wide biological diversity should be encouraged..*". Accordingly, a better equilibrium is needed among animal welfare, adaptability to the environment, biodiversity and productive performance. Several of the European Member States have provided a definition of slow growth based only on the daily weight gain. Others have identified these lines as female lines produced by the animal breeding industry, but the definitions of adaptability to extensive rearing systems and of the growth rate are still unclear. Factors such as animal health and welfare are key elements in alternative farming systems. It is necessary to consider not only growth rate but also other animal traits. Welfare can be viewed as a "state" with many aspects (Fraser et al., 1997). Behavioral observations can be used to determine the adaptation of animals as well as body status, which is an indicator of health and welfare (Tauson et al., 1993). From this perspective, the immune system of chickens can be modified by genetic selection and thus affects the adaptation of a genotype to a specific environment (Siegel, 2005). The relationship between animal welfare and performance is complex, but there are cases in which the improvement of welfare decreases the production costs (e.g., by decreasing disease and mortality) and increases people's perceptions of sustainable systems (Napolitano et al., 2013). In contrast, certain behavior patterns (e.g., kinetic and foraging activity) that are positively related to the welfare of the bird have negative effects on weight gain (Branciari et al., 2009). Given these considerations, the aim of the present study was to evaluate the welfare status of six different chicken meat-types through a multifunctional approach (behavior, tonic immobility, feather condition, the presence of body lesions, antioxidant and immune status and performance) to assess their adaptability to extensive rearing system.

2. Results

2.1 Behavioral observation and welfare

The ethogram of the six genotypes is summarized in Table 1. The fast-growing genotype (R) showed a smaller degree of initial interest; the moderately growing strains (NN and KR) reached intermediate values, whereas the slow-growing strains (L, A and CL) had the highest percentages. The slow-growing animals spent approximately 60% of their time budget outdoors and performed much of their behavior (20-50 m) from the shelter, exploiting all the available space (data not shown in table). Moreover, these birds showed a significantly higher initial interest towards the observers. The percentage of time spent moving was 70.0, 52.5 and 59.5% for the L, A and CL genotypes, respectively. In contrast, only 5.2% of the time budget was spent moving by the R genotype. On the contrary, eating and resting behaviors were less frequent in the slow-growing chickens and much more frequent in the R chickens (34.0% and 58.5%, respectively). Moderate growth rate birds (KR and NN) showed intermediate values for eating (17.8 and 19.9%, respectively). The, KR, NN and R chickens showed the highest value of resting (> 50%). The TI test, feather condition and footpad dermatitis (FPD) and breast blisters (BB) lesions of the six strains are

shown in Table 2. L and A showed a more rapid reaction time (< 40 sec); CL and NN showed intermediate times (approximately 60 sec); and KR and R showed the highest values (97 and 125 sec, respectively). The L, A and CL genotypes showed the best values of feather condition for all body regions considered and an absolute absence of FPD and BB lesions. A different situation was observed in the KR and R birds, which showed poor feather condition and a high level of carcass damage. NN showed intermediate results. The frequencies of FPD and BB lesions were dramatically higher in the birds with a moderate growth rate and in the rapidly growing birds than in the slow-growing chickens. Indeed, 70% of the fast-growing birds had a maximum FPD score, whereas a 0% FPD score was observed in the slowly growing birds. Of the moderate-growth genotypes, KR showed a high frequency of FPD, whereas a lower percentage (40%) of NN showed FPD.

2.2 Oxidative status, native immunity and blood parameters

The analysis of the *in vivo* oxidative status of the chickens (Table 4) showed that the ROS and TBARS values of the L birds were relatively high. These values were intermediate for A, CL, KR and NN and lower for R. Higher PAO was observed in the KR and NN birds. Blood α-tocopherol was higher in L and A, intermediate in CL and NN and lower in KR and R. Based on the findings for native immune status, the slow-growing birds showed higher values of HCA and lower values of lysozyme. The CL and R birds showed a lower HCA content. KR, NN and R showed a higher content of lysozyme. SBA did not show any trend, with all of the groups showing almost the same values. Lymphocytes, monocytes and eosinophils were lower in the fast-growing birds, intermediate in CL and KR and higher in the slow-growing birds. The opposite trend was observed for basophiles and heterophils. Accordingly, the H/L ratio was higher in the R birds. Hb was higher in the slow-growing birds and progressively lower in the moderate- and fast-growing birds. The Ancona birds, followed by the L birds, showed higher Ht values, whereas the CL, KR, NN and R genotypes had lower values. The RBC values did not show a clear trend.

2.3 Performance of different genotypes

As expected, the R birds reached the highest slaughter weight (4,500 g). The slaughter weights for KR and NN were intermediate (2,380 and 2,500 g, respectively), followed by CL (1,865 g), A and L (1,490 and 1,370 g, respectively) (Table 4). Consequently, all the other production traits followed this trend (feed intake, daily gain), whereas the feed-to-gain ratio increased in the slow-growing birds. The R chickens showed higher values of mortality and the culling rate, whereas the A and L chickens showed lower values. Mortality was primarily due to ascites and to sudden death syndrome.

3. Discussion

The analysis of behavioral patterns confirmed that the fast-growing genotypes had the poorest results in terms of initial interest and spent more time indoors than outdoors. In contrast, the slow-growing genotypes displayed a great variety of behavior patterns and exploited all of the available pasture area. The birds with a moderate growth rate had intermediate results in terms of initial interest, time spent outdoors and ethogram diversity. Lewis et al. (1997), comparing chickens with

different growth rates, observed marked differences in the behavior of the birds. In particular, the birds of the slow-growing genotype were much more active and more interested in the observer and made greater use of perches, and fewer birds were observed at rest. The slow-growing genotypes showed a superior ability to exploit the pasture, with many positive metabolic and qualitative consequences (Dal Bosco et al., 2011), confirming their adaptability to the alternative rearing system (Fanatico et al., 2005). In our previous study (Castellini et al., 2002), we observed that the level of activity was correlated with foraging behavior and that both are fundamental in this production system to allow the extensive use of the natural resources available. The fast-growing animals were less active, confirming the findings of Gordon and Charles (2002) and Lewis et al. (1997). Weeks et al. (1994) compared the behavior of Ross broilers reared under free-range conditions or maintained indoors and showed that the free-range birds made little use of pasture and tended to stay indoors and/or near the chicken house rather than foraging in the pasture. The authors attributed this behavior primarily to leg weakness, which prevented the birds from pasturing and behaving naturally. Gordon and Charles (2002) found slowly growing birds to be "moderately active" compared with the "inactive" fast-growing broilers. Fanatico et al. (2005) observed that fast-growing chickens did not venture outdoors as readily as slow-growing chickens. When they did go outdoors, the fast-growing birds did not appear to forage but rested in groups around the feeders. In our trial, eating behavior was less prevalent in the pure breeds and much more frequent in the Ross chickens. For decades, animal selection has focused on maximizing the production traits. Such selection pressure has induced farm animals to allocate a large portion of their resources to a particular production trait, reducing their ability to respond to other physiological demands (e.g., responses to environmental stimuli, immunity). Accordingly, even behavioral patterns have been affected. Indeed, selection for feed conversion efficiency has been found to be correlated with lower levels of activity (Dunnington and Siegel, 1996). Our results confirm this principle: the selected birds had a higher feed intake, which should compensate for the expenditure of energy on greater meat production. Additionally, the R genotype showed a greater percentage of resting behavior if reared in the free-range condition. In contrast, the slow-growing birds showed the lowest feeding efficiency and lowest feed intake and performed less resting and more kinetic (walking, running, foraging and exploring) activities. The TI test evaluates fearful behavior in the chicken. This behavior represents a terminal defensive reaction that can be used as a criterion to measure the well-being and stress levels of the birds. The fast-growing genotypes showed a greater reaction time. This result was consistent with the statement of Gallup (1974) that selection has a pronounced effect on the duration of TI and the finding of Jones (1986) that selection for productivity did not result in the elimination of fear and stress. In the context of feather condition, it is amply documented that feather pecking is a multi-factorial problem affected by environment and management and by the genetic background of the birds (Bilcik and Keeling, 2000). The poor feather score shown by the R birds confirms the last-mentioned genetic hypothesis. The fast-growing chickens showed severe difficulties in adapting to extensive system. These difficulties were caused by the high body and breast weights of the birds, which strongly reduced their mobility and forced the animals to rest on their bedding, especially during the last phase of rearing. A decrease in kinetic activity is a major cause of weakness in the legs, and long periods of rest in poor-quality litter produce skin lesions. As expected, the

differences in the kinetic activity of the slow-, medium- and fast-growing strains profoundly affected the oxidative metabolism of the body. Antioxidant power was relatively high in the medium-growth birds, whereas the values for the slow- and fast-growing birds were similar. The reasons for this peculiar trend are, most likely, related to the activity of the birds. The sedentary behavior of the fast-growing strain did not produce a significant oxidative burst, whereas the active behavior of the slow-growing birds increased their oxygen demand, accordingly, the plasma ROS and TBARs values increased. The PAO, an index of the body response to oxidative drive, acted to counterbalance this situation by activating a comparable antioxidant response. Plasma α-tocopherol followed the trend observed for kinetic and foraging activity: higher in the slow-growing birds, intermediate in the medium-growth birds and lower in the fast-growing birds. Because all of the birds ate the same feed, this trend was due primarily to the ingestion of grass, which is rich in vitamin E (Sossidou et al., 2010). Several studies in different animal species and in humans have reported that supplementation with vitamins C and E, other antioxidants or antioxidant mixtures can reduce oxidative stress as a result of exercise (Clarkson and Thompson, 2000). In our case, however, the increase in plasma tocopherol was unable to completely counteract the production of ROS and oxidative byproducts (TBARS) in highly active birds. Relationships among welfare, immunity and health have been considered by some authors (Mugnai et al., 2011). As previously stated, the general question concerns whether and how much the selection for productivity affects the ability of the animal to respond to environmental stressors. If the activation of the immune system is energetically expensive, the animals would exhibit a tradeoff between the production level and the immune response. Fast-growing birds, which are genetically programmed for high production, might have an impaired ability to achieve this tradeoff. As a result, they would be less capable of coping with environmental stress. Our results for innate immunity showed that HCA was higher in slow-growing birds and intermediate in birds with moderately or high growth rates and in CL. The reason for this finding could be that the antigenic pressure of the environment triggered the slow-growing birds to allocate resources to develop higher immune response (eg. HCA). High HCA levels indicate that the birds have not consumed the complement for specific immune reactions against various pathogens (Ricklin et al., 2010). The medium- and fast-growing birds also showed higher lysozyme levels, whereas the slow-growing strains had lower lysozyme levels, indicating a lower degree of acute and chronic inflammation. This trend is consistent with the results of Franciosini et al., (2011), who found a markedly low value for lysozyme in backyard turkeys. The rearing conditions for these birds were similar to the rearing conditions of the free-range slow-growing birds in the current study. Substantial breed-related differences in lysozyme have been reported in hens (Mugnai et al., 2011) and in broilers (Nath et al., 2002). Lysozyme is present in several cell types, and its concentration is related to granulocyte activities and the monocyte-macrophage system, indicating a state of inflammation (Gordon et al., 1979). The immune system reflects the ability to react against external stress. It is difficult to identify the traits that are best suited to adaptation, but we can hypothesize that the rapidly growing animals, which were genetically selected for high production, experienced difficulty in adapting to organic rearing. Under such conditions, the research of feed and other external factors (weather conditions) could be responsible for a stress situation (Franciosini et al., 2011). In terms of blood-related traits, it is widely known that stress increases heterophils and

reduces lymphocytes. Accordingly, the H/L ratio is an index of the response to a stressor (Maxwell et al., 1990). The slow- and medium-growth birds showed lower H/L ratios. Most likely, these lower ratios indicated a higher level of adaptation to the free-range system. In contrast, it has been reported that selection for higher body weight in turkeys is accompanied by a decreased immunological response. Avian heterophils act in the acute inflammatory response with high phagocytic specificity and accumulate in inflamed tissue (Campbell, 1995). Avian leukocytes are only transiently present in the blood (generally for 12-20 hours). After this period, they leave the circulation and migrate into the tissues, where they perform their functions. All types of leukocytes have functional and special actions. For example, lymphocytes are essential in protection against infection and in tumor rejection generating immune responses and retaining the memory of previous exposure to an antigen (Davison et al., 1991). Monocytes, heterophils, basophils and eosinophils are categorized as inflammatory leukocytes and produce substantial inflammatory effects. Monocytes constitute approximately 5-10% of the peripheral blood leukocytes, but this number varies in different chicken lines (Gordon and Taylor, 2005). In addition to modulating the immune response to infections by producing proinflammatory cytokines and chemokines upon activation, another important function of monocytes is their ability to produce tissue macrophages. Altan et al. (2000) reported a reduction in monocytes in birds subjected to heat stress, whereas Ajakaiye et al. (2010) found a decrease in the monocyte value of hens following transport. Eosinophils play a major phagocytic role in defense against parasitic organisms. The retention of normal levels of circulating eosinophils is associated with resistance to stress (Hohenhaus et al., 1998), and changes in blood eosinophils appear as a genotypic or phenotypic hallmark of physiological and psychological stress reactions (Hohenhaus et al., 1998). This hypothesis is consistent with the findings of the current study that eosinophilia occurred in the L, A and CL birds but not in the KR, NN and R birds. Bush (1991) noted that a low percentage of basophils in chickens could also cause a poor immunity response against disease and that such a low percentage can indicate a poor health state. Even if this observation appears to contradict all of the previous findings cited, a possible explanation could be lied to the strong age-related hematological profile observed in broiler strains and in different breeds of chickens (Islam et al., 2004). The changes observed in this study in hemochrome, hemoglobin and hematocrit are most likely linked to the previously described distinctive levels of exercise performed by the birds. It is widely known that exercise enhances [Hb] and VO_2 max, which is also proportional to the increase in the oxygen-carrying capacity of the blood (Calbet et al., 2006). The higher level of Ht in the slowly growing birds may have enhanced oxygen delivery to the tissues. Additionally, this increment is considered to play a role in increasing the number of RBCs as a response serving to increase the oxygen requirement of the body. Julian and Mirsalimi (1992) also found that oxygen saturation was greater in slowly growing chickens (91.6%) than in rapidly growing chickens (86.0%). These results imply an obvious genotypic effect on the behavior and native immune parameters analyzed. If the less productive birds are required to augment their natural defenses, the L, A and CL genotypes appear to be better adapted, most likely due to their lower productive performance, which allowed them to maintain physiological homeostasis. The data related to performance confirmed our previous findings (Castellini et al., 2002). In particular, these data conformed that the excessive weight of the birds was related to the culling rate and to leg

problems that prevented normal movement. Weeks et al. (1994) showed that approximately 80% of R birds had a gait abnormality at the 7th week of age. With age and increasing live weight, the leg joints of these animals are excessively stressed, and lameness, ascites and other related problems increase. The body weight of the L and A chickens was less than 2 kg, the minimum weight for organic products. The crossbred chickens almost attained this weight.

4. Conclusion

The results of this study support the conclusion that the slow-growing chickens showed a better welfare status and the greatest adaptability to the rearing conditions in the extensive system. Although the fast-growing chickens had the best productive performance, they appeared poorly adapted to the outdoor environment and showed high losses (culling and mortality). The position of pure breeds or their crosses as an non-conventional product could be strengthened if future research can demonstrate their ability to grow on feed of lower quality and to produce meat with well-differentiated qualitative characteristics. The medium-growth lines appear to represent a suitable compromise between adaptability and rusticity, which are key factors in pasture-based farming systems and in economic sustainability. A multicriteria analysis should be developed to obtain a global overview of the economic, ecological and qualitative performance of the poultry genotypes considered in this study.

Table 1. Ethogram (%) of different poultry genotypes

		L	A	CL	KR	NN	R	X^2
Initial interest*	%	65[c]	62[c]	60[c]	54[b]	52[b]	30[a]	20
Time spent outdoor	% of total time	60[c]	62[c]	55[b]	45[b]	52[b]	25[a]	152
Eating	% of observed behaviours	3.0[a]	1.8[a]	2.9[a]	17.8[b]	19.9[b]	34.0[c]	13.2
Moving	"	70.0[d]	52.5[c]	59.5[c]	14.3[b]	25.7[b]	5.2[a]	34.1
Resting	"	25.0[a]	33.3[a]	27.5[a]	66.5[b]	53.9[b]	58.5[b]	25.1
Comfort	"	2.0	4.5[b]	4.2[b]	1.4[a]	0.4[a]	2.0[a]	2.1
Others	"	0.0[a]	7.8[b]	5.9[b]	0.0[a]	0.0[a]	0.0[a]	1.2

N: 20 per genotype.

L: Leghorn; A: Ancona; CL: crossbreed Cornish x Leghorn; KR: Kabir; NN: Nacked neck, R: Ross.

[a,c] Values within a row with different superscripts differ significantly at $P<0.05$.

*: interest shown by the birds when the observer entered the pen

Table 2. Effect of poultry genotype on tonic immobility, feather condition and carcass damages.

		L	A	CL	KR	NN	R	Pooled Se
Tonic immobility	Sec.	37.7[a]	24.8[a]	62.5[b]	97.5[c]	61.6[b]	125.8[c]	24.2
Breast		4.0[c]	4.0[c]	4.0[c]	1.0[a]	2.0[b]	1.0[a]	1.6
Wings		4.0[b]	4.0[b]	4.0[b]	3.5[a]	3.5[a]	2.0[a]	0.2
Back		4.0[b]	4.0[b]	4.0[b]	3.8[a]	3.8[a]	2.0[a]	0.1
Tail		4.0[b]	4.0[b]	4.0[b]	3.7[a]	3.7[a]	2.0[a]	0.2
Vent/cloaca		4.0[b]	4.0[b]	4.0[b]	3.7[a]	3.7[a]	2.0[a]	0.1
Neck		4.0[b]	4.0[b]	4.0[b]	3.7[a]	-	2.0[a]	0.1
Total score		24.0[c]	24.0[c]	24.0[c]	19.4[b]	16.7[b]	11.0[a]	5.9
Footpad dermatitis	%	0.0[a]	0.0[a]	0.0[a]	30.0[c]	20.0[b]	60.0[c]	20.6
Breast blister	"	0.0[a]	0.0[a]	0.0[a]	25.0[b]	20.0[b]	40.0[c]	16.7

N: 20 per genotype.

L: Leghorn; A: Ancona; CL: crossbreed Cornish x Leghorn; KR: Kabir; NN: Nacked neck, R: Ross.

[a,c] Values within a row with different superscripts differ significantly at P<0.05

Table 3. Effect of poultry genotype on oxidative status, native immunity and blood parameters

		L	A	CL	KR	NN	R	Pooled SE
PAO	μM HClO ml^{-1}	66.19^{a}	73.78^{a}	89.03^{a}	170.60^{b}	151.16^{b}	75.28^{a}	37.59
ROS	mM H_2O_2	0.30^{c}	0.21^{b}	0.21^{b}	0.26^{b}	0.23^{b}	0.12^{a}	0.05
TBARs	μg/ml	2.14^{c}	1.55^{b}	1.59^{b}	1.46^{b}	1.45^{b}	1.24^{a}	0.18
α-tocopherol	"	5.33^{c}	6.09^{c}	3.37^{b}	1.89^{b}	2.75^{b}	1.03^{a}	0.36
Haemolityc complement assay	CH_{50}	81.86^{b}	82.64^{b}	76.45^{ab}	77.28^{ab}	78.47^{ab}	71.17^{a}	6.54
SBA	%	71.22	69.29	71.87	72.92	65.35	66.29	7.21
Lysozyme	μg/ml	1.51^{a}	1.45^{a}	2.12^{a}	5.20^{b}	5.07^{b}	6.89^{b}	1.59
Heterophils	%	44.75^{a}	48.00^{a}	53.50^{b}	54.14^{b}	50.11^{ab}	57.01^{c}	3.24
Lymphocytes	"	50.50^{b}	46.89^{ab}	44.67^{a}	43.14^{a}	46.33^{ab}	39.60^{a}	5.14
H/L	-	0.89^{a}	1.02^{a}	1.18^{ab}	1.25^{ab}	1.08^{a}	1.44^{b}	0.36
Monocytes	%	4.13^{c}	2.22^{b}	1.83^{b}	0.86^{a}	1.44^{b}	0.80^{a}	0.29
Eosinophiles	"	2.38^{b}	3.00^{b}	2.50^{b}	0.86^{a}	0.89^{a}	0.80^{a}	1.24
Basophiles	"	0.38^{a}	0.31^{a}	0.50^{ab}	0.86^{b}	0.60^{ab}	0.65^{ab}	0.19
Red Blood Cells	$10^{6}/ml^{-1}$	2.94	3.53	3.25	2.61	2.57	2.89	0.82
Hemoglobin	g/dl^{-1}	21.48^{b}	22.14^{b}	19.70^{ab}	18.17^{a}	17.64^{a}	17.48^{a}	2.16
Hematocrit	%	38.19^{b}	40.73^{b}	33.83^{a}	33.16^{a}	32.77^{a}	31.72^{a}	2.73
Platelets	"	5.50^{a}	7.78^{b}	5.83^{a}	6.43^{ab}	5.67^{a}	4.40^{a}	1.92

N: 20 per genotype.

L: Leghorn; A: Ancona; CL: crossbreed Cornish x Leghorn; KR: Kabir; NN: Nacked neck, R: Ross.

[a,c] Values within a row with different superscripts differ significantly at $P<0.05$.

Table 4. Productive performance of different poultry genotypes

		L	A	CL	KR	NN	R	Pooled SE/(*)X^2
Live weight	g	1370[a]	1490[a]	1865[ab]	2380[b]	2500[b]	4500[c]	120
Feed intake	g/d	65.3[a]	62.6[a]	75.7[a]	102.3[b]	100.8[b]	132.2[c]	19.2
Daily gain	"	16.7[a]	18.2[a]	22.9[b]	29.2[c]	30.7[c]	55.7[d]	0.09
Feed/gain ratio	-	3.81[b]	3.36[b]	3.24[b]	3.43[b]	3.22[b]	2.35[a]	0.28
Culled birds	%	0[a]	0[a]	2[b]	3[b]	2[b]	5[c]	3(*)
Mortality	"	4[a]	3[a]	4[a]	6[b]	5[ab]	14[c]	2(*)

N: 100 per genotype.

L: Leghorn; A: Ancona; CL: crossbreed Cornish x Leghorn; KR: Kabir; NN: Nacked neck, R: Ross.

[a..d] Values within a row with different superscripts differ significantly at P<0.05.

2nd CHAPTER:

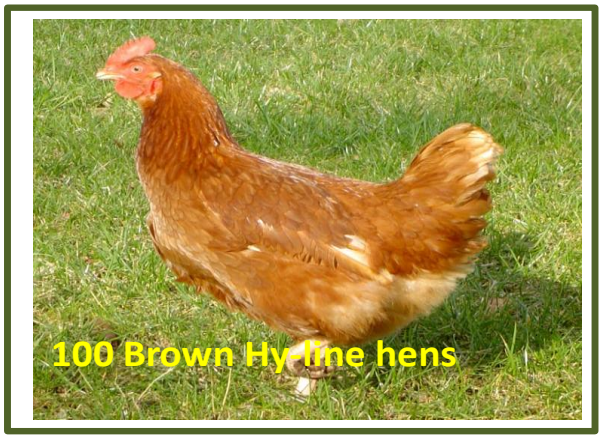
Products quality

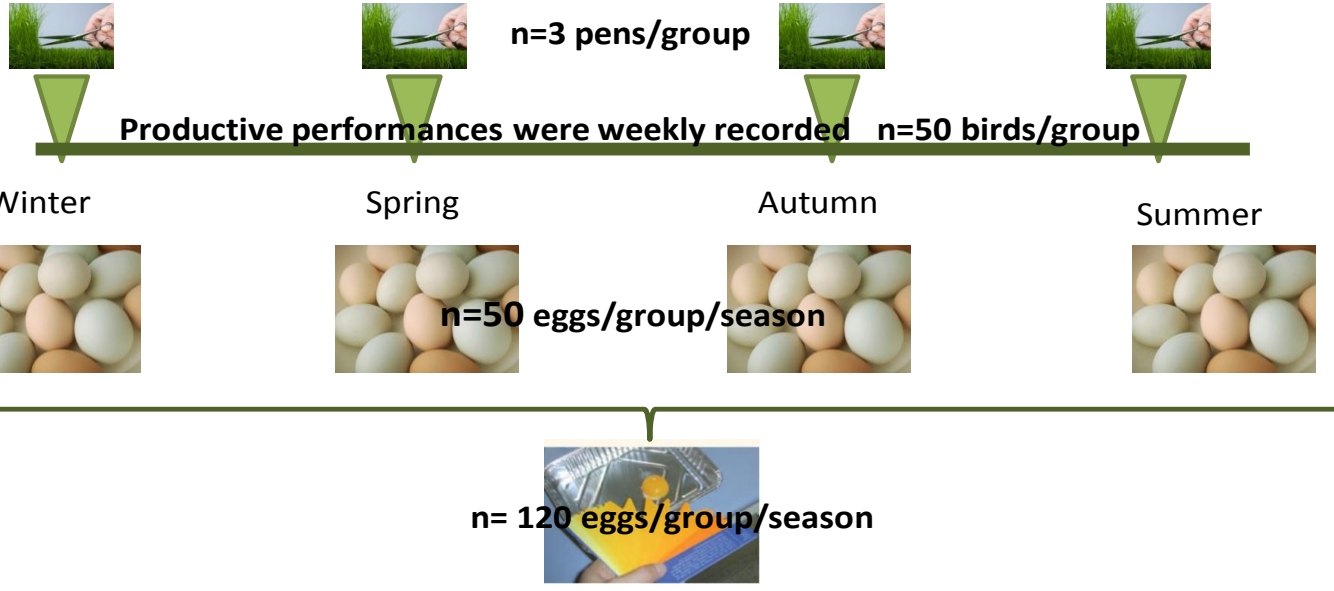
4.3 EXPERIMENT 3.

Testing of the effect of husbandry system on the grass intake and egg nutritive characteristics of laying hens.

Abstract. The aim of this study was to determine the adaptative response of two hens genotype in extensive farming system evaluating the grass intake and egg quality during different seasons. Forty eggs per group (vd 1st Chapter) were gathered (at 07.50 h) on three consecutive Tuesdays in winter, spring, summer and autumn. All the eggs (120 per group/season) were stored at 5◦C until the physical analyses (maximum 2 days after). Afterwards oxidative status and content in fatty acids and bioactive compounds were assessed, the following results/findings were identified. Ancona hens produced a lower number of eggs than commercial line, however they were markedly different from Hy-line ones, indeed the eggs had higher carotenoid, polyphenol and tocopherol contents which are bioactive compounds having a relevant effect on human health. This difference was due to the availability of green pasture. n-6/n-3 ratio was lower in Ancona hens compared to Hy-line, to witness a most linolenic acid intake through diet. The results showed that the grass intake was largely affected by genotype, and highlighted the seasonal effect of grass availability on the nutritional quality of eggs produced in non-conventional systems.


100 Ancona hens


100 Brown Hy-line hens


n=3 pens/group
Productive performances were weekly recorded
n=50 birds/group
Winter
Spring
Autumn
Summer
n=50 eggs/group/season
n= 120 eggs/group/season

1. Introduction

It is known that the welfare of the animal and the characteristics of the products largely depend on the adaptation of the genetic strain to extensive farming systems (foraging behaviour and resistance to environmental stresses). It has been reported that the most relevant role of grass in organic poultry is represented by the intake of several bioactive compounds (i.e. polyunsaturated fatty acids, vitamins and pigments (Morand-Fehr and Tran , 2001) that have a direct effect on the quality of the meat and eggs yet cannot be added in synthetic forms to organic diets. Lopez-Bote et al. (1998) showed that the egg yolk of Leghorn hens kept in a free-range system had increases in α-tocopherol and α-linolenic acid levels. However, there are many difficulties in determining forage intake, which itself has an unknown nutritive value; therefore, very little is known about the ability of layers to take advantage of forage to cover their nutritional needs. In extensive production systems, the potential contribution of vegetation, earthworms, insects and other food items from the outdoor area have been overlooked, presumably because the current production systems do not emphasise the utilisation of outdoor areas by poultry. (Walker and Gordon, 2003). However, grass could be considered a dietary source of energy, protein and vitamins and can also reduce the feed consumption to different degrees (5–10%, 30%; Walker and Gordon, 2003; Fanatico,1998). Gustafson and Antell (2005) indicate that hens foraging on oilseed, sunflower and wheat cropping systems are capable of supporting their entire nutritional needs. This observation is supported by studies on the crop content, indicating that free-range hens have a considerable intake of herbage and other accessible food items (Mwalusanya et al., 2002; Wood et al., 1963). It should be noted that not all breeds are adapted to alternative farming system. The EC Regulation 1804/99 and the final recommendation of Network for Animal Health and Welfare in Organic Agricultur (Hovi et al., 2003), suggest the utilization of pure breeds for their higher rusticity in order to assure a good welfare status. In fact, commercial strains selected to produce under highly controlled conditions, seem to be quite unsuitable for more extensive systems, such us the organic system, because the environment is less controlled and the rations are less equilibrated. The use of less selected strains, which still have natural behaviours, could also be a valuable alternative, particularly if they are in danger of extinction. Sundrum (2001) also ascribes added values such as biodiversity, species preservation, and environmental sustainability to Organic Agriculture. Italy is the country of origin of some egg type chicken strains which have seen a dramatic decline in number (Sponenberg and Christman, 1995); the Ancona breed which was widespread throughout Europe is an example. It has a good productivity (about 280 eggs/ year); the eggs are white and weigh 54-56g (Castellini et al., 1990). Analogous to other pure breeds, Ancona has been progressively replaced by hybrids, which are expressly selected for intensive production. Little information can be found in the literature regarding the effect of alternative rearing systems on the productivity and quality traits of eggs laid by pure breed hens (Lopez-Bote et al., 1998). For these reasons, it is important to determine the nutritional relevance of pasture to develop rations for free-range birds and to investigate the ability to pure breed and commercial line to transfer the above-mentioned compounds into the poultry products.

2. Results

2.1 Chemical composition of diet and pasture

The analysis of the floristic composition of the pasture (Table 2. Materials and Methods) showed a mixture of different species in all the seasons; in particular, *Lotus corniculatus, Sorghum halepense, Trifolium pratense, Lolium perenne, Diplotaxis erucoides, Malva moscheta, Coniza Canadensis* and *Amarantus retroflexus*. The most common species were *Lolium perenne, Lotus corniculatus* and *Trifolium pratense*; in summer and autumn *Sorghum halepensis* replaced *Lolium perenne* due to its greater resistance to dry periods. In general, grazing, compared to the feed showed, as was to be expected, higher levels of water, fiber, α-tocopherol, total carotenoids and polyphenols (Table 1.). The composition of the pasture has also felt the effects of the season, showing a strong increase of the dry matter in summer with consequent variation of the relative values of the other components. The comparison between the fatty acid profile of pasture and diet (Table 2) has highlighted significant differences concerning mainly the lower content of SFA and MUFA. In relation to PUFA was observed a greater presence of fatty acids of the n-6 series in the feed, due to the presence of soy (rich in linoleic acid - C18:2n-6), while in the pasture were more represented than those of n-3 (in particular α-linolenic acid - C18:3n-3) which are rich in legumes (Ouhayoun, 1998). This is achieved by a different n-6/n-3 ratio that was much higher in in the feed that the pasture (7.9 vs 0.2, 0.1, 0.2, 0.3 winter, spring, summer and autumn respectively).

2.2 Physical and chemical characteristics of egg

The chemical characteristics of the eggs (Table 3) were not affected by genotype, there were no significant differences in lipid and protein content of the eggs produced by commercial hens breed and Ancona (Hidalgo et al., 2008). The data reported in Table 4 show the differences in relation to the genotype: Ancona hens showed, for all analyzed variables (except the shell thickness), lower values, characteristic of this. The yolk/albumen ratio was higher in Ancona breed, because eggs have a greater yolk weight and, less albumen weight. Regarding the shell thickness, differences have showed in Ancona hens that, presented higher values. The Haugh index was average higher in Ancona breed, indicating a better internal quality of the eggs laid by the Ancona hens and providing a protection to the yolk. About yolk colour, the Hy-line eggs showed lower values than Ancona, except in summer ($P>0.05$), where both showed low values (8.1 and 7.0). This result can be due to productivity reduction in combination with vegetative growth, that brought a greater accumulation of carotenes in the yolk. In Ancona, colour (Roche value) showed a improvement, from 10.5 at the beginning to 13.4 at full productivity. This finding is in agreement with the assumption of grass and the content of carotenes of the yolk (Table 5). In both groups, the lower values were recorded in the summer, when high irradiation, confirming the biological action of these substances as protectors from solar radiation.

2.3 Bioactive compounds of egg

The variation of the content of carotenes of the yolk during seasons is linked to the recruitment of grass that, given its high content of carotenoids (Table 5), has favored accumulation in the yolk. In Ancona hens, the value of carotenoids has got 18.5 μg g-1 yolk in the autumn. This situation has

increased the yellow-orange color of the yolk until the highest obtainable with natural type xanthophylls lutein and zeaxanthin (15.2 μ g g-1 and 1.8 μ g g-1 of yolk, respectively). These pigments, highly bioavailable in the lipid matrix of yolk, make the egg in general, but in particular the Ancona eggs, an interesting dietary source of lutein and zeaxanthin.

2.4 Fatty acid content of egg

The fatty acid composition of the yolk was significantly affected (P<0.05) by the pasture availability (Table 6). The egg yolk from Ancona hens showed a lower concentration of PUFA n-6 (21,49 vs. 31,23%) due to less C18:2n-6 (19.11% vs. 24.91%) and a higher percentage of PUFA n-3 (8.32% vs. 3.84%) due mainly to a higher percentage of C18:3, C21:5 and C22:6n-3. Therefore, the n-6/n-3 PUFA ratio was lower (2.7 vs. 8.18). The total SFA value was not affected (P>0.05) because the increased amount of C16:0 was compensated for by the decrease in C18:0. The different levels of PUFA n-3 and n-6 precursors determined the modifications of their specific derivatives: a high level of linoleic (LA) acid resulted in an increase in arachidonic acid. In the Ancona eggs, the higher presence of a-linolenic acid (ALA) favoured the synthesis of DPA, and DHA in spring (87.5 and 468.0 mg/100g yolk, respectively). The atherogenic (IA) and thrombogenic (TI) indexes in all the groups were optimal under a nutritional point of view (Table 7). The Index of Nutritional Quality (INQ), which considers the amount (mg 100 g -1) of EPA and DHA with respect to the total energy (kcal 100 g -1) of the product, was consistently (exception in winter) higher (P =0.05) in the Ancona eggs. In spring, the INQ values of these eggs reached levels approximately three times higher than those of Hy-line in the same season. The HH index, calculated as the ratio between hypo- and hyper-cholesterolaemic fatty acids, was similar in all the eggs confirming the optimal nutritional value of the eggs. In our study, the cholesterol level showed a significant higher value in Ancona egg. Probably this result is correlated with the lower productivity of such breed (Hall and McKay, 1992). Regarding TBARS values, there aren't significantly differences. Even thought Ancona hens, seems to have an higher rusticity and exploratory attitude, and then produces more pro-oxidant compound, they balance this amount with antioxidant compound ingested with the pasture. Likewise, Hy-line hens, given their lower kinetic ability and grass intake, produce a lower amount of ROS and therefore are less susceptible to lipid peroxidation.

3. Discussion

The pasture availability widely affected the dietary intake of laying hens, in order to seasons. The grass, earthworms and insects ingestion incremented the assumption of biological compound as α-tocopherol, total carotenoids and polyphenols. The content in these components, in agreement with our previous studies (Castellini et al., 2008), has confirmed that this is a good dietary source of antioxidants, in particular during spring season. Even the fatty acids profile which was rich on essential fatty acids of n-6 series (feed) and n-3 (pasture), was particularly affected by seasons, in fact a n-6/n-3 ratio was much higher in the pasture in winter and decreased with the time (7.9 vs 0.2, 0.1, 0.2, 0.3 winter, spring, summer and autumn respectively). Given the different intake of pasture of two breeds, we have showed a great differences on chemical characteristics of the eggs. Indeed the higher weight value of yolk in Ancona hens was in agreement with Minelli et al. (2007) and Van Den Brand et al. (2004). Probably the low protein ration, reduced the production of eggs

and albumen weight, in fact Perez and Jensen (1991) affirmed that some physical parameters were strongly influenced by the level of protein 'food'. The shell weight of the commercial hens resulted higher than Ancona. This fact, in addition to nutritional and metabolic factors, was due to the effect of genetic selection operated on commercial genotypes in order to increase the egg weight (Singh and Panda, 1987; Narushin et al . 2001; Seker, 2004). Even the shell thickness was influenced by genotype, the higher shell weights recorded in Ancona eggs could have been due to the ingestion of tiny stones from the ground and to a higher synthesis of vitamin D_3 (Bar et al., 1999) as a result of a greater exposure to sunlight. Vitamin D is formed in the skin of animals through the following processes: provitamin D, previtamin D, Vitamin D; the conversion of provitamin D to previtamin D is a photochemical reaction requiring ultraviolet B photons (Wang and Quinn, 2000). In according to this theory, the shell showed a deposition rate tends lower than at the beginning. The Haugh index, also confirmed the better quality of Ancona eggs than Hy-lyne ones. Indeed the Haugh value evaluates the rigidity, the thickness and density of the albumen spread around the yolk (Haugh, 1937). Furthermore there are evidence (Benabdeljelil and Jensen, 1990; Franchini et al., 2002) that the quality of the albumen is improved by the presence of Vitamin C. This vitamin is water-soluble and widely distributed in the plant world (USDA) and therefore is expected that the intake of fresh grass from the pasture has greatly contributed. The yolk colour join with carotens content, pigmented compounds, further highlight the effect of seasons in outdoor farming system. In fact only in summer period commercial eggs has obtained similar values than Ancona ones. This result can be due to productivity reduction in combination with vegetative growth, that brought a greater accumulation of carotenes in the yolk. However such low Roche value can be due to the high solar irradiation, confirming the biological action of these substances as protectors. Therefore are likely that these hens have used the bioactive compounds for their biological processes, taking into account the reduced availability of grass and its lower content of carotenes in this period. The performance of the Ancona group is not agreement with those reported by several authors. There is a great evidences (Silversides and Scott, 2001), that the quality of the white eggs decreases with age increasing. Spackman (1987) in "pathogen free" hens, has verified that the effect of age on the quality of the albumen is zero or even reverse. Probably the better quality of Ancona egg in relation to the age is due to a good state of health, which can be explained by the high level of welfare of Ancona hens. Even cholesterol content could be effected by age (Naviglio et al., 2012), in our study cholesterol value not affected by breed, or age, probably there was a negative correlation with the productivity of hens (Hall and McKay, 1992). Some authors (Tolan et al., 1973; Scholtyssek, 1992) found higher levels of cholesterol in egg produced from hens kept under free-range conditions. Great importance should be given to the specific content of antioxidant molecules. We have found natural type xanthophylls, such pigments, highly bioavailable in the lipid matrix of yolk, make the egg in general, but in particular the Ancona eggs, an interesting dietary source of lutein and zeaxanthin. It should be noted that such eggs, while being significantly smaller, present a greater content (for whole egg) of bioactive compounds. In addition to quality parameters, the contents of egg carotenes is also a means of assessment welfare state of hens. In fact, many researchers define the carotenes as indicators of fitness animal (Moller et al., 2010; Surai et al., 1998; Horak and Saks, 2003; Mcgraw, 2004), because of their poor availability in nature ("foraging ability") and their decrease in all tissues. Based on this assumption

and considering the amount of carotenes in eggs transferred, it can be said that these chickens enjoyed of better conditions of fitness and thus greater well-being. In addition to affecting the yolk colour (Mugnai et al., 2009), carotenoids, tocopherols and flavonoids play a key role as antioxidants in the healthy development of chick embryos, immune response (Galobart et al., 2001b; Haq et al., 1996; Surai et al., 1996) assuring a stronger and result in a higher egg lipid stability (Galobart et al., 2001a). Further confirmation could be done from the high values of antioxidant capacity observed in these animals, despite the absence of synthetic vitamins in the food and the increased oxidative metabolism due to the kinetic activity. It is known that carotenes in the diet may have a high power scavenging of free radicals and are considered among the main elements of defense to coronary heart disease (Osganian et al., 2003). Same authors have highlighted the strong inhibitory power of lutein against lipid peroxidation in vitro. It should be added that these compounds inhibit the oxidation of LDL, which represent the first step towards the processes of atherosclerosis. Greene et al. (2006), underline the great importance of carotenoids in terms of eye health and the protective effect against the degeneration of the organ that can lead to blindness phenomena. In particular, lutein and zeaxanthin have a filter effect and protect the macula sensory (a small region of the retina) from degenerative, oxidative and photochemical damages. Although the yolk egg containing smaller amounts of lutein than some vegetables (i.e. spinach) presents a higher bioavailability due to the lipid matrix in which these pigments are deposited. To underline the importance of these compounds can be cited other studies (Chung et al., 2004) in which they are actually evaluated the effects of consumption of lutein-enriched eggs. Burke et al. (2005) studying the effects of food supplements of lutein on its blood concentration and status of the macular pigment, found that the best situations of optical density of pigment in the macula were always associated with high levels of dietary intake of lutein and zeaxanthin. The levels of lutein found in yolk egg of Ancona breed produced in the spring are comparable or even higher than that found by Handelman et al. (1999) in commercial eggs added with corn oil; this finding is of great interest since, as already said, the eggs from extensive farming are smaller than the commercial ones. According to the authors mentioned about the consumption of an egg a day (1.3 yolks) increases blood levels of lutein by 39%. The level of polyphenols increased through the recruitment of grass, with maximum value in the spring on Ancona breed (65.1 mg 100 g-1 whole egg). The role of polyphenols is to contribute to the egg stability, protecting the yolk components and albumen oxidative stress (Winkel-Shirley, 2002). The α-tocopherol content of eggs in Ancona breed was higher than commercial line in all seasons, due to increased intake of grass, rich in this compound (Lopez-Bote et al., 1998). These findings are in according to those reported by numerous authors (Jiang et al., 1994; Surai et al., 1997; Gebert et al., 1998; Meluzzi et al., 2000; Franchini et al., 2002) compared to "excellent ability of hen to transfer α-tocopherol from feed to egg" (Cherian et al., 1996; Galobart et al., 2001a, b). Furthermore the Ancona eggs could be considered a good source of others compounds essential to the human health, like fatty acids of n-6 and mainly n-3 series. The n-3 profile confirmed that DHA was the major LCP n-3 and that egg is one of the most important terrestrial feed sources of DHA for human and becomes very important during the growth of the chick (Speake et al., 1999). Indeed, DHA in standard or enriched meat of several animal species (pork, chicken, rabbit) is poorly represented whereas EPA is found much more. Increases in the DHA value were reported to be higher in eggs produced from hens fed diets

with 5% extruded linseed or *Sativa camelina* (a plant rich in linolenic acid)(Shapira et al., 2007) Moreover, these results confirmed the efficiency of the hens (Mugnai et al., 2009; Donaldson, 1996; Cherian and Sim, 2001) to elongate and desaturate LNA into n-3 LCP and to transfer these fatty acids to their eggs. The dietary strategies for increasing the nutritional properties of animal products (Grashorn, 2007) mainly consist of enriching diets with ingredients rich in LNA (e.g. linseed, colza; Van Elswyk, 1997; Jiang, 1991) or directly with LCP (e.g. fish oils, algae, water plants; Hargis et al., 1991; Huopalahti et al., 2007). The supplementation of LNA produces less dramatic LCP increases, the levels of which are also affected by the n-6/n-3 ratio of the diet and hen metabolism; however, such a strategy is physiological and dependent on the oxidative status of the animals and products (Kassis et al., 2010). Indeed, high levels of PUFA, if not adequately protected, can transform the dietetic advantages of an LCP enrichment into risks upon the formation of unstable and extremely injurious by-products (Halliwell and Gutteridge, 1999). Contrary to past reports, recent epidemiological studies have indicated that eggs are not a predisposing factor for the risk of cardiovascular pathologies, as confirmed by our results (n-6/n-3 ratio, PI, AI, TI and IQN indeces). Dietary recommendations (Huopalahti et al., 2007; British Nutrition Foundation, 1999; Hu et al., 1999; Qureshi et al., 2007) have underlined the necessity for humans to consume products with an n-6/n-3 ratio equal to or less than 6, though Simopoulos (1991) indicated 4:1 the as optimal ratio. The increase in n-6 fatty acids observed in modern human diets can represent a risk factor for tumours, diabetes and cardiovascular disease. Interestingly, the Ancona eggs consistently showed an n-6/n-3 value smaller than 4, and the eggs produced in spring showed values equal to 1.9. Regarding the n-6/n-3 ratio, this parameter is very important because it affects the efficacy of desaturation/elongation. When hens are fed diets rich in linolenic and/or n-3 fatty acids, the conversion of linoleic acid to arachidonic acid drastically decreases (Jiang et al., 1991), with linoleic acid favouring platelet aggregation and immuno-suppression (Wander et al., 1997).

4. Conclusion

Ancona hens produced a lower number of eggs than commercial line, however they were markedly different from Hy-line ones, indeed the eggs had higher carotenoid, polyphenol and tocopherol contents which are bioactive compounds that have a relevant effect on human health. This difference was due to the availability of green pasture. Even n-6/n-3 ratio was lower in Ancona hens compared to Hy-line, to witness a most linolenic acid intake through diet. Also long-chain products derived from n-3 showed larger quantities, confirming the ability of the hen to elongare and desaturate linolenic acid (Lopez-Bote et al., 1998; Donaldson, 1996) and to put in place efficient mechanisms for the transfer of n-3 long-chain inside the egg, where exert a very important role during the growth of the chick (Speake et al., 1999). Moreover, rearing an autochthonous genotype under alternative conditions, with wide pasture availability, matches the expectations of consumers regarding the higher quality of eggs and the maintenance of biodiversity.

Table 1. Chemical composition of feed and pasture (Mean ± Std.Dev.)

Chemical composition		**Feed**	**Pasture**			
			Winter	Spring	Summer	Autumn
Dry matter	%	88.7± 1.9	24.0 ± 1.7	22.0 ± 0.9	35.1 ± 2.1	29.5 ± 2.3
Crude protein	% D.M.	17.5 ± 1.6	18.4 ± 2.0	16.4 ± 0.8	10.5 ± 1.6	11.3 ± 1.7
Lipid	"	4.7 ± 0.9	2.5 ± 0.2	1.7 ± 0.1	2.6 ± 0.2	3.9 ± 1.6
Crude Fibre	"	3.6 ± 1.0	16.9 ± 1.8	15.5 ± 2.0	20.2 ± 3.2	22.6 ± 2.8
Ash	"	15.6 ± 1.6	9.2 ± 0.1	12.5 ± 1.5	7.5 ± 0.6	5.9 ± 0.3
ME*	MJ kg^{-1} D.M.	13.0 ± 1.0	6.0 ± 1.0	8.9 ± 1.6	6.6 ± 0.1	5.7 ± 1.1
Carotenoids	μg g^{-1} D.M.	3.2 ± 0.6	67.1 ± 6.5	75.6 ± 7.9	26.7 ± 3.0	33.2 ± 2.8
α-tocopherol	mg kg^{-1} D.M.	62.8 ± 3.5	133.6 ± 31.2	235.8 ± 45.8	177.2 ± 26.5	153.0 ± 32.5
Polyphenols	μg g^{-1} D.M.	170.0 ± 26.9	446.0 ± 56.1	578.0 ± 62.3	368.0 ± 45.2	599.0 ± 54.9
Lutein	mg g^{-1}	1.1 ± 0.3	19.2 ± 2.1	15.4 ± 2.8	6.1 ± 1.3	5.5 ± 0.6
β-carotene	"	n.d.	12.6 ± 2.5	22.5 ± 5.4	13.9 ± 3.9	1.9 ± 0.6
Zeaxantin	"	0.1 ± 0.1	1.1 ± 0.2	1.2 ± 0.4	0.6 ± 0.2	0.4 ± 0.1

ME*: Metabolized Energy, by Carrè et al., 1989.

Table 2. Fatty acids content of feed and pasture (Mean ± Std.Dev.)

		Feed	Pasture			
			Winter	Spring	Summer	Autumn
C16:0	mg 100 g^{-1}	601.8 ± 102.1	420.1 ± 70.3	289.2 ± 33.2	356.6 ± 37.6	405.2 ± 25.4
C18:0	"	330.6 ± 50.5	54.6 ± 7.5	42.4 ± 6.8	45.0 ± 10.5	58.3 ± 15.4
Other	"	43.5 ± 3.7	23.0 ± 5.6	14.2 ± 2.4	18.6 ± 4.9	22.1 ± 15.4
Σ SFA	**"**	**975.9 ± 146.3**	**497.7± 66.2**	**345.8 ± 43.0**	**420.2 ± 88.8**	**485.6 ± 115.5**
C16:1(n-7)	"	16.7 ± 2.3	12.1 ± 2.8	7.2 ± 2.1	7.9 ± 3.7	10.8 ± 3.5
C18:1(n-9)	"	1126.6 ± 408.8	250.1 ± 26.8	178.8 ± 31.9	219.5 ± 30.5	269.4 ± 43.2
Other	"	62.7 ± 10.8	24.2 ± 8.1	17.0 ± 4.5	20.6 ± 10.6	25.3± 10.0
ΣMUFA	**"**	**1206.0 ± 147.3**	**286.4 ± 32.7**	**203 ± 42.1**	**248.0 ± 58.7**	**305.5 ± 78.2**
C18:2(n-6)	"	1345.1 ± 121.1	360.3 ± 19.8	242.4 ± 21.6	300.8 ± 24.3	380.5 ± 98.4
Other	"	12.8 ± 4.0	4.3 ± 2.2	3.6 ± 0.4	3.7 ± 0.5	5.0 ± 2.2
Σ (n-6)	"	1357.9 ± 310.2	364.6 ± 50.3	246.0 ± 4.0	304.5 ± 23.9	385.5 ± 122.8
C18:3(n-3)	"	145.2 ± 32.2	1128.3 ± 159.1	1332.6 ± 173.2	934.7 ± 147.8	1207.0 ± 182.5
Other	"	20.1 ± 4.0	2.1 ± 0.6	4.0 ± 1.2	2.0 ± 0.3	2.3 ± 0.4
Σ (n-3)	"	165.3 ± 26.9	1130.4 ± 176.2	1336.6 ± 190.9	936.7 ± 111.1	1209.3 ± 218.9
Σ PUFA	**"**	**1522.3 ± 239.4**	**1495 ± 290.8**	**1582.6 ± 277.7**	**1241.2 ± 187.4**	**1594.8 ± 245.8**
n-6/n-3	"	7.9	0.2	0.1	0.2	0.3

Table 3. Chemical composition of eggs

		Ancona	**Hy-line**	DSE
Dry Matter	%	20.3	20.5	3.0
Crude Protein	"	13.3	13.0	2.3
Lipid	"	8.9	9.0	1.5
Metabolized Energy	Kcal 100 g^{-1}"	137.8	138.5	25.6

N = 22 pool (5 eggs) per group and season

Table 4. Physical characteristics of eggs in different seasons

		Ancona				Hy-line				
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	DSE
Egg weight	g	51.4^{a}	52.6^{a}	52.8^{a}	54.6^{a}	61.5^{b}	62.7^{b}	63.7^{b}	64.8^{b}	8.1
Yolk weight	"	18.1^{ab}	18.3^{ab}	18.9^{ab}	19.0^{ab}	18.8^{b}	20.6^{b}	20.4^{b}	20.8^{b}	3.4
Albumen weight	"	28.0^{a}	29.0^{a}	28.8^{a}	30.3^{a}	33.5^{b}	33.2^{b}	33.9^{b}	35.0^{bc}	4.8
Shell weight	g	4.0^{a}	4.1^{a}	4.2^{a}	4.1^{a}	7.0^{b}	6.8^{b}	7.1^{b}	7.0^{b}	9.8
Shell thickness	mm	0.40^{b}	0.38^{ab}	0.40^{b}	0.41^{b}	0.34^{a}	0.37^{a}	0.37^{ab}	0.40^{b}	0.16
Yolk	% p.o.	34.8	34.3	36.8	35.0	31.8	33.5	31.5	31.2	5.1
Albumen	"	54.3	54.8	54.0	55.6	54.9	53.3	54.3	54.2	4.3
Shell	"	8.9^{a}	8.8^{a}	9.0^{a}	7.9^{a}	11.8^{b}	11.0^{b}	10.4^{b}	11.1^{b}	0.8
Haugh UNIT		87.8^{a}	90.4^{ab}	96.4^{b}	109.2^{c}	87.0^{a}	87.9^{a}	89.4^{ab}	90.1^{ab}	22.1
Yolk colour	Scale Roche	10.1^{b}	13.0^{c}	8.1^{a}	13.4^{c}	8.2^{a}	8.4^{a}	7.0^{a}	9.4^{b}	1.5

N = 220 per group and seasons; a..b.: P<0.05

Table 5. Content of some bioactive compounds of eggs.

		Ancona				Hy-line				
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	DSE
Carotenoids	mg egg	253.8^{b}	319.5^{c}	215.1^{b}	367.3^{c}	128.8^{a}	161.9^{a}	110.3^{a}	157.3^{a}	31.6
"	mg g^{-1} yolk	12.4^{bc}	16.2^{b}	9.6^{b}	18.5^{c}	5.9^{a}	6.7^{a}	4.6^{a}	6.7^{a}	4.2
Lutein	mg egg	222.8^{c}	273.3^{c}	187.3^{ab}	329.7^{d}	99.3^{a}	126.2^{a}	84.8^{a}	121.1^{a}	70.1
"	mg g^{-1} yolk	11.5^{b}	14.2^{b}	9.6^{b}	16.4^{c}	4.4^{a}	5.0^{a}	3.4^{a}	5.3^{a}	3.9
Zeaxantin	mg egg	25.2^{ab}	32.9^{ab}	18.9^{a}	22.3^{a}	16.8^{a}	18.6^{a}	19.5^{a}	20.6^{a}	4.2
"	mg g^{-1} yolk	1.2	1.7	1.0	1.2	0.7	0.7	0.8	0.9	0.5
Polyphenols egg	mg 100 g^{-1}	53.4^{b}	65.1^{b}	23.4^{a}	53.0^{b}	16.2^{a}	22.3^{a}	19.8^{a}	21.1^{a}	15.4
Plyphenols albumen	"	13.1^{b}	14.4^{b}	10.2^{ab}	8.0^{a}	7.4^{a}	11.0^{ab}	7.5^{a}	12.0^{ab}	3.4
α-tocopherol	mg g^{-1} egg	30.8^{b}	43.0^{c}	32.2^{b}	42.3^{c}	17.1^{a}	17.8^{a}	15.7^{a}	16.9^{a}	5.4
"	mg g^{-1} yolk	80.1^{b}	115.4^{c}	89.3^{b}	100.3^{bc}	60.4^{a}	58.1^{a}	59.3^{a}	57.4^{a}	19.9

N = 110 eggs per group and season (22 pool of 5 egg/yolk per group and season); a.b.: P<0.05

Table 6. Fatty acids content of eggs (mg 100 g-1 yolk)

	Ancona				Hy-line				
	winter	spring	Summer	autumn	winter	spring	summer	autumn	DSE
C14:0	83.4	88.5	87.5	79.7	92.5	87.6	98.7	86.6	28.8
C16:0	5003.7	4678.9	5287.0	5191.4	4274.2	3932.2	4303.5	4292.1	147.1
C18:0	2061.9	1737.3	1907.2	1933.1	2252.0	1907.9	1767.5	1797.7	95.8
Other	180.5	176.0	189.9	209.5	149.3	163.2	149.5	135.4	40.5
SFA	**7328.4^{ab}**	**6681.7^{ab}**	**7471.5^{ab}**	**7414.8^{ab}**	**6768.0^{ab}**	**6091.9^{a}**	**6318.2^{a}**	**6312.8^{a}**	**191.9**
C16:1n-7	756.3	697.1	769.6	715.2	913.5	1066.9	1281.0	955.6	56.0
C18:1n-9	10812.6^{b}	11393.1^{b}	10642.5^{b}	10849.2^{b}	10091.8^{ab}	10325.6^{ab}	10057.4^{ab}	10328.2^{ab}	99.3
Other	45.1	55.1	40.8	55.1	88.6	90.6	88.7	77.6	18.0
MUFA	**11613.1^{b}**	**12145.2^{b}**	**11452.9^{b}**	**11619.5^{b}**	**11092.9^{b}**	**11483.1^{b}**	**11426.1^{b}**	**11361.4^{b}**	**367.6**
C18:2n-6	1999.1^{a}	1746.1^{a}	1971.8^{a}	1927.2^{a}	2310.7^{b}	2600.6^{b}	2493.2^{b}	2561.1^{b}	150.1
C20:4n-6	52.0^{a}	111.1^{a}	63.6^{a}	82.6^{a}	477.7^{b}	548.4^{b}	496.4^{b}	463.9^{b}	161.8
Other	119.7^{a}	281.2^{b}	135.2^{a}	107.2^{a}	116.4^{a}	155.3^{a}	126.6^{a}	144.3^{a}	53.1
Σ n-6	2170.8^{a}	2139.3^{a}	2170.8^{a}	2116.6^{a}	2905.0^{b}	3300.4^{b}	3116.3^{b}	3168.7^{b}	195.1
C18:3n-3	191.3^{ab}	238.9^{b}	204.8^{ab}	196.8^{ab}	143.3^{a}	188.1^{ab}	114.6^{a}	154.3^{a}	45.6
C20:5n-3	69.6^{c}	104.2^{c}	70.6^{c}	87.6^{c}	26.9^{b}	26.9^{b}	23.9^{b}	23.9^{b}	11.9
C22:5n-3	69.6^{b}	87.5^{b}	70.6^{b}	69.8^{b}	12.9^{a}	15.9^{a}	12.0^{a}	14.9^{a}	5.5
C22:6n-3	126.5^{a}	468.0^{c}	222,7^{b}	317.8^{bc}	105,5^{a}	117.4^{a}	100.7^{a}	111.5^{a}	53.7
Other	87.3	121.9	105.4	122.0^{b}	85.6	83.6	85.7	86.6	118.8
Σ n-3	594.8^{b}	1136.8^{c}	731.0^{b}	866.1^{b}	374.4^{a}	432.3^{a}	337.5^{a}	391.0^{a}	101.7
PUFA	**2765.2**	**3275.9**	**2901.5**	**2982.8**	**3279.0**	**3733.1**	**3454.2**	**3559.5**	**145.6**
n-6/n-3	3.5^{a}	1.9^{a}	3.0^{a}	2.4^{a}	7.8^{b}	7.6^{b}	9.2^{bc}	8.1^{b}	1.7

N = 220 per group and season (22 pool of 5 yolk); a.b.: P<0.05

Table 7. Nutritional indices of eggs in different seasons

		Ancona				Hy-line				
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	DSE
Peroxydability Index (PI)		47.26^{a}	78.65^{b}	55.50^{a}	64.27^{a}	81.27^{b}	92.92^{c}	91.83^{bc}	84.97^{b}	13.2
Atherogenicity Index (AI)		0.38^{ab}	0.34^{a}	0.41^{b}	0.39^{ab}	0.33^{a}	0.29^{a}	0.33^{a}	0.32^{a}	0.28
Thrombogenicity Index (TI)		0.82^{ab}	0.62^{a}	0.81^{ab}	0.76^{a}	0.81^{ab}	0.68^{a}	0.74^{a}	0.73^{a}	0.46
Nutritional quality (QNI)		1.46^{a}	4.70^{c}	2.41^{ab}	3.33^{c}	1.27^{a}	1.37^{a}	1.20^{a}	1.30^{a}	0.97
FA hypochol/hyperchol (HH)		2.58	2.92	2.43	2.53	2.98	3.39	2.99	3.08	0.91
Cholesterol	mg egg	226,4	239,2	226,4	225,3	215,9	239,9	234,7	251,8	30,4
	mg 100 g^{-1} yolk	1194.6^{b}	1246.4^{b}	1192.2^{b}	1156.1^{b}	1039.8^{a}	1044.6^{a}	1048.8^{a}	1127.8^{ab}	77.7
TBARs	mg MDA kg^{-1} yolk	0.24^{a}	0.40^{b}	0.36^{b}	0.39^{b}	0.32^{ab}	0.39^{b}	0.40^{b}	0.35^{b}	0.07

N = 22 pool of 5 yolk/group/season; a.b.: P<0.05

5. Article published (experiment 3)

The results presented in experiment 3 have been published on Journal Science Food Agriculture, as shown below. The article described the effect of the farming system (conventional and alternative) on products quality of Ancona laying hens. In this context, there were studied three experimental groups:

- Control: Ancona hens were kept in cages under standard housing conditions (single-bird cages, 0.75m^2) of three-tier batteries that were provided with a linear automatic feeder and drinker; an artificial photoperiod of 16 h per day of light was applied at 17 weeks of age.
- Organic: Ancona hens were kept in four (one per season) adjacent covered, straw-bedded houses (6 hens/m^2)with access to four separate pens (one per season) with natural pasture; each pens was divided into three sub-pens (4 m^2/hen).
- Organic Plus: Ancona hens were kept in four identical (one per season) adjacent covered, straw-bedded houses (6 hens/m^2) with access to four separate pens (one per season) with natural pasture; each pen was divided into three sub-pens (10 m^2/hen).

4.4 EXPERIMENT 4.

Comparison of oxidative status and fatty acid composition of meat in different poultry genotypes reared under extensive system.

Abstract. The fatty acid profile and the oxidative status of meat of six different chicken genotypes extensively reared were compared. The genotypes used were Ancona (A) and Leghorn (L) as pure slow-growing, Cornish x Leghorn crossbreed (CL), Naked Neck (NN), Kabir (KR), as medium-growing and Ross 308 (R) as fast-growing strains. Slow-growing strains have showed a lower pHu values, in relation to the muscle metabolism of this genotype. Even the WHC and cooking loss values are correlated with the pH. On the contrary the muscle lightness of A and L, were lower, due to the higher oxidative metabolism of these strains. The chemical composition of meat have detected a difference in the lipid content in commercial genotypes, explained by the substantial consumption of food and the small movement. KR has produced tenderness meat but more fat, while L breed has showed best nutrient meat qualities but low technological qualities (drip loss, lightness). Genotype affected the main fatty acids and the antioxidant profile of meat. Concerning the content of total polyunsaturated fatty acids (PUFA), the highest value was observed in A and L chickens, in particular, n-3 FA was lower in favor of EPA and DHA concentrations. Such results confirmed a different lipid metabolism between breeds. Among commercial strains (NN, KR, R) have been showed the lowest SFA values in NN, whereas the KR showed intermediate values. The NN chickens exhibited lower concentrations of linolenic acid, but higher long chain PUFA derivatives. However, the meat of these chickens showed a lower lipid stability despite a higher antioxidant content probably due to the kinetic behavior and the resulting high oxidative metabolism. This was evident in A, and CL breeds, where the TBARS value increased due to higher kinetic activity and to the antioxidants content which was still higher. The scope of the current work is to identify, through a multi-criteria analysis, which are breeds that best reach a compromise between productivity and quality in an extensive farming system. In this regard, the medium-growing strains seems to have the best response between adaptability and quality, in particular NN, following by CL and pure breed.

Slow-growing strains

Medium-growing strains

Fast-growing strains

Start 1 days

Starter diet

21 days

+ Finisher diet

End 81 days

Slaughtering n=20 bird/strains

Productive performance n= 50 birds/strain

Physical and chemical analysis

1. Introduction

The consumer attention to healthy foods and to the welfare conditions of animals, has favoured alternative farming system that is supposed as an environmentally friendly production method, sustaining animals in good health and with high welfare standards (Sundrum, 2001). Some slow-growing poultry products have a long history in Europe as for example the French Label Rouge program, which requires outdoor access and a growing period of at least 81 d. This typical product captures 30% of the French poultry market despite selling products for twice the price of conventional poultry products (Westgren, 1999; Fanatico and Born, 2001). In Europe, over 4 million laying hens and 1.8 million broilers are kept according to the organic principles. Although the regulations are the same for every country, there is a large diversity in farm sizes and farming systems (Bestman and Maurer, 2006). Italy has the largest organic farmland in Europe, with 1.23 million hectares and 60,509 farms. Nevertheless, there is a shortage of organic animal products and significant amounts are imported from other European countries. Rigorous rule (Reg. EC 834/07) avoids the use of chemical products for assuring the absence of residues and the safety of food; however, the effects of organic protocol on the qualitative characteristics of meat are lacking and the few studies show conflicting results. Some authors (Castellini et al., 2002a; Lewis et al., 1997) showed that one aspect of free-range animals could be the lower oxidative stability of the meat due to the higher motor activity that improves the oxidative metabolism and the free radical production of chicken. Different adaptation of genetic strains to larger space availability and to outdoor environment greatly affect such assessment by modulating the intake of grass (Castellini et al., 2002a), muscle metabolism (Branciari et al., 2009) with implications on the lipid profile and oxidative stability of meat (Castellini et al., 2005). In order to assure a good welfare status, the EC Regulations and the final recommendation of Network for Animal Health and Welfare in Organic Agriculture (Hovi et al., 2003), suggest to utilize slow-growing birds (daily weight gain < 35g; Guéméné et al., 2009) for their higher adaptability to poorer environment. On the contrary, fast-growing strains selected to produce under highly controlled conditions, seem to be quite unsuitable for extensive systems because the environment is less controlled and the too heavy weight at older ages. In this contest, the use of purebred strains could also be a valuable alternative, particularly if they are in danger of extinction (Sundrum, 2001). Breeds with a slow growth rate have been selected by several breeders, e.g. Isa-Hubbard, Sasso, Kabir (Guéméné et al., 2009) but it should be also considered that the maintenance of biodiversity is one of the main goals of organic farming (IFOAM, 1997). According to this the Italian Agricultural Ministry funded some projects oriented towards the valorisation of local poultry purebred for organic production. Unfortunately, such birds showed very poor productive performance and it is unprofitable to widespread such strains in commercial field. Possible solutions could be the creation of crossed lines in order to increase live weight and feed efficiency. Thus, the aim of this study was to evaluate the performance, carcass and meat quality of pure, cross and commercial breeds extensively reared.

2. Results

2.1 Carcass traits

In Table 1. was reported the carcass characteristics of each genotype. The characteristics of the carcasses were overall good. The commercial breeds have produced the best yields and heavier carcasses. Although the yields of the breast and the muscle/bone ratio was higher in KR and NN. The most low muscle/bones value recorded in L type was due to the very lightness of the carcass (live weight 1,370g, data reported in experiment 2). Abdominal fat was lower in native breeds at higher kinetic activity (L, A, CL), then in slow-growing strains.

2.2 Physical characteristics

The physical characteristics of breast are shown in Table 2. The lowest pH values were found in native genotypes (A and L) on breast meat. At the same meat with lower pH values had less WHC and, therefore, the highest declines of cooking loss. The analysis of the color of the breast showed that the meat was brighter than commercial genotypes. The other colorimetric parameters have appeared homogeneous for all genetic types, even if the thigh showed higher results than the breast (data not shown), due to the different composition in red fibers. The breast showed the higher values of redness index (a*) (CIELAB, 1976) in L and A genotypes, to indicate an increased oxidative metabolism.

2.3 Chemical composition and oxidative parameters

In Table 3. were showed the chemical composition of breast meat. Significantly was the lipid content in commercial genotype, explained by the substantial consumption of food and very little movement of them. It can be said that the KR has produced fatter meats, but more tender, while L chickens, provided poor quality meat from the technological point of view, with high cocking loss. The analysis of the fatty acid composition of meat (Tables 4 and 5) showed a clear differentiation of genotypes. The native genotypes appeared characterized by a fatty acid profile very similar to each other and different from commercial ones. There were no significantly differences in the total content of saturated fatty acids, though, NN, KR and R genotypes showed higher levels of short-chain fatty acids (C14:0 and C16:0). On the contrary, in the L and A meats were detected higher amounts of stearic acid (C18:0). Differences were found in the MUFA content that were represented in some commercial genotypes (KR and NN), with a higher content of oleic (21.63 and 23.03%) acid and palmitoleic (3.26 and 3.10%). The muscles of pure genotypes were characterized by a high concentration of PUFA, both in the total content that in the different fractions. Despite the increased consumption of fresh grass, hitting the low linolenic acid, explained by the greater presence of EPA and DHA. The analysis of the nutritional indices of meat (Table 5) has revealed interesting aspects, in particular slow and medium-growing groups showed good amount of PUFA and favorable indices of AI and TI, while the PI was higher than medium and fast-growing.
About the oxidative stability was observed some variability. The TBARs value seems to be high in A then commercial lines (174.48 vs 159.60, 177.50, 111.13 ng MDA/g of meat in KR, NN and R respectively). Nevertheless the total tocopherols content was higher only in slow-growing strain (1165.21 vs 1066.19, 782.38 and 636.38 ng/g of meat in KR, NN and R respectively).

3. Discussion

Given the higher selection of commercial lines for productive performance, such breeds have produced the best yields and heavier carcasses. The lowest pH values founded in native genotypes, could be due to a faster drop in pH (Berri et al. 2005), or a different genotype-dependent glycolytic potential, as noted by Gardzielewska et al. (1995). Many authors have investigated the correlation between pH and physical characteristics of meat, such as color, drip loss, cooking loss, WHC and tenderness, since the lowering of the pH changes the electrostatic forces of the protein able to retain water in muscle fibers (Offer and Knight. 1988). Debut et al. (2003) have investigated the relationship between meat quality and susceptibility of animals to stress pre-slaughter, concluding that the slow-growing animals are more responsive than the selected lines. Therefore such breeds suffer an increase in the concentration of corticosteron in the blood, which impacts on acidification of the muscle with an increased rate of the pHu, particularly in the thigh muscle, correlated to greater water losses. About tenderness, native genotypes showed increasingly higher values in breast and thigh, due to the different composition of the muscle fibers (Branciari et al., 2009). This situation indicates a considerable maturity of meat (Baeza et al., 2010). Compared to the shear stress, should also be noted that a higher lipid content helps to reduce the toughness of the muscle (Nishimura et al. 1999), at the same time improving the sensory characteristics (Zerehdaran et al., 2004). However the higher kinetic activity of pure breed (Dal Bosco et al., 2009), produced an increase of redness index (a*), indicating an increased oxidative metabolism and probably a greater degree of oxidation of myoglobin contained in the muscle. The chemical composition of breast in commercial lines, reflected the higher feed consumption and very little movement especially in the last phase of farming; these two factors are definitely translated into accumulation of fat in the tissue. The higher kinetic attitude of slow-growing strains, in particular Ancona, was confirmed by oxidative parameters of meat. The TBARS showed that the oxidation of the pectoral muscle suffered a diversified trend in the different groups of animals. In particular Ancona breed showed an higher value, even mach higher than commercial lines. However this strains given the greater kinetic aptitude, took an large amount of tocopherols, which can be balance the pro-oxidant compound formed. In previous studies carried out on poultry breeds in slow, medium and fast-growing, Castellini et al (2004) observed different levels of oxidative stability (Castellini et al., 2002b; Castellini et al., 2002c) probably due to genetic differences adaptive muscle cell movement and oxidative stimuli. The restriction of movement reduces oxidation *in vivo* by producing a greater oxidative stability on the meat. It is known that physical exercise increases the number of mitochondria in the fibers α-W and therefore favors the replacement of those glycolytic with α-R (Ouhayoun and Dalle Zotte, 1993), thereby also increasing the endowment of heme-Fe (Hoffmann, 1999), in particular in the oxidative muscles (O'Brien et al., 1992), however a short period of confinement is not sufficient to reduce it (Castellini et al. 2004). The results in terms of chemical composition of the meat confirm that they are largely related to the genotype (Berri et al., 2005). From the nutritional point of view there are relevant differences regarding the fat content of meat and the relative FA profile. *Pectoralis major* of rural strains have lower lipid content and a higher PUFA n-3 which result in a lower n-6/n-3 ratio. Optimal dietary intakes of the n-6/n-3 ratio should be around 1–4:1. However, according to the nutritional changes in the Western diet , this ratio has now increased to be within the range of 10:1 to 20:1 (Olivier et al., 2011). In parallel, there are

coinciding increases in the incidence of diseases involving inflammatory processes such as cardiovascular disease, obesity, rheumatoid arthritis, and cancer (Patterson et al., 2012).
It should be noted that at despite the increased consumption of fresh grass of pure lines, the linolenic acid was low, but such value could be explain by the greater presence of EPA and DHA, products of ALA. This result confirms a different lipid metabolism between genotypes.

4. Conclusion

In conclusion the results of this study indicate that medium-growing strains seems to have the best response between adaptability and meat quality, in particular NN breed. The crossbreed have showed intermediated results between pure breeds and commercial ones. CL are much lower productive performance than meat-type birds (Farran et al., 2000). However slow-growing products are qualitatively better, as demonstrated by antioxidant compound and lipid composition of meat. Although autochthones breed, which have a slow-growth are not competitive for productive performance and feed conversion than fast-growing strains, the crossbreed or some medium strains (NN) seems a suitable compromise between the adaptability and rusticity which are key factors in pasture-based farming system and economic sustainability. Furthermore, such birds have showed a product quality similar to pure breed as demonstrated by PUFA and tocopherols content, especially about CL. Further study are be necessary to deepen a global overview of the economic, ecological and qualitative performance of the poultry genotypes considered in this study.

Table 1. Carcass characteristics of genotype

		L	A	CL	KR	NN	R	SEM
Cold carcass	g	1158^{a}	1273^{a}	1633^{b}	1793^{b}	2018^{c}	2975^{d}	110
Head legs yield	"	78.7^{a}	79.0^{a}	81.6^{b}	81.1^{b}	81.0^{b}	99.6^{c}	8.9
Bust	"	989^{a}	1105^{b}	1433^{c}	1556^{c}	1779^{d}	2201^{d}	9.5
Bust yield	%	68.9^{a}	68.6^{a}	71.6^{b}	70.3^{b}	70.9^{b}	74.3^{bc}	6.3
Abdominal fat	%	0.1^{a}	0.4^{a}	0.9^{b}	1.4^{c}	2.1^{d}	3.9^{e}	0.5
Yield breast	%	11.1^{a}	12.3^{b}	13.4^{bc}	14.7^{c}	14.6^{c}	24.3^{d}	1.1
Yield bobbins (2)	%	18.1^{d}	15.7^{b}	14.2^{a}	17.5^{c}	16.4^{c}	16.4^{c}	0.8
Muscle / bone		1.8^{a}	2.3^{b}	2.8^{c}	2.7^{c}	2.8^{c}	2.3^{b}	0.3

On the same row: a..e: P<0.05.

Table 2. Physic characteristics of carcass

Breast		L	A	CL	KN	NN	R	SEM
pH		5.70	5.67	5.71	5.87	5.78	5.60	0.65
Tenderness	Kg/cm^2	1.53^{b}	1.71^{bc}	1.45^{b}	1.05^{a}	1.17^{a}	1.85^{bc}	0.27
WHC	%	50.53^{a}	50.94^{a}	52.62^{a}	56.41^{b}	54.82^{b}	55.2^{b}	1.36
Drip Loss	"	44.23^{c}	44.56^{c}	43.26^{c}	33.25^{b}	30.80^{b}	25.83^{a}	2.45
Colour								
L*		48.10^{a}	48.31^{a}	52.47^{a}	62.74^{c}	61.68^{c}	56.38^{b}	3.17
a*		13.39^{d}	9.74^{c}	7.27^{a}	10.27^{c}	9.65^{c}	8.37^{b}	0.89
b*		-3.25^{c}	-2.93^{b}	-4.23^{d}	-0.76^{a}	-0.57^{a}	-0.82^{a}	0.25

On the same row: a..d: P<0.05.

Table 3. Chemical composition of meat

Breast		L	A	CL	KR	NN	R	LSD
Humidity	%	77.11	75.81	75.81	76.30	77.01	75.80	3.01
Protein	"	20.63	21.72	21.72	21.53	21.03	21.21	2.23
Lipid	"	0.32[a]	0.91[b]	0.91[b]	0.85[b]	0.73[b]	1.42[c]	0.25
Ash	"	1.95	1.56	1.56	1.33	1.24	1.57	0.45

On the same row: a..c: P<0.05.

Table 4. Fatty acids profile of breast meat

	L	A	CL	KR	NN	R	LSD
C14:0	0.48	0.66	1.04	1.68	1.56	1.31	0.52
C16:0	28.77	28.29	29.53	32.54	30.35	31.2	1.57
C18:0	13.28	13.41	11.04	8.76	10.11	12.3	2.31
Others	4.53	3.47	2.07	1.86	1.97	2.41	1.69
SFA	47.06	45.83	43.68	44.84	43.99	47.3	2.96
C14:1n-6	0.09	0.10	0.01	0.18	0.04	0.03	0.14
C16:1n-7	0.60	0.58	1.41	3.26	3.10	1.32	1.24
C18:1n-9	16.98	18.53	17.99	21.63	23.03	21.7	2.67
Others	0.18	0.18	0.17	0.24	0.22	0.15	0.18
MUFA	17.85	19.39	19.58	25.31	26.39	29.7	1.89
C18:2n-6	20.64	18.49	25.76	22.27	20.90	21.2	1.20
C20:3n-6	0.15	0.17	0.16	0.24	0.24	0.27	0.09
C20:4n-6	9.80	10.65	8.14	5.33	6.34	5.12	2.01
tot n-6	**30.68**	**28.54**	**36.59**	**30.75**	**26.51**	**27.81**	**12.36**
C18:3n-3	0.64	1.03	1.03	0.87	0.93	1.21	0.33
C20:5n-3	0.17	0.17	0.09	0.08	0.12	0.13	0.05
C22:5n-3	1.34	1.37	1.02	0.45	0.54	0.31	0.40
C22:6n-3	0.94	1.32	0.20	0.09	0.14	0.48	0.39
Others	0.75	0.68	0.28	0.44	0.41	1.58	0.21
tot n-3	**3.09**	**3.89**	**2.34**	**1.49**	**1.73**	**2.13**	**0.15**
PUFA	34.43	33.88	36.68	29.77	29.55	30.3	4.62
n-6/n-3	9.93	7.34	15.64	20.64	15.32	13.06	0.38

Table 5. Nutritional indices and oxidative profile

			L	A	CL	KR	NN	R	LSD
PI	mg/100 g		78.14	83.43	69.13	49.86	53.85	51.16	15.26
AI	"		0.60	0.60	0.58	0.69	0.67	0.69	0.15
TI	"		1.27	1.19	1.19	1.33	1.33	1.40	0.25
TBARs		ng/g	106.42	174.48	108.40	159.60	177.50	111.13	25.24
Tocoferols		ng/g	1013.73	1165.21	1038.47	1066.19	782.38	636.38	189.15

PI: Peroxidabilty index; AI: Atherogenicity index; TI: Thrombogenicity index.
L, Leghorn; A, Ancona, CL, Cornish x Leghorn crossbreed; KR, Kabir; NN, Naken Neck; R, Ross.

5. Article published (experiment 5)

Data about the commercial genotypes (KR, NN and R) have been published on Italian Journal of Animal Science, evaluating the use of commercial lines in a extensive farming system. The following research, focused on medium (NN and KR) and fast-growing strains (R) meat quality, during two different slaughter ages (70 and 81 days). The reason for this choice is due to the high weight at sacrifice achieved in particular by R strains, that worsened health status.

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PAPER

Effect of slaughtering age in different commercial chicken genotypes reared according to the organic system: 2. Fatty acid and oxidative status of meat

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Abstract

The fatty acid profile and the oxidative status of meat of three different commercial chicken genotypes organically reared and slaughtered at two different ages (70 and 81 days) were compared. The genotypes used were Naked Neck (CN1 strain), Kabir (KR4 strain) and Ross 308 (R). All animals were raised in the facilities of a big Italian company, in field conditions. Genotype and slaughtering age affected the main fatty acids and the antioxidant profile of meat. Concerning the content of total saturated fatty acids (SFA), the highest value was observed in R chicks. The CN1 birds showed the lowest SFA values, whereas the KR4 showed intermediate values. Polyunsaturated fatty acids (PUFA) showed a different trend at the two slaughter ages. At 71 days medium-growing chickens had lower values, while at 81 days CN1 birds reached the highest value. The CN1 chickens exhibited lower concentrations of linolenic acid, but higher long chain PUFA derivatives. However, the meat of these chickens showed a lower lipid stability despite a higher antioxidant content probably due to the kinetic behaviour and the resulting high oxidative metabolism. This finding is of importance since health concerns over fatty acid profile are among the main factors contributing to the decline of meat intake. Regarding the slaughtering age, the results of this trial demonstrate that at older age chickens showed a better fatty acid profile under a nutritional point of view even if the oxidative status worsened.

Introduction

The organic system requires the use of strains appropriate to free range mainly because of foraging behaviour and immune response. This notwithstanding, commercial poultry farms often use fast-growing genotypes not suitable for the organic system for economic reasons [high body weight (BW), carcass and breast yield].

Previous studies (Dal Bosco *et al.*, 2012; Fanatico *et al.*, 2005) underlined that fast-growing chicks are not appropriate for extensive rearing conditions, as they exhibit muscular-skeletal problems, very low motor activity and foraging behaviour (Castellini *et al.*, 2002a, 2002b; Dal Bosco *et al.*, 2010; Sirri *et al.*, 2010). On the contrary, slow-growing strains generally have a remarkable consumption of fresh forage which implies a reliable intake of antioxidant compounds (tocopherols, tocotrienols and carotenoids; Kerry *et al.*, 2000) and α-linoleic acid (C18:3n-3, ALA) which is partly converted to long-chain derivatives [C20:5n-3, eicosapentaenoic acid (EPA) and C22:6n-3, docosahexaenoic acid (DHA)]. Indeed, fatty acids of the meat derive from dietary uptake, and/or bioconversion; specifically, the bioconversion of long-chain polyunsaturated fatty acids (LCP) of the n-3 series includes endoplasmic D^6-desaturation, chain elongation and D^5-desaturation of ALA to EPA, which is subsequently converted to docosapentaenoic acid (C22:5n-3, DPA). The final metabolite, DHA, is synthesised by chain elongation, D^6-desaturation and peroxisomal b-oxidation of DPA (Poureslami *et al.*, 2010).

In a previous study (Dal Bosco *et al.*, 2014a), welfare, carcass traits and meat quality of three commercial genotypes reared under organic system and slaughtered at two different ages were investigated. In the present trial we analyse the fatty acid profile and oxidative status of meat from chickens reared in field condition according to the organic system.

Materials and methods

Animals, housing and feeding

The trial was conducted in the facilities of an European supplier of organic broilers in Central Italy. The used genotypes were Naked Neck (strain CN1), Kabir (strain KR4) and Ross 308 (R); all the birds were furnished by a commercial hatchery (Avicola Berlanda, Carmignano di Brenta, Italy). Kabir and CN1 were of both sexes, while R were only females due to the too high BW reachable by males.

The trial was carried out from April to June 2012 in the facilities of the company in production units of 3000 birds wich were also vaccinated against Marek, Newcastle diseases and coccidiosis (Paracox[3]-8). At 21 days of age all the birds were put in 9 covered shelters (0.10 m^2/bird) with straw litter and access to a grass paddock (4 m^2/bird); feeders and drinkers were available both outdoor and indoor (three replications). Chickens were fed *ad libitum* the same starter (1-21 days) and finisher (22 days to slaughter) diets, containing 100% certified organic ingredients (Table 1). Fatty acids, tocopherol and carotenoid profile of the diets are presented in Table 2. Chemical analyses of diet were done according to AOAC methods (1995).

Blood sampling

Before slaughtering, blood samples were taken from the brachial vein in ten chickens per group and collected in heparinised vacutainers and centrifuged at 1500 g for 10 min at +4°C, to measure the *in vivo* oxidative status. After collection, blood samples were immediately sent to the laboratory of the Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy where they were centrifuged and frozen at -80°C until analysis.

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Carcass dissection and sampling

At 70 and 81 days of age, 20 chickens per genotype were slaughtered in the processing plant of the farm, 12 h after feed withdrawal. Chickens were stunned by electrocution (110 V; 350 Hz) before killing. After killing, carcasses were plucked, eviscerated (non-edible viscera: intestines, proventriculus, gall bladder, spleen, oesophagus and full crop) and stored for 24 h at +4°C. Head, neck, legs, edible viscera (heart, liver, gizzard), and fat (perivisceral, perineal and abdominal) were removed in order to obtain the ready-to-cook carcass (Romboli *et al*., 1996). From the carcass, the *Pectoralis major* muscles were excised for successive analysis.

Analytical determinations

Feed and meat fatty acids were quantified as methyl esters (FAME) with a Mega 2 Carlo Erba Gas Chromatograph, model HRGC (Carlo Erba Agents, Milan, Italy), using a D-B wax capillary column (0.25 mm Æ, 30 m long). Fatty acid methyl ester peaks were identified by comparing the retention time with the commercially available FAME standards. The fatty acid compositions were calculated using the peak areas and expressed on percentage basis. The average amount of each fatty acid was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

Peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai (1986):

PI=(% monoenoic 0.025)+(% dienoic 1)+(% trienoic 2)+(% tetraenoic 4)+(% pentaenoic 6)+(% hexaenoic 8)

The amount of each fatty acid was used to calculate the indexes of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991), and the hypocholesterolaemic/hypercholesterolaemic ratio (HH), as suggested by Santos-Silva *et al.* (2002):

AI=(C12:0+4 C14:0+C16:0)/[(MUFA+Σ(n-6)+Σ(n-3)];

TI=(C14:0+C16:0+C18:0)/ [(0.5 MUFA+0.5 (n-6)+3 (n-3)+(n-3)/(n-6)];

HH=[(C18:1n-9+C18:2n-6+C20:4n-6+C18:3n-3+C20:5n-3+C22:5n-3 +C22:6n-3)/(C14:0+C16:0)]

The extent of plasma and muscle lipid oxidation was evaluated by a spectrophotometer set at 532 nm (UV-2550; Shimadzu, Kyoto, Japan) which measured the absorbance of thio-barbituric acid-reactive substances (TBARS), and a 1,1,3,3-tetraethoxypropane calibration curve in sodium acetate buffer (pH=3.5; Dal Bosco *et al*., 2009). Oxidation products were quantified as malondialdehyde index (mg MDA/g muscle). Tocopherol content and retinol of plasma and meat were quantified by high-performance liquid chromatography (HPLC) (Hewavitharana *et al*., 2004). Briefly, 5 mL of distilled water and 4 mL of ethanol were added to 2 g of sample and then vortexing for 10 sec. After mixing, 4 mL of hexane containing butylated hydroxytoluene (200 mg/L) were added and the mixture was carefully shaken and centrifuged. An aliquot of supernatant (3 mL) was dried under a stream of nitrogen and then redissolved in 300 L of acetonitrile. 50 µL were injected into the HPLC (PU-1580, equipped with an autosampler sistem AS 950-10; Jasco Int. Co., Tokyo, Japan) on a Ultrasphere ODS column (250 4.6 mm internal diameter, 5 µm particles size; CPS Analitica, Milan, Italy). Tocopherols (α-tocopherol and its isomers β+γ and δ) were identified using a FD detector (FP-1525, excitation and emission wavelength of 295 nm and 328 nm, respectively; Jasco) and quantified using external calibration curves prepared with increasing amounts of pure tocopherols in ethanol.

Statistical analyses

A linear model (StataCorp, 2005; GLM procedure) was used to evaluate the interactive effect of genetic strain and slaughtering age. Differences were assessed by ANOVA test with a Bonferroni multiple *t*-test. Differences with at least a $P<0.05$ value were considered statistically significant.

Results and discussion

The fatty acid and antioxidant profile of the finisher diet is shown in Table 2.

Polyunsaturated fatty acids represented the main class of fatty acids and linoleic acid (LA) was the main n-6 PUFA (47.7%). Oleic acid was the main MUFA (24.9%) and palmitic acid the principal SFA (12.9%). Although α-tocopherol was added in the diet as additive (30 mg/kg), γ-tocopherol was the main tocopherol isoform because it is the isomer mostly represented in corn (Rocheford *et al*., 2002) and soybean (Seguin *et al*., 2009). The same applies to lutein and zeaxanthin, the main carotenoids in finisher diets, mostly due to corn meal.

The fatty acid profile of breast meat is

Table 1. Formulation and chemical composition of the finisher diet.

	Finisher diet
Ingredients, %	
Corn	46.0
Full fat soybean	12.5
Wheat	20.0
Soybean meal*	14.0
Alfalfa meal	2.8
Gluten feed	2.0
Vitamin-mineral premix†	1.0
Dicalcium phosphate	1.0
Sodium bicarbonate	0.5
NaCl	0.2
Chemical composition	
DM, %	90.80
CP, % DM	18.05
EE, % DM	4.98
CF, % DM	4.01
Ash, % DM	5.59
NDF, % DM	10.11
ADF, % DM	5.06
Cellulose, % DM	3.56
ADL, % DM	1.11
Hemicellulose, % DM	5.05
ME,‡ MJ kg^{-1}	12.98

DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent liquid; ME, metabolisable energy. *From conventional crops. †Amounts per kg: vitamin A, 11,000 U; vitamin D_3, 2000 U; vitamin B_1, 2.5 mg; vitamin B_2, 4 mg; vitamin B_6, 1.25 mg; vitamin B_{12}, 0.01 mg; α-tocopheryl acetate, 30 mg; biotin, 0.06 mg; vitamin K, 2.5 mg; niacin, 15 mg; folic acid, 0.20 mg; pantotenic acid, 10 mg; choline chloride, 600 mg; Mn, 60 mg; Fe, 50 mg; Zn, 15 mg; I, 0.5 mg; Co, 0.5 mg. ‡Estimated following Carrè and Rouo (1990).

Table 2. Antioxidant and fatty acid profile of the finisher diet.

Antioxidants, mg/100 g	
Lutein	1.03
Zeaxanthin	0.40
α-tocopherol	6.11
δ-tocopherol	2.54
γ-tocopherol	10.25
α-tocotrienol	2.03
γ-tocotrienol	4.10
SFA, % of total fatty acids	
C14:0	1.2
C16:0	12.9
C18:0	3.9
Total	18.0
MUFA, % of total fatty acids	
C16:1	0.6
C18:1	24.9
Total	25.5
PUFA, % of total fatty acids	
C18:2n-6	47.7
C18:3n-3	8.8
Total	56.5

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

shown in Table 3. Both genotype and slaughtering age affected the main fatty acids of meat. The higher content of SFA was observed in the R chicks, mainly due to the higher amounts of C16:0 and C18:0. The CN1 birds showed the lower SFA values, whereas KR4 was intermediate.

Monounsaturated fatty acid concentration, mainly represented by C18:1n-9, showed the highest levels in CN1 chickens. The low MUFA level, which in chickens depends both on the endogenous synthesis and the gut absorption from the diet, was significantly lower in R birds and at 81 days of age.

Polyunsaturated fatty acids showed a different trend at the two slaughter ages considered: at 70 days of age medium-growing chickens (CN1 and KR4) showed the lowest values, while at 81 days the highest ones. The total n-3 fatty acid and, above all, the LCP derivatives were higher in the medium-growing than in fast-growing strain (R). The lower n-3 amount of these latter birds could be due to different factors: the scarce/null intake of grass and the lower Δ^6-desaturase activity in line with slow-growing lines (Dal Bosco *et al.*, 2012). Indeed, it is widely known that the rate-limiting step in the enzymatic LCP biosynthesis is thought to be Δ^6-desaturase (Yamazaki *et al.*, 1992). On the other hand, medium-growing lines probably eat much more grass than fast-growing ones (Castellini *et al.*, 2002b) and the competition for LCP synthesis is more advantageous for n-3 series since grass major PUFA is ALA. Indeed, ALA and LA elongation and desaturation require the same desaturation pathways (Lands, 1992) and higher ALA intake could contribute to the different n-3 profile of medium-growing chickens.

Moreover, the CN1 meat exhibited lower concentrations of ALA, but higher LCP derivatives. As observed in a previous study (Dal Bosco *et al.*, 2012), there is no direct correlation between grass intake and ALA level in the meat. Naked Neck chickens probably ingested more ALA but simultaneously had a higher conversion of ALA into LCP as confirmed by the higher level of EPA, DPA and DHA in the meat. Also, Ponte *et al.* (2008) showed that forage consumption in broiler chickens do not contribute to improve ALA levels in breast meat, while desaturation and elongation of this precursor contribute to improve LCP derivatives and n-6/n-3 ratio.

Older birds showed higher LCP and total PUFA levels (Table 4. These results are in line with Poureslami *et al.* (2010) whose study of the effect of age on fatty acid metabolism revealed that chickens slaughtered at 42 days of age had higher values for PUFA intake, PUFA apparent digestibility and ALA and LCP derivates accumulation when compared with the 7-14 d age period, lied to lower values for b-oxidation. Authors justified this trend with the fact that young birds had a higher metabolism rate compared to the older ones.

Naked Neck chickens showed the best values of total PUFA/total SFA and n-6/n-3 ratio at both considered ages; even peroxidability, atherogenicity and thrombogenicity indexes, as well as hypocholesterolaemic/hypercholesterolaemic fatty acid ratio showed a similar trend among genotypes but without a clear trend with the slaughtering ages.

The tocopherols, tocotrienols, carotenoids and the oxidative status of plasma are presented in Table 5. α-tocopherol was the most represented vitamin E isoform in blood and it is considered as the most active antioxidant. The other two isoforms play a role in reduction of inflammation (Singh *et al.*, 2005). α- and γ-tocotrienol were the only tocotrienols detected in plasma. Tocotrienols, apart for their antioxidant property, are well known for their hypoc-

Table 3. Fatty acid profile of *Pectoralis major* muscle at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
SFA							
C14:0	0.87^{a}	1.56^{b}	1.64^{b}	0.84^{a}	1.69^{b}	1.31^{ab}	0.41
C16:0	29.2	30.1	30.7	28.9	30.0	31.2	3.35
C18:0	9.18^{a}	10.1ab	11.0^{ab}	10.5^{ab}	11.1ab	12.3^{b}	2.89
Others	3.32^{c}	2.06^{a}	1.99^{a}	2.65^{bc}	2.19^{a}	2.41^{b}	0.48
Total	42.5^{a}	43.8^{a}	45.3bc	42.9^{a}	45.0^{b}	47.3^{c}	2.59
MUFA							
C14:1n-6	0.12^{b}	0.04^{a}	0.01^{a}	0.13^{b}	0.05^{a}	0.03^{a}	0.06
C16:1n-7	3.35e	3.18^{d}	1.41^{a}	2.71^{b}	2.85^{c}	1.32^{a}	0.13
C18:1n-9	24.3bc	23.5^{b}	22.6^{a}	22.5^{a}	21.9^{a}	21.7^{a}	1.87
Others	0.22^{b}	0.22^{b}	0.17^{a}	0.23^{b}	0.15^{a}	0.15^{a}	0.07
Total	27.9^{b}	26.8^{b}	24.1^{a}	25.5^{ab}	24.9^{a}	23.2^{a}	2.06
Polyenoic n-6							
C18:2	20.8^{ab}	20.5^{a}	20.7ab	22.3^{b}	21.3^{ab}	21.2^{ab}	1.95
C20:2	0.84^{b}	0.34^{a}	0.28^{a}	1.35^{c}	1.55^{c}	0.39^{a}	0.18
C20:3	0.26^{b}	0.24^{b}	0.16^{a}	0.23^{b}	0.31^{b}	0.27^{b}	0.11
C20:4	4.06^{ab}	5.07^{b}	7.42^{c}	3.05^{a}	3.21^{a}	5.12^{b}	1.51
Total	25.9^{a}	26.1^{a}	28.5^{b}	26.9^{a}	26.3^{a}	26.9^{a}	1.29
Polyenoic n-3							
C18:3	0.62^{a}	0.93^{b}	0.58^{a}	0.75^{ab}	0.90^{b}	1.21^{c}	0.21
C20:5	0.18^{b}	0.12^{ab}	0.09^{a}	0.59^{d}	0.26^{c}	0.13^{ab}	0.08
C22:5	0.94^{c}	0.64^{b}	0.28^{a}	1.05^{d}	0.92^{c}	0.31^{a}	0.07
C22:6	0.95^{c}	0.67^{b}	0.60^{b}	1.29^{d}	0.85^{c}	0.48^{a}	0.12
Total	2.72^{c}	2.41^{b}	1.57^{a}	3.76^{d}	3.00^{c}	2.16^{b}	0.36
Total PUFA	28.6	28.5	30.1	30.6	29.3	29.1	2.68

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; SFA, staurated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. $^{a-e}$Different letters in the same column denote significant differences ($P<0.05$). Values are expressed as percentage of total fatty acids.

holesterolemic, anti-cancer and neuroprotective effects that are often not exhibited by tocopherols (Qureshi *et al.*, 1996).

Despite the high level of non-α-tocopherol isoforms in diets (about 75% data not shown), in plasma the α-tocopherol represents about 87% of all vitamin E isoform. It should be underlined that oral supplementation of α-tocopherol reduces all the other isoforms because only α-tocopherol is selectively bound to a transfer protein (Oram *et al.*, 2001). The presence of such a protein that preferentially selects α-tocopherol seems to explain why all other vitamin E isoforms have a lower biological activity than α-tocopherol. Moreover, tocotrienols belong to a group of phenolic compounds with a lower tissue retention and half-life in respect to α-tocopherol (Qureshi *et al.*, 1996). The plasma of more active chickens (CN1 followed by KR4) had a higher amount of malondialdehyde (Castellini *et al.*, 2006) despite the higher antioxidant content. This fact could be due to the higher oxidative metabolism and free-radical production of this strain which is not fully counterbalanced by the response of organism (Alessio *et al.*, 2000). More kinetic chicks, despite the higher antioxidant intake (Dal Bosco *et al.*, 2010, 2014b), require further antioxidant protection to protect the high LCP level of the body. The antioxidant profile and the oxidative status of *Pectoralis major* are presented in Table 6. The meat followed the same trend of blood plasma: more active chickens had a considerable amount of malondialdehyde (Castellini *et al.*, 2002b; 2006) despite the higher antioxidant content. In agreement with Hewavitharana *et al.* (2004), α-tocopherol was the principal isomer of chicken meat, followed by γ-tocopherol, α-tocotrienol, α- and γ-tocotrienol. β-tocotrienol was coeluted with γ-tocotrienol, while δ-tocotrienol was present only in trace. Ponte *et al.* (2008) did not found any difference in the antioxidant profile of broiler meat when chickens were supplemented with dehydrated forage: the use of fast-growing chickens at very early age (28 days) could explain the difference obtained.

Table 4. Fatty acid indexes of *Pectoralis major* muscle at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
P/S	0.67^{ab}	0.65^{ab}	0.66^{ab}	0.71^{b}	0.65^{ab}	0.62^{a}	0.08
n-6/n-3	9.54^{a}	10.85^{c}	18.19^{e}	7.16^{a}	8.79^{b}	12.48^{d}	0.26
Peroxidability index	53.50^{a}	54.28^{a}	59.15^{b}	55.43^{ab}	51.00^{a}	51.07^{a}	3.65
Atherogenicity index	0.58	0.66	0.69	0.57	0.68	0.70	0.12
Thrombogenicity index	1.44^{b}	1.11^{ab}	1.39^{b}	1.07a	1.23^{ab}	1.42^{b}	0.23
HH	1.72	1.62	1.61	1.73	1.55	1.54	0.31

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. **Different letters in the same column denote significant differences (P<0.05). Values are expressed as percentage of total fatty acids.

Table 5. Oxidative status of plasma at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
TBAR	24.5^{c}	20.3^{b}	21.9^{bc}	20.4^{b}	17.8^{a}	18.7^{a}	2.04
α-tocopherol	10.1	6.81	6.74	10.8	10.6	9.48	5.80
δ-tocopherol	0.29	0.30	0.30	0.35	0.37	0.32	0.06
γ-tocopherol	0.78	0.62	0.53	0.44	0.50	0.44	0.32
α-tocotrienol	0.25^{ab}	0.18^{ab}	0.15^{a}	0.30^{ab}	0.28^{ab}	0.27^{ab}	0.10
γ-tocotrienol	0.10	0.11	0.09	0.12	0.11	0.10	0.02
Lutein+zeaxantin	31.2^{ab}	28.3^{a}	29.4^{a}	41.0^{c}	34.6^{b}	34.9^{bc}	3.41

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; TBAR, thio-barbituric acid-reactive substances. **Different letters in the same column denote significant differences (P<0.05). Values are expressed as nmol/mL.

Table 6. Oxidative status of *Pectoralis major* muscle at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
TBAR, mg/g	0.16^{a}	0.15^{a}	0.11^{a}	0.25^{b}	0.16^{a}	0.13^{a}	0.04
α-tocopherol, ng/g	469.2^{ab}	418.5^{a}	435.2^{a}	589.9^{b}	526.8^{b}	525.4^{b}	45.8
δ-tocopherol, ng/g	22.0^{c}	12.9^{a}	16.8^{b}	34.1^{e}	15.4^{ab}	15.7^{ab}	2.69
γ-tocopherol, ng/g	31.8^{a}	25.7^{a}	51.0^{b}	38.4^{b}	56.8^{cd}	61.7^{d}	5.02
α-tocotrienol, ng/g	23.7^{c}	16.5^{a}	18.9^{a}	34.6^{d}	32.4^{d}	31.3^{d}	2.30
γ-tocotrienol, ng/g	14.4^{c}	11.5^{ab}	10.0^{a}	14.7^{c}	12.6^{b}	12.5^{b}	1.25
Lutein+zeaxantin, ng/g	31.2^{ab}	28.3^{a}	29.1^{a}	41.0^{c}	34.6^{b}	34.9^{b}	3.41

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; TBAR, thio-barbituric acid-reactive substances. **Different letters in the same column denote significant differences (P<0.05).

More consideration to antioxidant stability of organic meat should be devoted by improving the pasture allowance or by adding antioxidants to the diets.

Conclusions

In conclusion, the results of this study indicate that both genotype and age affect the fatty acid content of chicken breast. In organic farming, chicken genotype plays a fundamental role in meat nutritional value (fatty acid, antioxidant, oxidative stability) owing to its peculiar foraging behaviour, metabolism and kinetic activity. This finding assumes considerable importance as health concerns over fat intake are among the main factors contributing to the decline of meat intake. Regarding the slaughtering age, the results of this trial demonstrate that older chickens show a better fatty acid profile from a nutritional point of view even if the oxidative status gets worse. These results open new research perspectives on management and nutrition techniques in order to maintain a good oxidative status and ultimately provide an optimal fatty acid profile to the consumer.

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3rd CHAPTER:

LIPID METABOLIC ACTIVITY

4.5 EXPERIMENT 5.

Fatty acid composition of meat and estimated indices of lipid metabolism in different poultry genotype reared under organic system

Abstract. According to EC regulation 889/08 different European countries should draw up a list of slow-growing strains adapted to an organic system. and in the meantime provide this information to poultry operators and to European Union. Thus the aim of the present work was to evaluate the effect of poultry genotype on fatty acid composition and lipid indices of poultry meat. Six poultry genotypes (100 birds each) with a different growth rate (slow growing: Leghorn, Ancona, Cornish × Leghorn; medium-growing: Kabir, Naked Neck; fast growing: Ross) were reared under an organic system. Breast meat fatness, fatty acid composition and indices were largely related to genotype, as slow-growing strains had higher elongase, thioesterase and Δ5/Δ6 desaturase indices accompanied by a lower Δ9. Differences in the fatty acid profiles were observed by varying contents of total saturated fatty acids with a higher value seen in Leghorn chickens and a lower value seen in commercial lines. On the contrary. Leghorn and Ancona chickens revealed higher amounts of stearic acid and total polyunsaturated fatty acids compared with commercial genotypes both in the total content and in the different fractions (total n-3 and total n-6). Despite the increased consumption of fresh forage, the lower linolenic acid in meat of the slow-growing strain could be explained by the higher conversion of this fatty acid to its long-chain derivatives.

Slow-growing strains

Medium-growing strains

Fast-growing strains

Slaughtering n=20 bird/strains

↓

Fatty acids profile of breast meat

↓

Estimation of indices of fatty acid metabolism

Elongase = C18:0/C16:0

Thioesterase = C16:0/C14:0

Δ9-desaturase (18) = [C18:1/(C18:1+C18:0)]x100

Δ9-desaturase (16+18) = [C16:1+C18:1/(C16:1+C16:0+C18:1+C18:0)]x100

Δ5+Δ6-desaturase* = [C20:2n-6+AA+EPA+C22:5n-3+DHA/LA+ALA+C20:2n-6+AA+EPA+C22:5n-3+DHA]x100

*LA: C18:2n-6; ALA: C18:3n-3; AA: c20:4n-6;EPA: c20:5n-3; DHA: C22:6n-3

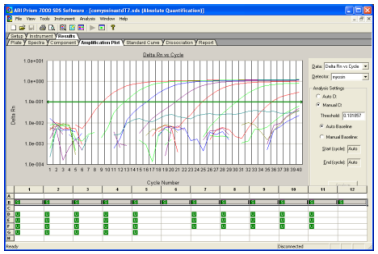
4.6 EXPERIMENT 6.

Evaluating of the mRNA expression and Δ6-desaturase activity (capacity to desaturate LNA to EPA and DHA) in three chicken genotypes.

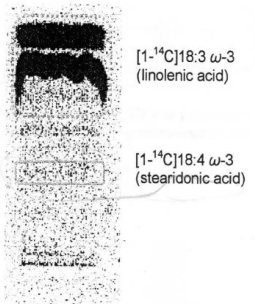
Abstract. Domestic animals are unable to synthesize long chain-PUFA, but they can convert dietary linoleic and α-linolenic acid through a pathway catalyzed by elongating and desaturating enzymes. The relatively low efficiency of the desaturating enzymes in poultry allows for further consideration on the selection of genotypes able to synthesize a higher PUFA amount. In this study the liver messenger RNA expression and enzyme activity of the Δ6-desaturase were evaluated in three chicken strains (slow-SG, fast-growing-FG and SG x FG crossing-SFG). Chickens were slaughtered at hatch, and liver was taken and analyzed. A relationship between genotype and desaturating ability was evidenced. SG chicken in comparison to FG strain showed a higher desaturase activity ($P<0.05$). Whereas there were no significantly differences between SG and SFG Δ6-desaturase enzyme value. Even mRNA expression was widely affected by genotype. Indeed, SFG showed a much higher mRNA level, while SG not showed significant differences. Data herein reported showed that the Δ6-destaurase activity is strongly correlated with the genotypes. However, several other factors involved in the process of expression/translation, can be assessed. Gene expression may follow certain design principles for optimal evolutionary fitness as demonstrated by the high mRNA expression of the SFG.

At hatching, 5 chicks/group were sacrificed and the liver was taken.

RNA expression of Δ6-desaturase



Δ6-desaturase activity



1. Introduction

In chickens, as in all animals, body fatty acids (FA) are derived from dietary uptake, *de novo* synthesis and/or bioconversion. Among the different fatty acid classes, n-3 long-chain PUFA (n-3 LC-PUFA) are of particular interest due to their beneficial role on human health (Ruxton et al., 2005). Meat from different animal species is characterized by different FA composition; within the same specie the FA profile reflects the endogenous biosynthesis as well as the composition of the diet. This relationship is stronger in monogastrics (pigs, poultry and rabbits) than in ruminants, where dietary fatty acids are hydrogenated in the rumen (Kouba and Mourot, 2011). In vertebrates, α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) cannot be biosynthesized *de novo*, but derived from the diet and can be converted to longer and more unsaturated n-3 and n-6 LC-PUFA, respectively. This bioconversion of n-3 LC-PUFA includes the intervention of two enzyms: Δ5 and Δ6-desaturase. Both take part to the transformation of ALA to EPA (eicosapentaenoic acid, 20:5 n-3), which is subsequently converted to DHA (dochosaesaenoic acid, 22:5 n-3) by chain elongation and likewise LA to AA (arachidonic acid, 20:4 n-6). Even the n-6/n-3 ratio has a relevant role in nutrition because human diets are retained unbalanced with a too low proportion of n-3. Since the two PUFA series compete for the same desaturation enzymes the n-6/n-3 ratio is relevant for the relative enzyme availability. Recent studies assessed that humans are rather poor EPA and DHA synthesizers; as a result some LC-PUFA might be almost essential (Robinson et al, 2013; Jeppesen et al, 2013). Thus, dietary guidelines suggest the replacing of dietary SFA with PUFA, mainly of n-3 series (Lands, 1992). The absolute amount of 18:3n-3 intake is of prime importance to the efficiency of conversion to the LC-PUFA. However, the production of n-3 LC-PUFA, particularly DHA, from ALA is limited in the human body (Burdge and Calder, 2005; Brenna et al., 2009). Therefore LC-PUFA seems to be essential in the human health and they should be adequately supplied by the diet. In order to optimize the FA composition of foods derived from farmed animals, knowledge of their LC-PUFA metabolism is required. Several trials have demonstrated that it is possible to enrich poultry products (meat and eggs) with n-3 LC-PUFA through dietary strategies (Rossi et al., 2013; Fraeye et al., 2012; Woods and Fearon, 2009; Meluzzi et al., 2001). In recent papers Sirri et al. (2010; 2011) evidenced that LC-PUFA composition of chicken meat was affected by genotype. In particular, slow-growing (SG) seems to have a higher n-6 and n-3 PUFA content in the breast muscle than medium-growing (MG) and fast-growing (FG) chickens, suggesting a different expression of genes encoding for the desaturating enzymes. On the other side, several authors (Castellini et al., 2003) showed that SG and MG lines eat much grass than FG lines; accordingly the different desaturating ability could be masked by a higher ALA intake which is the major PUFA in the grass. Many factors can be also influence the LC-PUFA content in chicken meat, for instance Poureslami et al. (2010) found an accumulation of 18:2n-6 and 18:3n-3 increased and β-oxidation decreased with age, while the gender had a low effect.

In this study the RNA expression and Δ6-desaturase activity in the liver of three chicken strains were evaluated, and compared in SG, FG and SG x FG crossing (SFG) eating the same diet with no access to pasture.

2. Results

In Table 1 fatty acids composition of diet is report. Given the large content of soybean meal (Table 4 on Materials and Methods) in the diet, the amount of LA (49.5%) and the n-6/n-3 ratio is high.

Desaturating enzyme relative gene expression in the three strains is reported in Figure 1. The FG strain showed a lower mRNA expression of FADS2 genes than the SG one ($P>0.01$). However, RNA expression was two- or three-hundred times higher in SFG than SG and FG strains, respectively ($P<0.01$).

Even the enzyme activity confirmed when reported by mRNA (Figure 2). The Δ6-desaturase activity was significantly higher in SG than FG (172.0 vs 63.56 pmoli in 30min/mg prot), whereas in SFG was intermediate (136.66 pmoli in 30min/mg prot). However, the enzyme activity of SFG was not significantly different from SG ($P>0.01$).

3. Discussion

Δ6-desaturase activity appared more influenced by genotype than by diet. As previously showen (Experiment 5), SG strains had a higher value of estimated Δ5/Δ6-desaturase than FG. The same results we have been obtained through the study of the "real" enzyme activity ($P>0.01$). Little knowledge exhixsts about Δ6-desaturase acitivity on 1-old chick. Poureslami et al. (2010) reported that the accumulation of LA and ALA increased and β-oxidation decreased with age. Apparent desaturation activity was higher at 7–21 d than at 21–42 d of age. This data corresponds to the fact that young chickens have a higher metabolism rate compared with the same at higher age. Indeed, a high requirement during avian embryo development is the supply of certain LC-PUFA to the developing tissues in order to maintain appropriate functional development (Nobel and Speake, 1997; Speake et al., 1998).

The above mentioned trend could justify the not similar enzyme activity in the SFG and SG chicks, contrary to what occurs in adult chickens (Sirri et al., 2010, 2011).

With regard to a quantitative description of gene expression, several studies comparing mRNA and protein levels concluded that the correlation is poor (De Sousa Abreu et al., 2009; Maier at al., 2009).

In our study the mRNA expression of FADS2 gene in SFG was higher than others strains. There is emerging evidence which suggests that mRNA expression patterns are necessary but are by themselves insufficient for the quantitative description of biological systems. This evidence includes discoveries of post-transcriptional mechanisms controlling the protein translation rate (Harford et al., 1997), the half-lives of specific proteins or mRNAs (Varshavsky, 1996), and the intracellular location and molecular association of the protein products of expressed genes (Vrlinger, 1997). mRNA and protein levels result from coupled processes of synthesis and degradation. Schwanhäusser et al. (2013) found that mRNA levels of different murine genes, explain only 40% of the variability in protein levels. De Sousa Abreu et al. (2009) and Maier et al. (2009) in other mammals reported a lower values.

A much lower correlation has been showed in *Saccharomyces cerevisiae*, indeed for about 40 on 106 genes studied, while the mRNA levels were of the same value, the protein levels varied by more than 20-fold (Gygi et al., 1999). In particular, in sea bass, despite expressing an apparently active Δ6-desaturase enzyme, the activity of the LC-PUFA biosynthesis pathway in both hepatocytes and

enterocytes was very low (Mourente and Dick 2002; Mourente et al., 2005b), and considerably lower than the activities measured in salmon hepatocytes and enterocytes (Tocher et al., 2002; Zheng et al., 2005b).

It should be noted that the SFG cross hereby studied is produced by the SG female and FG male bird crossing. Considering that gene expression is a multistep process that involves the transcription, translation and turnover of mRNAs and mammalian proteins, we can be hypothesize a missing evolutionary effect in the adaptation and selection process of this crossbreed. Gene expression may follow certain design principles for optimal evolutionary fitness. Intriguingly, genes with certain combinations of mRNA and protein half-lives share common functions, indicating that they evolved under similar constraints. One of these constraints may be the energy efficiency (Wagner, 2005). A second constraint may be the ability of genes to respond quickly to a stimulus. Many transcription factors and genes with cell-cycle-specific function have unstable mRNAs and proteins, predisposing them to rapid transcriptional and/or translational regulation (Schwanhäusser et al., 2013).

4. Conclusion

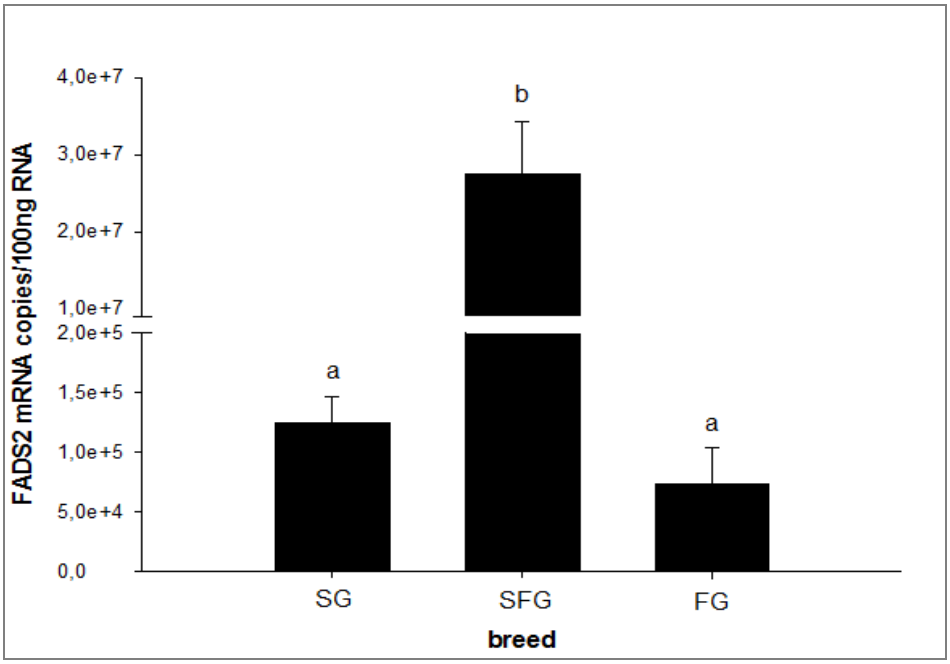
The changes in mRNA expression of FADS2 gene and Δ6-desaturase activity previously observed between chicks with different growing rates can be due to the selection rate of these strains. SG breeds, not selected for growing traits, showed a higher activity of the Δ6-desaturase enzyme. This is an important goal for the liverstock production, because the low productivity can be compensated by the particular lipid metabolism which improves the nutritional quality of the products.

Crossing birds, nevertheless a much higher mRNA expression, seem to have a little lower ability to desaturate essential fatty acids in LC-PUFA than SG. Probably, the absence of long-term selection on this breed has contributed to the increase in not-translated mRNA.

Table1. Main fatty acid profile of the chicken diet.

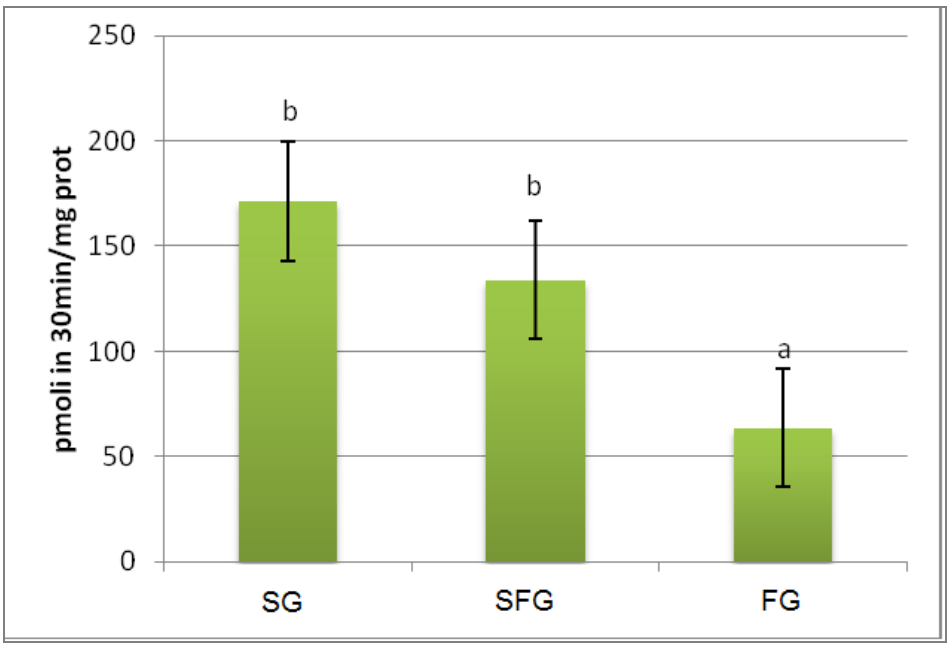
% fatty acid		Feed
C14:0	%	1.0
C16:0		13.2
C18:0		4.3
Σ SFA		**18.5**
C16:1		0.9
C18:1		24.3
Σ MUFA		**25.2**
C18:2 n-6		49.5
C18:3 n-3		6.8
Σ PUFA		**56.3**
n-6/n-3		7.3

Figure 1. mRNA expression of Δ6-desaturase in three chicken genotypes (mean+SEM)



a,b: P<0.01

Figure 2. Δ6-desaturase enzyme activity in three chicken genotypes (mean+SEM)



a,b: P<0.01

4.7 EXPERIMENT 7.

Effect of maternal dietary n-3 fatty acids supplementation on fatty acid composition, Δ6-desaturase expression and activity of chicken liver.

Abstract. Mammals, like fish and probably all other vertebrates, are unable to endogenously synthesize polyunsaturated fatty acids (PUFA). Accordingly, these compounds are required in the diet. If a dietary deficiency occurs, the animal stops growing and reproducing and develops various pathologies and eventually dies. The PUFA in question are termed "essential fatty acids" (EFA) and they include members of both the n-6 and n-3 series typified by linoleic acid (C18:2n-6-LA), and α-linolenic acid (C18:3n-3-ALA). The aim of this study was to check if it is possible to influence lipid metabolism by a maternal dietary supplementation of linseed (ALA) or fish oil (EPA and DHA). A total of 60 laying hens belonging to two genetic lines (Leghorn and Ross) fed 3 experimental diets were studied: control, LNA (10% lineseed) and LCP (3% fish oil) group. The liver was collected by chicks at hatching, to assess the lipid composition, mRNA expression and Δ6-desaturase activity. The results showed that diet lightly affected lipid metabolism whereas the genetic effect was confirmed. Data reported suggested that the expression and activity of Δ6-desaturase is strongly correlated with the genotype, so reaching an important objective for the food industry, since dietary modifications do not seem to be able to greatly change the metabolism of birds.

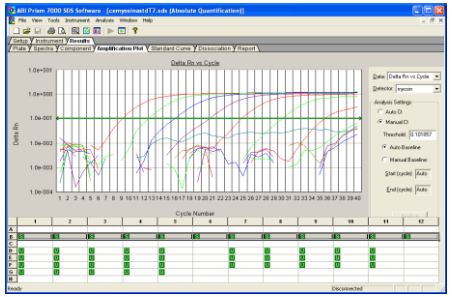
Control diet

LNA groups:10% Linseed diet (large amount of LNA - *PRECURSOR*)

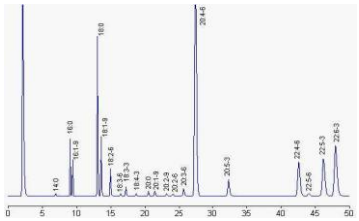
LCP groups:3% Fish oil (large amount of EPA/DHA - *PRODUCTS*)

To hatch, **5 chicks/group** were sacrificed and the liver was taken.

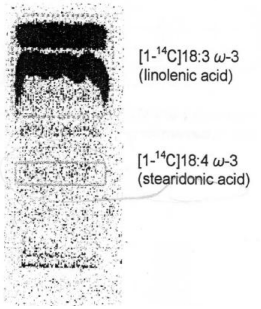
RNA expression of Δ6-desaturase



fatty acids profile



Δ6-desaturase activity



1. Introduction

Animals are unable to synthesize long-chain PUFA (LCP ≥20 carbon atoms) from acetyl-CoA, but they can convert the linoleic acid (LA;18:2 n-6) and α-linolenic acid (ALA 18:3 n-3) supplied by the diet to LC-PUFA (Tocher, 2003). If a dietary deficiency occurs, the animal stops growing and reproducing. It develops various pathologies and eventually dies (Das, 2006). The PUFA in question are termed "essential fatty acids" (EFA) and they include members of both the n-6 and n-3 series typified by linoleic acid, (18:2n-6-LA), and α-linolenic acid (18:3n-3-ALA) (Das, 2006). Both LA and ALA may be converted by elongation and desaturation into their long-chain metabolites (Gregory et. al., 2011). This process is catalyzed by the elongating and desaturating enzymes. Delta-6 (Δ6) and delta-5 (Δ5) desaturases, which introduce double bonds in EFA to obtain LC-PUFA, are encoded by FADS2 and FADS1 genes, respectively (Innis, 2003; Nakamura and Nara, 2004). Δ6 and Δ5 are retained the rate-limiting enzymes in the synthesis of arachidonic acid (ARA; 20:4 n-6), eicosapentaenoic (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) from their dietary precursors LA and ALA, respectively (Cho et al, 1999a; Cho et al, 1999b; Wood et al., 2004). Furthermore, another desaturating enzyme exists, i.e. the delta-9 (Δ9) or stearoyl-CoA desaturase, which converts palmitic (16:0) and stearic acid (18:0) to palmitoleic (16:1) and oleic acid (18:1), and is encoded by the SCD1 gene (Ntambi and Miyazaki, 2003). As a result fish and terrestrial animals developed a different ability to convert ALA in LCPn-3 and the extent of conversion greatly depends on elongase (ELOVL2-5) and desaturase (Δ5-Δ6) enzymes (Tocher, 2010). All fishes showed a Δ6 activity, required for the initial desaturation of LA and ALA, whereas Δ5 activity, necessary to desaturate 20:4 n−3 to EPA, have only been found in the diadromous/freshwater species (Reuss and Poulsen, 2002; Tocher, 2010). The lipid composition of animal body tissues largely depends on the feeding background of the meat producing non-ruminant animals (Wood, 2004). There has been an increased interest in the substitution of animal fat sources with vegetable oils or fish oil in animal nutrition. Grass and vegetable oils have been attributed with reducing the level of saturation in animal fat tissue due to their unsaturated fatty acid concentration when compared with animal fat (Enser et al., 2000; Kloareg et al., 2007; Trattner et al., 2011; Gruffat et al., 2011). Since some meats naturally have a P/S ratio of around 0.1 (Wood, 2004), meat has been implicated in causing the imbalanced fatty acid intake of today's consumers. Thus, the recommendation ratio should be increased to above 0.4.

In addition, some vegetable oils are rich in n-3 PUFA, mostly 18:3 n-3. Increasing the n-3 content in animal meats can be achieved by including fish oil or fish meal in the diet, or in more sustainable way adding vegetable oils rich in ALA. Diet rich in ALA results in an increased level of ALA, EPA, and DPA in the meat (Vatansever et al., 2000), while in most cases no effect on DHA level was observed (Enser et al., 2000).

Human diets are retained unbalanced in term of PUFA n-6 and n-3 with a too low proportion of the latter (Kuipers et al., 2010). Several studies assessed that humans are rather poor EPA and DHA synthesizers (Muskiet et al., 2004), despite ARA and DHA are especially abundant in the brain and the retina and have relevant role in many physiological pathways and pathological disorder like: CHD, reproductive dysfunctions and depression (Muskiet et al., 2004; Simopoulos, 1991). As a result, it seems that at least some LCP might be almost essential to humans (Robinson

et al, 2013; Jeppesen et al, 2013). Such assessment could be corroborated by a high dietary LCPn-3 intake by our hominid ancestors might have precluded the need to conserve a highly sophisticated expression of genes coding for the enzymatic conversion of ALA to DHA (Kuipers et al., 2010). Since the two PUFA series compete for the same desaturation enzymes the n-6/n-3 ratio is relevant for the relative enzyme availability. Thus, dietary guidelines suggest the replacing of dietary SFA with PUFA, mainly of n-3 series (FAO, 2010). The dietary aspect has been widely investigated even if some studies showed different conclusion about ALA elongation/desaturation in several species. According to these findings anamals could be grouped according to the possibility of product or only accumulate LCP n-3.

In this study we have tested the possibility to modify lipid metabolic pathway with maternal dietary supplementation of n-3 precursor (linolenic acid) or directly LC-PUFA (EPA and DHA). Accordingly, the effect of dietary supplementation of n-3 sources (precursor and derivatives) was evaluated in two chicken strains. The fatty acid composition, Δ6-desaturase expression and Δ6-desaturase activity on chick liver hatched from hens fed linseed or fish oil was estimated.

2. Results

In Tables 1 and 2 the formulation of diets and fatty acids profile are reported. Given the supplementation of 10% extruded linseed the LNA diet showed a higher value of C18:3n-3 (54.3%) then LCPn-3 (3.5%). On the contrary, the percentage of C20:5n-3 (EPA) and C22:6n-3 (DHA) is detectable only in LCPn-3 group. LNA and LCPn-3 diets had a total n-3 fatty acids value much higher than control as demonstrated by the n-3/n-6 ratio (7.2 vs 0.19 and0.34 in the control diet, LCPn-3 and LNA respectively).

The fatty acids profile of chick liver is showed in Table 3. There were no statistically significant differences between breeds used (within the same feeding), but only between dietary treatments. The SFA amount of liver was higher in the control group than in the n-3 groups. Even the MUFA content was higher in control chicks, due to the higher level of C18.

The LA amount followed the dietary trend. It was the highest in control followed by LNA and LCPn-3 groups independently on strains. At the same time, the AA amount was higher in control than others groups where there are no significant differences ($P>0.05$). The supplementation of extruded lineseed increased the ALA content (9.10 and 8.80% in SG and FG respectively). Likewise, the amount of LC-PUFA n-3 (EPA, DPA and DHA) was higher in LCPn-3 chick liver. As a result the total PUFA n-3 was about the same in both n-3 groups and n-6/n-3 ratio was lower than the control.

In Figure 1 is reported the mRNA expression of FADS2 gene. It was evident that there is no significant dietary effect, but there was a strong difference between SG and FG strains. In LCPn-3, and especially in LNA group, the mRNA expression of SG appeared lower than control whereas the opposite trend was shown by FG ($P>0.01$).

The enzyme activity of Δ6-desaturase is reported in Figure 2. The activity value was very high in the SG fed control diet and decreased with the n-3 dietary enrichment. The same result was showed in FG but with a lower trend. However, the enzyme activity seem to show a few higher in FG than SG when they fed n-3 diets.

3. Discussion

The lipid profile show that diets rich in PUFAn-3 reduced liver SFA content, compared with the control, due to the high amount of PUFA. Schumann et al. (2000), found a reduction in total liver lipid content in laying hens fed with flaxseed, flax oil, or n-3 fatty acid supplement, respect to a mixture of animal and vegetable oil. Similar results were found in rats fed fish oil (Lambert et al., 1998). This reduction of liver lipid content by dietary n-3 fatty acids could be the consequence of the higher oxidation rate of these fatty acids (Madsen et al., 1999; Fu and Sinclair, 2000). Lambert et al. (1998), in their study with rats, suggested a reduction on lipid lipogenesis produced by these fatty acids.

Our results were in agreement with Cherian and Sim (2001) which reported that flaxseeds in the diet of laying hens increased the total n-3 fatty acids in the egg yolk lipids until 13.0% compared with 2.3% in control diet.

Even the fatty acid composition of microsomes reflected the fatty acid of diet and the probable increase of n-3 in yolk. It is not surprising, because during the 21d developmental period, there is a large accumulation of yolk lipid in embryo liver (Noble and Cocchi, 1990). Extensive removal of yolk lipids and fatty acids during the third week of incubation is associated with a marked increase in PUFA in chick embryonic tissues (Cherian et al., 1997).

The content of ALA, EPA, DPA, and DHA were higher in microsomes of LCPn-3 and LNA groups when compared to control chicks. The high level of LC-PUFA n-3 and n-6, especially DHA and AA, reflects a unique role of embryonic liver in supplying LC-PUFA to the developing chick. An emphasis by the developing embryo for higher levels of C20 and C22 fatty acids is clearly indicated by the preferential incorporation of C20 and C22 fatty acids into the embryo liver (Noble and Cocchi, 1989; Cherian and Sim, 1993). In avians, liver microsomes are the main sites of PUFA synthesis. Results from the present study indicated that liver microsomes of hatched chicks respond to different PUFA in the diet and to the role of maternal lipids in providing substrates for PUFA synthesis.

The LNA chicks had less Δ6-desaturase enzyme. The Δ6 desaturase is the rate-limiting step in the synthesis of 20:4n-6 from 18:2n-6 and 20:5n-3 from 18:3n-3. Therefore, a decrease in desaturase activity would indicate inhibition of 20:4n-6 derived from 18:2n-6. This inhibition is evident from the reduced content of 20:4n-6 when ALA added. Christiansen et al. (1991) reported reduction of Δ6, Δ5 and Δ9- desaturase activities in rat livers when diets were high in 20- and 22-carbon n-3 fatty acids from fish oil. Even in our study in LCPn-3 chicks there was reduction of activity especially in SG strain ($P<0.01$). It should be mentioned (unpublished results) that the preference for n-3 or n-6 seems strain-dependent and that SG has less specificity for n-6. Accordingly, it is sound that the dietary addition of preformed n-3 reduced the specific Δ6 activity.

LA and ALA share the desaturase enzyme for elongation of 20- and 22-carbon n-6 and n-3 fatty acids. There is a competition between the n-6 and n-3 fatty acids in which n-3 fatty acids are used as the preferred substrate in the desaturation and elongation pathway (Lands, 1992). Thus, the increase in LC-PUFA in the liver microsomes may be attributed to the use of ALA as the preferred substrate over LA. The conversion of ALA into EPA and DHA is competitively lowered by LA supply (Schuchardt et al., 2010).

Indeed, in the control group, the enzyme activity was higher than other ones, expecially for SG, due to the high amount of LA in the diet and chick liver. In control diet the SG seem to have a 4-fold higher Δ6-desaturase activity of FG. The same is suggested by the mRNA expression of the control group, whereas in the other ones there were not statistically significant differences.

The reason for such discrepancy is probably due to the choice of substrate; indeed, in the present study, [1-^{14}C]18:2 n-6 was used as Δ6 substrate. Further experiments with n-6 and n-3 substrates (18:2n-6 and 18:3n-3) may be warranted for further understanding of the role of maternal dietary n-3 and n-6 PUFA.

The effect of dietary fatty acids on desaturase activity has been reported in rats (Garg et al., 1988; Brenner, 1989; Christiansen et al., 1991; Giron and Suarez, 1996). Hofacer et al., (2011) reported that rat liver Δ6-desaturase expression and activity indices are negatively regulated by dietary n-3 fatty acids.

The presence of n-3 fatty acids in the egg yolk and its effect on desaturase activity in the newly hatched chick has never been depth. The developing chick embryo requires PUFA for the synthesis of membrane lipids and eicosanoids, as evidenced by an increase in PUFA content and desaturase activity in the embryonic chick liver toward hatching (Cherian and Sim, 2001). There is much interest at present in the role of dietary fatty acids in controlling gene expression. Jiang et al. (1990) reported increased tolerance of dietary cholesterol in hatched chicks when eggs were loaded with cholesterol through hen's diets. The same phenomenon has been reported in other species, suggesting biological programming of enzymes involved in lipid and PUFA metabolism (Reiser and Sidelman, 1972; Chapman et al., 2000). If the activity of enzymes involved in PUFA synthesis could be set by diet in early life, PUFA metabolism and eicosanoid-related functions, such as immune health of birds, could be manipulated in later life. Recently Da Costa et al., (2014) suggested that dietary silage level influences the hepatic fatty acid metabolism in a breed-dependent manner in beef cattle, through changes in the expression of genes encoding for enzymes associated with the desaturation and elongation pathway. Even in seawater Atlantic salmon, Miller et al., (2008), have showed that the high concentrations of (n-3) LC-PUFA found in fish fed EPA and DHA rich diet were not attained by increased metabolism of fish fed diets rich in ALA or stearidonic acid (C18:4 n-3).

4. Conclusion

In summary, the present study confirms the role of liver in PUFA metabolism. In addition, the results indicate a breed modulation of hepatic desaturation of fatty acids, possibly through the differential expression of genes encoding for enzymes involved in the desaturation and elongation pathway.

The maternal n-3 dietary supplementation does not seem to modulate the genetic expression of Δ6 desaturase enzyme, thus, such results can be obtained only with the use of not selected breeds. However, it is known that the feeding of laying hens influence the chemical and nutritional composition of the egg and consequently of the chick. For this reason, further studies needed to understand the reason of such little modulation of Δ6-desaturase activity and FADS2 gene expression by dietary supplementation.

Table1. Formulation (%), chemical composition (%t.q.) and nutritional value of diets

	Starter	Finisher		
Ingredients (%)		Control	LCPn-3	LNA
Maize	51.0	60.00	60	58
Soybean meal(1)	40	31.00	31.00	23.00
Alfalfa meal	3	3.00	3.00	3.00
Extruded flaxseed	-	-	-	10
Oil soybean	3.0	3.0		
Nordos fat n-3	-	-	3.0	-
Vitamin-mineral premix	1.00	1.00	1.00	1.00
Calcium Carbonate	.3	.3		
Dicalcium phosphate	1.00	1.20	1.20	1.20
Sodium bicarbonate	0.50	0.4	0.40	0.40
NaCl	0.20	0.2	0.2	0.20
L-lisine	.02	.02	.02	.02
Methionine	.02	.02	.02	.02
Chemical composition				
Dry matter (%)	90.9	90.9	90.9	90.6
Crude protein (%)	22.3	18.4	18.43	18.43
Ether extract (%)	4.20	4.09	4.4	4.45
Crude fibre (%)	3.67	3.09	2.88	3.87
Ash (%)	5.76	9.14	9.10	9.37
NDF - Neutral Detergent Fibre (%)	10.7	9.86	9.55	11.0
ADF – Acid Detergent Fibre (%)	5.58	4.52	4.18	5.37
Cellulose (%)	4.22	3.33	3.04	3.79
ADL – Acid Detergent Liquid (%)	1.03	0.86	0.81	1.25
Hemicellulose (%)	5.16	5.34	5.37	5.67
Metabolizable Energy (Mj/kg DM)	12.5	12.0	11.0	12.1

Table 2. Main fatty acid profile of the chicken diet

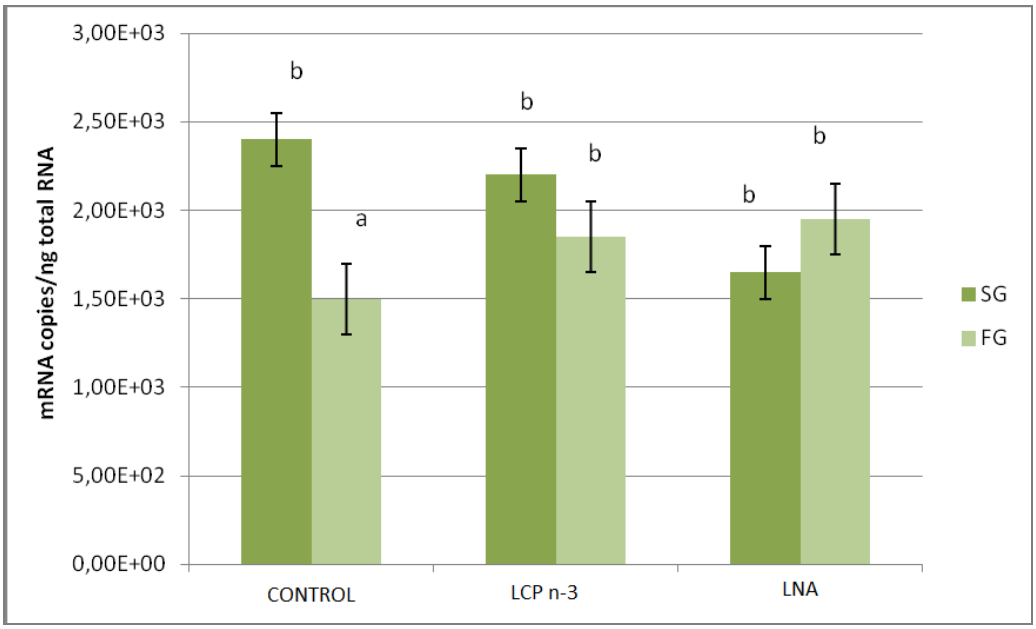
	Control	LCPn-3	LNA	SED
C14:0	1,1	1,05	1.05	0.776
C16:0	13.1b	20.6c	5.5a	2.706
C18:0	4.3b	5.9c	2.1a	0.987
Σ SFA	**18.5b**	**27.5c**	**8.9a**	**3.421**
C16:1	0.9a	9.5b	0.9a	0.740
C18:1	24.3c	12.6a	17.3b	4.527
Σ MUFA	**25.2b**	**22.2a**	**18.2a**	**5.938**
C18:2 n-6	49.5c	7.9a	18.6b	9.846
C18:3 n-3	6.8b	3.5a	54.3c	0.055
EPA	0a	9.5b	0a	0.140
DPA	0a	10.4b	0a	0.033
DHA	0a	19.0b	0a	0.018
Σ PUFA	**56.3a**	**50.3a**	**72.9b**	**9.048**
n-6/n-3	**7.2b**	**0.19a**	**0.34a**	**0.52**

Table 3. Fatty acids profile of chick liver

	Control		LCP n-3		LNA		
	SG	FG	SG	FG	SG	FG	SEM
C14:0	0.52b	0.49b	0.50b	0.52b	0.40a	0.41a	0.02
C16:0	26.3b	25.1b	17.8a	17.8a	18.8a	18.2a	1.96
C18:0	19.6b	18.7a	21.8c	21.2c	22.2c	21.8c	0.70
SFA	**46.4d**	**44.3c**	**40.1a**	**39.5a**	**41.4b**	**40.4a**	1.36
C16:1	3.9c	4.02c	3.3b	3.55b	1.65a	1.83a	0.52
C18:1	27.4c	28.5c	20.9b	21.8b	18.4a	19.7a	2.09
MUFA	**31.3c**	**32.5c**	**24.2b**	**23.4b**	**20.0a**	**21.5a**	2.60
C18:2 n-6	15.7c	16.48c	8.80a	9.20a	13.1b	13.5b	1.60
C20:4 n-6	3.80b	4.30b	1.10a	1.15a	1.27a	1.33a	0.73
PUFA n-6	**19.5c**	**20.8c**	**9.90a**	**10.4a**	**14.4b**	**14.8b**	2.25
C18:3 n-3	0.24a	0.20a	0.85a	0.78a	9.10b	8.80b	2.18
C20:5 n-3 (EPA)	0.33a	0.28a	9.9c	9.6c	6.7b	6.40b	2.14
C22:5 n-3 (DPA)	0.99a	0.86a	7.00c	6.60c	4.10b	3.85b	1.31
C22:6 n-3 (DHA)	1.22a	1.07a	8.10c	7.80c	4.30b	4.20b	1.52
PUFA n-3	**2.78a**	**2.41a**	**25.9b**	**24.8b**	**24.2b**	**23.25b**	**5.67**
PUFA	**22.3a**	**23.2a**	**35.8b**	**35.1b**	**38.6b**	**38.1b**	3.71
n-6/n-3	**7.01b**	**8.62ba**	**0.38a**	**0.42a**	**0.59a**	**0.63a**	1.905

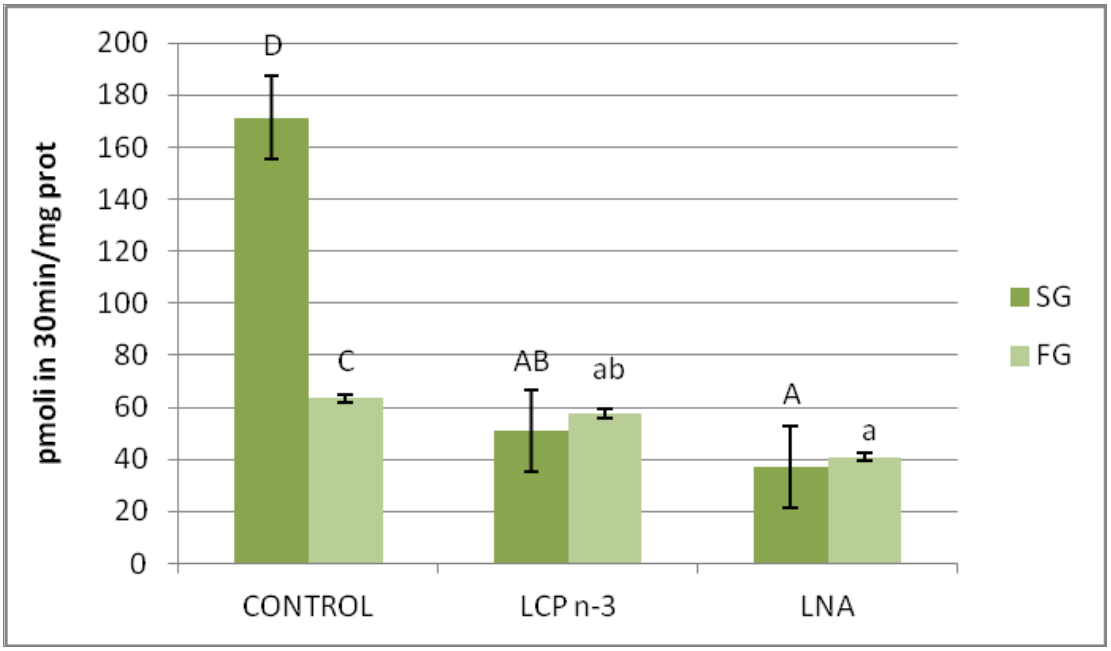
a…d : P<0.05 on the same row

Figure 1. mRNA expression of Δ6-desaturase in three dietary groups and genetic lines (mean+SEM)



a,b: P<0.01

Figure 2. Δ6-desaturase activity in three dietary groups and genetic lines (mean+SEM)



A,D: P<0.01; a,b: P<0.05

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4th CHAPTER

GENERAL CONCLUSIONS

In recent decades, intensive livestock have emerged in response to a growing demand for animal products. However there are serious doubts about the long-term sustainability of intensive farming (Zhang et al., 2012; Veleva et al., 2001; Cerutti et al., 2011). For this reason, many studies have been done for finding a suitable compromise between human food needs, environmental preservation, animal welfare, economic resources and quality of life.

In this productive background, the consumption of poultry meat is increased of 25% consequently requiring a significant increase of production (UNA-Italy).

Modern consumers are aware of the relationship between product quality, safety and animal welfare (Hermansen, 2003; Grunert, 2005) and more consumers believe that extensive farming system (organic, free-range) represents a suitable point of equilibrium. Thus, the recent development of extensive farming in Europe is not only a matter of productive changes (Michelsen, 2001) but it also represents the perception/inclusion of major changes in public opinion into agriculture.

Such complex vision implies a strong effort in term of government support, technical changes and scientific research (Lund and Algers, 2003).

The current PhD work is an original contributes to this purpose. In particular the adaptative response of some chicken genotypes on extensive farming system has been studied in detail. Great attention has been given to autochthones breeds (namely Ancona and Leghorn) to highlight the importance of genetic preservation of biodiversity.

The results confirmed that native breeds, are more adapted to alternative farming systems. They show a higher proportion of natural behavior (walking, running) that results in a major intake of bioactive compounds through grass, earthworms or insects. However, the high kinetic activity of these breeds, from one side reduces the efficiency of conversion of feed into meat and egg and on the other side causes a higher production of pro-oxidant compounds (ROS), that triggers radical reactions and enlarges lipid and protein oxidation. However, given their higher ingestion of antioxidant compounds (tocopherol, carotenes, polyphenols) with the pasture and accumulation in tissues, their health and product quality are not affected. Indeed, eggs and meat of these strains are rich in antioxidant compounds. Even the fatty acids composition is better than commercial products, with a lower SFA content and higher PUFA (n-3, n-6 and LCPUFA).

Although pure breeds show a lower productivity, caused by high behavioral activity, they develop a better health status and a higher resistance to disease. The commercial poultry lines (egg- and meat-type) are more productive but show a difficulty to adapt to non-conventional system (welfare, immune state, mortality rate).

Medium-growing strains and crossbreeds (local x hybrids) show a good welfare and better behavioral activity than fast-growing one. Indeed, the meat of such strains could be considered a good compromise between nutritional quality and farmer profit. These strains seem to maintain

certain adaptability to extensive farming system, as demonstrated by the grazing attitude, kinetic activity, body structure and immune response.

Slow-growing genotypes require a longer fattening period and are well adapted to outdoor farming conditions (Fanatico et al., 2005). Their meat has intrinsic lipid characteristic that increases the nutritional value. They showed a higher capacity to desaturate essential fatty acids (n-3 and n-6) in their long chain derivative (AA, EPA and DHA) which greatly enhances the quality of the product. The same results cannot be obtained with appropriate dietary supplementation.

This finding assumes great importance because of the poor economic availability of farmers and encourages them to use highly productive lines to increase yield. The observed differences may be sufficient to study in depth the local poultry genotype in order to reach a suitable compromise between economic sustainability, market requirement and biodiversity management.

In conclusion, this PhD work suggests that:

- Autochthon birds and their crosses are more adapted to extensive farming system. On the contrary, commercial hybrids seem to suffer from poor environmental conditions.
- The use of high productive hybrids in extensive farming system (organic, free range) is not consistent with the principles sustainability (welfare, health, environmental sustainability and biodiversity).
- Pure breeds have a lower productivity, however their products are markedly different from commercial ones. Indeed, they can be enhanced as products with high nutritional value, with several benefits to human health.

The above-mentioned summary features involve not just the behavioral activity since autochthones breeds show a distinctive lipid and antioxidant metabolism that can positively affect the quality of their products. Accordingly, the position of native strains as unique source of genetic variability could be strengthened.

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