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IMPROVEMENTS OF POULTRY QUALITY PRODUCTS BY GENETIC MEANS

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ABSTRACT

In Europe, although poultry products are diverse from conventional to Label chicken, the general trend for poultry meat is for portioned and further processed products to increase their market share. In the Egg sector, due to new European legislation, conventional cage system should be given up in 2012 for alternative systems which might increase risk of egg contamination. In this context, technological quality of poultry meat and the natural egg defence properties are our priorities. The purpose of this report is to evaluate whether they are under genetic control and for meat, linked to growth rate or stress susceptibility. Within genotypes, most meat quality parameters are highly heritable but influenced by stress at slaughter. Ultimate pH values are highly negatively correlated with glycogen stores and with meat quality traits (luminance, exudate, cooking loss and toughness). By contrast, low correlations are observed between ultimate pH and early pH, and between growth and meat quality traits. Therefore, ultimate pH can be considered a selection criterion to improve meat colour, water holding capacity and texture. Because of their role during eggshell formation and their anti-bacterial properties, eggshell proteins are thought to be involved in natural egg defences by reinforcing the mineral structure and by expressing antibacterial activities which preserve the hygienic quality of eggs during its formation. The strategy of targeting a candidate gene approach (MAS) at some egg shell matrix proteins appeared promising for improving eggshell quality. On the other hand, the heritability of antimicrobial activity of egg white was low but significant. Lowering cost of search for Single Nucleotide Polymorphisms and the recent development of high-throughput methods combined with the chicken genomic sequence should facilitate in near future use of Marker assisted selection by breeders to improve quality of poultry products.

Keywords: Poultry Meat, Egg Defence, Genetic, Heritability, SNP.

General Trends of the European Poultry Meat and Egg Industry

Meat. Due to genetic improvement and progress in nutrition and bird management, meat-type chickens may exhibit very high growth rate and food efficiency. At the same time, poultry production has been greatly diversified, with the use of various genetic types reared in either intensive or more extensive conditions. While standard fast-growing birds are mainly used for portions or further-processed products, in some countries alternative productions using intermediate or slow-growing genotypes are also developing. The quality of poultry meat products can be decomposed into several attributes: mainly, the sensory (colour, tenderness, flavour, juiciness) and the physical (muscle yield, water holding capacity, cooking loss) attributes of chicken carcasses and

meat, which vary with growth rate and body composition. The first concern is to produce a carcass of good quality, specifically with a limited fatness, and a maximal meat yield. Given the general trend for consuming more chicken cuts and further processed products, rather that whole carcass, an important aspect today is the technological quality of the meat.

Eggs. An estimated 70-80% of the world's laying hens and 85% of European hens are kept in conventional un-enriched cages. By 2012, EU directive 1999/74 defining minimum standards for the protection of laying hens plans to abolish the conventional cage system in favour of furnished cages or non-caged system (free range, barn, perchery) to improve the welfare of hens. In this very competitive sector, it is expected that conventional cage producers will move more likely towards furnished cages because the impact on cost of this system should be lower (<10%) than that expected for non-cage egg production systems (free range: 20% increase in cost, 12% for barn production; SANCO SPC.2003258, 2004). Our objectives are therefore to optimize the quality and safety of table eggs in new production systems designed to improve the welfare of hens. This is achieved by understanding the factors which cause variation in quality, by reinforcing the natural defences of eggs which prevent bacterial penetration and growth. That has been carried out by understanding the origin of eggshell defects and by selecting hens for high eggshell quality (using marker assisted selection), and high antibacterial egg white activity by conventional selection.

Genetic control of poultry meat quality

Biological Basis of Poultry Meat Quality.

Post mortem metabolism of the muscle tissue influences the characteristics of the meat. In particular, the rate and the amplitude of acidification have a strong impact on both organoleptic and technological parameters of meat quality. After bleeding, the cessation of oxygen supply modifies the muscular metabolism during the installation of rigor mortis. The muscle relies on the anaerobic glycolitic pathway to use the glycogen stores for ATP production, which leads to the accumulation of lactic acid and protons. Therefore, the acidification process depends upon the amount of glycogen stores (estimated by the glycolytic potential) and the rate of the glycolysis.

In the chicken, normal pH values at 15 minutes (pH_{15}) post mortem are around 6.2 to 6.5 (Berri et al., 2005), while normal ultimate pH values are around 5.8 (Fletcher, 1999). If the pH value is low (below 6.0) at 15 minutes when the muscle is still warm, the proteins are subject to denaturation, which leads to a decreased water holding capacity and a decolouration of the meat. These meats are often qualified as Pale, Soft and Exudative by analogy with those described in pigs. They do indeed show exudative water loss and a decreased technological yield (Fernandez et al., 2002; Molette et al., 2003). When raw they show a rather soft texture while they tend to be less tender after cooking as a consequence of excessive exudation (McKee and Sams, 1997; Molette et al., 2003).

Acid meats are characterized by a low ultimate pH (pHu<5.7) which induces structural alterations in the muscle with an impairment of the technological processing ability. Artificially acidifying turkey meat (Barbut, 1997) induces a destructuration of the myofibrillar network, which also induces a marked decrease in water holding

capacity. At the other extreme, meats with a high ultimate pH also show defects in their colour, texture and water holding capacity. They are called Dark, Firm and Dry. This type of meat can occur in poultry (Mallia et al., 2000), darker meat show enhanced water holding capacities but also an increased sensitivity to microbial development (Allen et al., 1997).

Relation between growth rate and meat quality

Several studies have shown that selection for body weight or muscle development has induced histological and biochemical modifications of the muscle tissue. By comparing 2 genotypes divergently selected for high or low growth rate or for higher breast muscle development and a control genotype, it was shown that increased breast muscle development was associated with a marked increase in muscle fibre size and that change in muscle fibre number was modest (Remignon et al., 1995). Alterations in muscle biochemistry have also been observed. The meat from the genotypes with higher muscle mass showed a decreased content of haeminic pigments and a decreased level of glycogen stores (Berri et al., 2001). As a result, those genotypes showed a paler breast meat (lower redness and increased luminance) with higher pH values.

Another study was designed to relate breast muscle development, including muscle fibre size, to the *post-mortem* metabolism and the further breast meat quality (Berri et al., 2004). As the fibre size increased, both the glycogen reserve at death (glycolytic potential) and the *post-mortem* glycolytic activity of muscle decreased. As a consequence, breast muscles with the largest fibres exhibited the highest pH_{15} and pH_u. Therefore, breast muscles with the largest fibres exhibited lower luminance, drip and thawing-cooking losses and higher tenderness after cooking.

By comparing 2 genotypes of chickens divergently selected for fatness and leanness at the same body weight, we observed that lean chickens showed a comparatively lower level of glycogen stores than fat chickens, with decreased amplitude of acidification of the meat post-mortem and decreased exudation (Berri et al., 2005). This suggests that selections for increased muscle yields and against fat deposition could exert cumulative effects on muscle metabolism, decreasing glycogen storage and thereby reducing the amplitude of acidification post-mortem. Finally, the water holding capacity of the meat and therefore its processing yield were ameliorated.

Altogether, these studies did not evidence any antagonism between growth rate or muscle development and breast meat quality parameters such as water retention and processing ability.

Heritability of technological parameters of meat quality

Heritability values and genetic correlations have been measured in an experimental line of chickens selected for improved breast meat yield and decreased fat deposition (Le Bihan-Duval et al., 2001). All recorded parameters (pH15, pHu, luminance, redness, yellowness and water loss) showed high heritability values ranging from 0.35 to 0.57. In this model, the highest genetic correlations were between pHu and luminance (-0.91), pHu and drip loss (-0.83), luminance and drip loss (+0.81), in agreement with data obtained in other species like pigs. Notably, a rather high negative correlation between pHu and abdominal fat was also recorded (-0.54). By contrast, the correlations with pH15 were rather low, due to the relatively high values for this

parameter observed under experimental conditions. The correlations between growth and meat quality traits were also rather low, suggesting that the genetic mechanisms underlying those traits could be different, and further indicating that selection for growth rate or breast meat yield would not negatively alter meat quality. A comparable study was performed on turkeys (Le Bihan-Duval et al., 2003), where similar data were obtained. In this last study, however, the heritability values were lower, possibly because the birds had been slaughtered in a commercial plant. In a commercial strain of heavy broilers Debut et al. (2005), confirm the correlations observed in the experimental strain, and significant heritability values for meat parameters (from 0.25 to 0.35). Three additional traits, glycolytic potential, cooking loss and toughness of cooked meat also showed high heritability values (from 0.34 to 0.43). Notably, the glycolytic potential was negatively correlated to the pHu with a value close to -1. Luminance, exudate, cooking loss and toughness were negatively correlated with pHu (-0.65 to -0.89), while only luminance and exudate were negatively correlated with pH15 (about -0.5). Again, no correlation was observed between pHu and pH15 suggesting a distinct genetic control of these two parameters.

In conclusion, the genetic studies suggested that pHu was a highly heritable character which was highly correlated with several meat quality traits. It could therefore be considered as a good selection parameter to improve meat colour, water holding capacity and texture. One strategy could be selecting for an optimal window of pHu values avoiding too high or too low values.

Impact of stress by genotype interactions on meat quality

Studies conducted in different species indicate that stress during transportation and at the slaughter house has a strong influence on meat quality. A comparison of a commercial fast growing and slow growing broilers submitted to transport or acute heat stress (Debut et al., 2003) firstly showed a muscle specific effect of stress, breast muscle being less sensitive to stress than thigh muscle. The slow growing birds showed a higher level of struggle during shackling, and an accelerated rate of post-mortem glycolysis detrimental to the quality of their breast meat. The birds of two fast growing genotypes (Debut et al., 2005) were handled with minimal stress, submitted to a hanging stress of 2 minutes, or to 3.5 h of heat stress followed by hanging. Blood levels of corticosterone confirmed the additive effects of both stress conditions. Observation of the birds during shackling with recording of vocalizations showed that the slow growing line was the most active and the heavy line was the less active, while the fast growing line was intermediate. Both genotypes and stress altered post mortem acidification of the meat. The values of early pH increased for the heaviest birds in relation to their lower level of activity on the shackle line, while they decreased with the hanging duration. Heat stress induced no further decrease. Within each genotype, we observed a strong negative correlation between pH15 and wing flapping duration. The values of ultimate pH were lowest for the slow growing genotype, highest for the standard fast growing genotype, and intermediate for the heavy genotype and they decreased following the heat plus hanging stress. Within each genotype, the pHu was negatively correlated with the glycolytic potential (-0.42 to -0.66). Finally the technological yield was mostly altered by stress in the slow growing genotype while it was almost unaltered in the other two genotypes.

In conclusion, we have accumulated data showing that variations in growth rate between genotypes have an impact on meat quality parameters but that there is no antagonism between growth rate or muscle development and breast meat quality parameters such as water retention and processing ability. Part of the differences between genotypes might result from the differences in their susceptibility to pre-slaughter stress. As quality traits are highly heritable, this suggests a possibility to select for meat quality without altering growth rate because of their low genetic correlation. We have shown that pHu could be a target for selection but because of its requirement for slaughtering birds and selecting on collaterals we believe that there is a need for identifying molecular markers allowing for marker assisted selection. This approach has been recently carried out using divergently selected lines of broiler with high or low growth rate, or with high or low adiposity at the same body weight (F2 population) and looking for identification of QTL governing growth and body composition (Lagarrigue et al., 2005). Simultaneously, microarrays are being used to compare global gene _expression profiles (Cogburn et al., 2003). The combination of both strategies is expected to identify double functional and positional candidates, as a step toward the identification of the genes explaining the differences between genotypes in order to propose to the breeder's companies marker assisted selection schemes.

Reinforcing natural egg defence by genetic

Even if the egg is a complex biological system which provides aseptically safe environment to the embryo, infections can occur, especially when eggs are in contact with faeces which might occur more frequently in alternative systems of egg production. Internal egg content can be contaminated after bacteria penetration (Messens *et al*, 2005). Two major natural antimicrobial mechanisms have been identified in egg. Firstly, the eggshell, together with the cuticle constitutes a physical barrier against bacteria penetration. Alteration in eggshell integrity is directly related to increased risks of egg contamination. The second defence is the chemical barrier composed of several proteins present in the eggshell membranes and in the albumen that exhibit antimicrobial activities. Our strategy has been to look at the feasibility of reinforcing these systems by genetic (quantitative genetic and candidate gene approach).

Mechanical defences: identification of eggshell matrix components

The avian eggshell is made of an organic matrix (3.5%), comprising the eggshell membranes, the cuticle and some constituents embedded in the layer of calcium carbonate. Since 1990, numerous efforts have been carried out to identify and characterize the protein components of the calcified shell. These previously identified matrix proteins can be divided in 3 groups according to their characteristics (Nys et al., 2004).

1) The major egg white proteins have been identified in the eggshell. They are ovalbumin (Hincke, 1995), lysozyme (Hincke et al., 2000) and ovotransferrin (Gautron et al., 2001a). These proteins are synthesized and secreted by the uterus (organ of eggshell calcification) and are mainly localized in the basal layer of the shell.
2) The second group is made of ubiquitous proteins widely expressed in other tissues. Osteopontin is a phosphorylated glycoprotein present in the bone, kidney.

This protein was shown to be expressed in the chicken uterus and localized in the shell (Pines et al., 1994). A widely secretory protein, clusterin, was also identified as an eggshell and egg white protein (Mann et al., 2003). It is thought to function as an extracellular chaperone.

3) The third group is constituted of specific eggshell matrix proteins. These proteins are only synthesized by oviduct regions where eggshell takes place (red isthmus and uterus) and are unique to the eggshell. Ovocleidin-17 was the first eggshell protein purified from the shell (Hincke et al., 1995). Ovocleidin-116 (OC-116), (Hincke et al., 1999) found to be the protein core of a 120-/200- kDa eggshell dermatan sulfate proteoglycan (Carrino et al., 1997). Ovocalyxin-32 (OCX-32) is predominately found in the upper part of the calcified shell (Gautron et al., 2001b). Ovocalyxin-36 corresponds to a 36 kDa component of the uterine fluid, eggshell and eggshell membrane (Gautron et al., 2006). OCX-36 mRNA _expression was 17-fold higher in uterine tissue collected during eggshell formation, compared to the period when no shell was in formation. Finally, two additional proteins (Ovocalyxin-25 and Ovocalyxin-21) have also been cloned and identified as novel eggshell matrix proteins (Gautron, Murayama, Hincke, Nys, in preparation).

The recent publication of 90-95% of the chicken genome makes it possible to explore the egg proteome using mass spectrometry-based high-throughput methods as shown recently for the acid-soluble organic matrix of the chicken calcified eggshell layer (Mann et al., 2006). Using these methods 520 different proteins were identified as constituents of the eggshell matrix, including all matrix proteins known before. The highly abundant group corresponded to 32 proteins including all previously identified specific eggshell proteins, known as ovocleidins and ovocalyxins. It is noteworthy that the range of proteins relative to their previous identification and function was very large. The complex mixture contained egg white proteins, extracellular growth factors and many other signal transduction chain components, lipid-binding proteins, immune system-related and antimicrobial proteins and also some previously uncharacterized proteins of unknown origin and function.

Mechanical defences: Putative function of matrix proteins

Eggshell matrix proteins are involved in the fabric of the eggshell and consequently influence its resulting mechanical properties. This hypothesis was confirmed by numerous experimental observations i.) Presence in eggshell of novel proteins only secreted by tissues where eggshell calcification takes place and that are highly stimulated during the calcification process. ii.) Different profile of eggshell matrix proteins in the uterine fluid at each stage of eggshell calcification process that demonstrated an adaptation of the matrix composition depending of the calcification process. iii.) *In vitro* modification of calcite crystal morphology when crystals are grown in presence of matrix components showing an interaction of mineral and organic compounds (Dominguez-Vera et al., 2000). A similar effect was observed when isolated matrix proteins (lysozyme and ovotransferrin) were added into the milieu (Hincke et al., 2000; Gautron et al., 2001a). iv.) Furthermore a relationship between changes in eggshell mechanical properties induced by hen aging or moult and level of matrix components were established *in vivo* (Ahmed et al., 2005).

Some egg white proteins (ovotransferrin and lysozyme), well known for their antimicrobial properties, have been identified in egg shell (Gautron et al., 2001a; Hincke et al., 2000). We have cloned Ovocalyxin-36, a novel eggshell specific matrix protein that showed significant identities with lipopolysaccharide binding proteins, bactericidal permeability increasing proteins and the PLUNC family of proteins (Gautron *et al.*, 2006). These proteins are often described as "first-line host defence proteins" and could be involved in the innate immune response.

Mechanical defenses: Marker assisted selection

Recent efforts to improve eggshell quality by genetic selection have been based on breaking strength, but this does not describe all the components of the shell which prevent breakage or bacterial penetration. Methods to measure these components may be too difficult or time consuming to perform and are not possible for sires. We have therefore adopted a candidate gene association analysis approach (Dunn et al., 2006) with alleles of ovocleidin-116, osteopontin, ovocalyxin-32, ovotransferrin, ovalbumin and ovocalyxin-36, genes which contribute to the organic matrix of the eggshell to discover potential markers for use in selection. A number of novel and traditional measurements which included acoustic resonance, quasi static compression and the thickness of the mammillary and palisade layers of the eggshell, were measured in 1980 pedigree Rhode Island red hens and used in the analysis. Single nucleotide polymorphism (SNP) markers were found in all the genes including SNPs causing non-synonymous amino acid changes in ovocleidin-116 and ovocalyxin-32. Significant associations were found between alleles of i) ovalbumin and quasi-static compression measurements (P=0.012-0.031), and total shell thickness (0.007) and thickness of the mammillary layer (0.011) ii) ovocleidin-116 and mammillary layer thickness (P=0.003), and shell stiffness (P=0.019) iii) ovocalyxin 32 and quasi-static compression measurements (P=0.001-0.038), the ratio of effective thickness to mammillary layer thickness (P=0.05) and mammillary layer thickness (P=0.025). The effects of the markers are up to 30% of trait standard deviations calculated as allele substitutions and may be useful to improve eggshell quality.

Heritability of egg white antimicrobial activity

Our aim was to test the putative efficiency of a selection to increase antimicrobial egg's natural defences. The repeatability and heritability of growth inhibition by egg albumen of two major pathogenic bacteria, a gram-negative (*Salmonella* Enteritidis, SE) and a gram-positive (*Staphyloccocus* Aureus, SA) and of two antimicrobial albumen proteins, lysozyme and ovotransferrin quantified by ELISA, were estimated in commercial pedigreed hens. Repeatability for bacterial growth in albumen ranged from 0.29 to 0.39 for S.E. number one day post inoculation (p.i.) but was lower and more variable at 5 days p.i or for S.A. number. Repeatabilities were low and variable for total egg white protein or lysozyme and ovotranferrin levels (0 to 0.22). Negative phenotypic correlations were observed between lysozyme or ovotransferrin levels and S.E number. Heritabilities were inferior to 0.1 for protein traits. They were equal to 0.1 for S.A. number and to 0.16 for S.E. number one day p.i.. It seems therefore more efficient to select on global bacterial growth than on specific antimicrobial proteins, the most promising trait being the number of S.E one day p.i..

In conclusion, the recent development of high-throughput methods and the availability of the chicken genome sequence will allow the identification of hundred of novel minor components of the egg. It is obvious that the list of egg proteins and genes

coding for them by oviduct tissues will increase and this screening step might be achieved in a very near future. The following step will be to assess the physiological functions of these proteins, through the analysis of their biological activities (enzymatic or antimicrobial activities for example) or using bioinformatic analysis of protein sequences for the identification of bioactive domains or motifs that defines properties and activities of proteins. The lowering cost of gene sequencing and identification of SNP should allow the development of marker assisted selection. The study of functions of proteins and of their potential for industrial application should be the main challenge for the next years.

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